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## Conifer Biodiversity in Mongolia and Adjacent Regions of Russia Using Morphological, Karyological and Genetical Features

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### Abstract

Research into the intraspecific biodiversity of four species of conifers was carried out in different regions of Mongolia and adjacent regions of Russia and Kazakhstan (Markakol, Zaysan). The structures of *Larix sibirica*, *L. gmelinii*, *Pinus sylvestris*, and *P. sibirica* populations were studied using morphological, karyological and biochemical features. Striking similarities in intraspecific polymorphism of conifer populations in Mongolia and in Southern Zabaikalje were revealed. At the same time, some specific structural characteristics of Mongolian populations were observed. Studies of variability of morphological features in conifers have shown that in most cases these parameters were close to those in adjacent regions of Russia, in particular in Zabaikalje. Only some insignificant differences were noted. Populations of *L. sibirica* and *L. gmelinii* in Mongolia and South Siberia (Southern Zabaikalje) exhibited similar karyological features. The chromosomes of *P. sylvestris* from Southern Zabaikalje differ from those of Bogdo-Ula, however the differences are insignificant. Investigations on genetic polymorphism of *L. sibirica* from Mongolia and adjacent regions in Russia showed that the level of genetic variability varied in the larch populations studied, but the differences between populations were insignificant. Isolated steppe forests of *P. sylvestris* near Mongolia had significant genetic variability.

**Key words:** morphological, karyological and genetical, features, biodiversity, conifers, structure of populations

### Introduction

The southern border of the boreal zone is found in Mongolia and is made up of a distribution of many forest-forming coniferous species such as *Larix sibirica* Ledeb., *Pinus sibirica* Du Tour, *P. sylvestris* L., *Picea obovata* Ledeb., *Abies sibirica* Ledeb.. Milyutin *et al.*, (1988) studied species variability regularities near the southern borders of the plants' range and discovered important information on the evolutionary and genetic features that has been used to improve breeding and seed production.

This research is also of interest because these isolated (marginal) populations of *Larix sibirica*, *Pinus sylvestris*, *P. sibirica*, *Picea obovata*, *Abies sibirica* are near the southern borders of their range. In such populations the effect of selection and the formation of population structures are specific to each species (Scotch pine in Southern Siberia, 1988). As a whole, marginal populations of woody plants are characterized by specific structural features and levels of polymorphism (Milyutin, 1991). The purpose of this study is to analyze variability of forest-forming conifer species of Mongolia using morphological, karyological and genetical (isoenzymes) features.

### Materials and methods

#### Plant materials

The biodiversity of conifers in Mongolia and adjacent territories in Russia was studied using different methods: morphological, karyological, genetical (isoenzyme analysis). The morphological features of generative and vegetative organs of *Larix sibirica*, *L. gmelinii*, *L. czekanowskii*, *Pinus sylvestris*, *P. sibirica* were analyzed mainly in East Khentii (Milyutin *et al.*, 1988).

This paper deals with data from karyological studies of *L. sibirica* provenances in Central Khangai (Tosontsengel, 1800-2000 m ASL, floodlands of the r. Tchuluut, 2200m ASL, mountain pass Solgotoin-Daba, 2400m ASL), and the regions of Khakasia and Tuva. *L. gmelinii* populations near Bayan-Ula (mountain ridge Eren-Daba, 1000m ASL), and Southern Zabaikalje (Muratova, 1994) were also studied. *P. sylvestris* was studied by A. V. Suntsov (Milyutin *et al.*, 1988) in a nature reserve in Bogdo-Ula (1500m ASL) in Mongolia and by E. N. Muratova and A. V. Suntsov in Southern Zabaikalje (Buryatia) and Tuva (Scotch pine in Southern Siberia, 1988). The locations of studied populations are presented in Fig. 1.

#### Morphological observation

Morphological features were studied using standard methods: mean values and levels of variability of quantitative features were determined; the frequency in

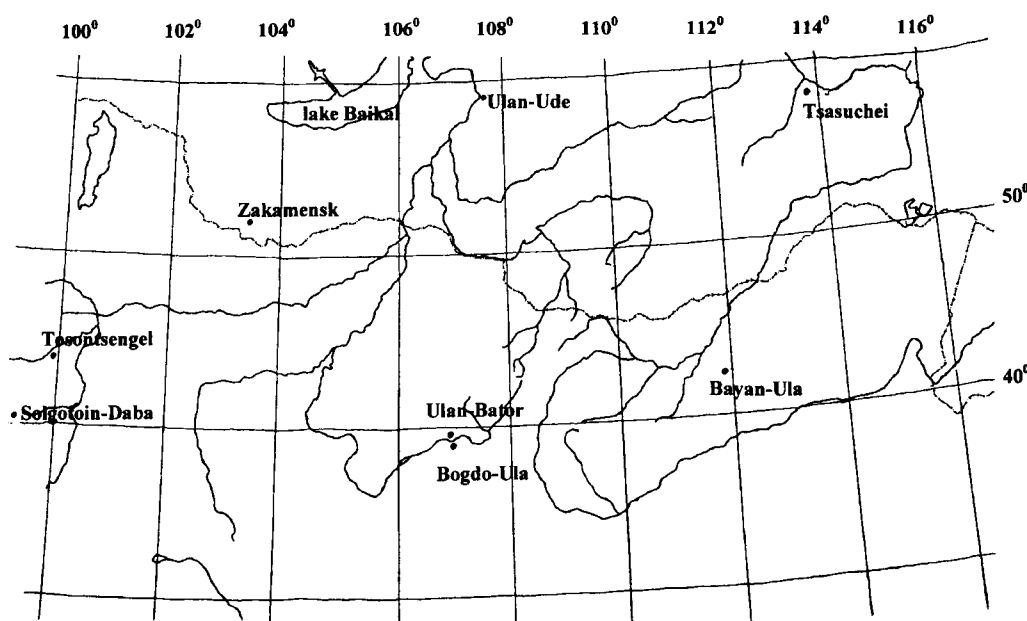


Fig. 1. The location of studied populations of *Larix gmelinii*, *L. sibirica*, *Pinus sylvestris*, *P. sibirica*.

populations of qualitative features was studied. Special hybrid index method (Anderson, 1949) was used in certain cases, for example, as part of the study of the structure of *L. czekanowskii* hybrid populations

#### Karyological investigation

For karyological investigations, seeds were germinated in Petri dishes under laboratory conditions (25-27°C). The materials were prepared and analyzed according to generally accepted techniques for coniferous plants (Pravdin *et al.*, 1972) with some modifications in pretreatment and staining.

The germinating seeds i.e. root tips were pretreated in a 0.5% colchicine solution for 6-8 hours, fixed in 3:1 ethanol:acetic acid mixture and stained with acetohematoxylin. Root tip meristem cells were used for the study, and slides were prepared using the improved squash technique. Suitable cells were selected for analysis and photographed.

The chromosomes were measured on photomicrographs. The following parameters were determined: absolute length of each chromosome ( $L^a$ , in micrometers); the total diploid complement chromosome length ( $\Sigma L^a$ , in micrometers), relative chromosome length ( $L^r$ , the ratio of absolute length to the total chromosome length, %); centromeric index ( $I^c$ , the ratio of the short arm length to absolute chromosome length, %) and localization of secondary constriction (sc, %). To stain nucleoli a 50% solution of silver nitrate was used (Muratova, 1995).

#### Electrophoresis of isoenzymes

Studies of genetic polymorphism of *L. sibirica* from Mongolia and adjacent regions of Russia (Khakasia, Buryatia, Irkutsk Priangarje – basin of Angara river in Irkutsk region) were carried out by electrophoresis of isoenzymes. Populations included for investigation were represented by bulk seed lots, each of which were

collected from more than 30 trees. The macrogametophytes of seeds were used as material for isoenzyme analyses. Seeds were germinated until the appearance of a root. Then the hard covers were discarded and the macrogametophytes were homogenized in extraction buffer (0.1M tris-HCl pH 7.5).

Electrophoretic separation of homogenates was conducted in vertical chambers on polyacrylamide and starch gels. 100-200 macrogametophytes were analyzed in each population. Histochemical detection of the isoenzymes was performed according to conventional protocols (Brewer, 1970) with minor modifications. Five enzyme systems: leucine aminopeptidase (LAP, EC 3.4.11.1), acid phosphatase (APH, EC 3.1.3.2), esterase (EST, EC 3.1.1.1), malate dehydrogenase (MDH, EC 1.1.1.37), isocitrate dehydrogenase (IDH, EC 1.1.1.42) encoded by 12 loci were included in the investigation.

For the study of *P. sylvestris* genetic polymorphism in an isolated population in the southern part of East Zabaikalie near Mongolia (Tsasuchaysky pine forest in the Chita region) vertical and horizontal starch gel electrophoresis were applied. In this population nine enzyme systems: leucine aminopeptidase (LAP, EC 3.4.11.1), malate dehydrogenase (MDH, EC 1.1.1.37), isocitrate dehydrogenase (IDH, EC 1.1.1.42), alcohol dehydrogenase (ADH, EC 1.1.1.1), 6-phospho gluconate dehydrogenase (6-PGD, EC 1.1.1.44), glutamate dehydrogenase (GDH, EC 1.4.1.2), aspartate aminotransferase (AAT, EC 2.6.1.1), phosphor glucomutase (FGM, EC 2.7.5.1), diaphorase (DIA, EC 1.6.4.3) encoded 19 loci were assayed. Experiments were performed with seeds harvested from individual trees.

The level of genetic variability in the populations studied was estimated according to indices commonly used in population genetics: percentage of polymorphic

loci at 99% polymorphism criterion ( $P_{99}$ ), the mean number of alleles per locus ( $A$ ), the effective number of alleles per locus ( $n_e$ ), the average expected ( $H_e$ ) and the average observed ( $H_o$ ) heterozygosity. To determine the level of gene diversity the parameters of G-statistics ( $H_t$  – the total gene diversity,  $H_s$  – the average gene diversity within each population,  $G_{st}$  – relative magnitude of gene differentiation among populations) were calculated (Nei, 1972, 1973). Nei's genetic distance coefficient ( $D$ ) was used to quantify the degree of differentiation between populations; it is the generally accepted method for analysis of gene diversity.

## Results and Discussion

### Variability of morphological features

Research on the morphological variability of conifers showed that in most cases the parameters of these features were close to ones in adjacent regions of Russia (southern region of Buryatia and Chita region), in particular Zabaikalje. Only some insignificant differences were noted. For example, variability of cone length for *L. sibirica* in East Khentii was more pronounced depending upon zone altitude. Cone length in a high-altitude zone was smaller. Some populations of *L. sibirica* (the Bogdo-Ula reserve) had an unusually high frequency of polyembryonal seeds (in 94% of the trees studied). An extreme variability in seed quality was noted in Mongolian populations of *L. sibirica*. For example, the coefficient of variation of seed weight amounted to 12% in a certain East Siberia region – southern parts of Krasnoyarsk Territory and Irkutsk region, Khakasia (Iroshnikov, Milyutin *et al.*, 1974). At the same time in East Khentii, this parameter was 32%. Other indices of seed quality were different too: germinative energy was 60% (East Siberia) and 90% (East Khentii); germinating ability was 65% and 93%; percentages of full seeds were 30% and 39%, respectively.

Some features of interspecific interrelations of *L. sibirica* and *L. gmelinii* were revealed in Mongolia. *L. sibirica* is dominant but in other regions *L. gmelinii* dominates. One small population of *L. gmelinii* is found

in Mongolia (Bayan-Ula region in northeastern Mongolia). *L. sibirica* is a widely distributed in Mongolia. It forms different intraspecific taxons, for example, climatic ecotypes (climatypes). The differences in larch climatotypes in Mongolia were published by C. Zham'yansuren (1992).

Studies on the intraspecific variability of *P. sylvestris* in Mongolia were done by A. V. Suntsov (Milyutin *et al.*, 1988). It was shown that the structure of Mongolian populations of this species had many features similar to ones in Southern Siberia and Zabaikalje. Lower seed quality and a reduction in seed-lobe numbers were noted in the isolated populations of Mongolia (Bogdo-Ula).

The variability in morphological features in *P. sibirica* was studied by A. V. Suntsov in the Bogdo-Ula reserve (Milyutin *et al.*, 1988). It was shown that there were no significant differences from those in Southern Siberia. *P. sibirica* grows in pessime conditions near the steppe border. Therefore, in A. V. Suntsov's opinion the selection of drought-resistant forms of this species is possible in Mongolia.

### Karyotype analysis

Karyological studies on Mongolian populations of *L. sibirica*, *L. gmelinii* and *P. sylvestris* showed the presence of 24 chromosomes ( $2n=24$ ) in diploid sets. Karyotypes of two larch species (*L. sibirica* and *L. gmelinii*) contained 6 pairs of long symmetric (metacentric) chromosomes and 6 pairs of short asymmetric (submetacentric) ones. Chromosomes of these two groups as a rule can't be identified.

Chromosome parameters of *L. sibirica* are given in Table 1. The chromosomes had the following parameters: I – VI  $L^a = 14.7 \pm 0.14 \mu m$ ,  $L^r = 4.6 \pm 0.03\%$ ,  $I^c = 47.0 \pm 0.24\%$ ; VII – XII  $L^a = 10.1 \pm 0.08 \mu m$ ,  $L^r = 3.1 \pm 0.02\%$ ,  $I^c = 32.3 \pm 0.26\%$ .

Among metacentric chromosomes there were two pairs (III – IV) of nucleolar chromosomes in *L. sibirica*. They had secondary constrictions located in the distal regions of the arms.

In the *L. gmelinii* karyotype secondary constrictions occurred in III-IV and VII pairs of chromosomes. All nucleolar chromosomes had secondary constrictions in

Table 1. Morphometric characteristics of *Larix sibirica* Ledeb. Chromosomes.

| Chromosome numbers | Absolute length ( $L^a$ ), $\mu m$ |       | Relative chromosome length ( $L^r$ ), % |       | Centromeric index ( $I^c$ ), % |       | Localization of secondary constriction (sc), % |       |
|--------------------|------------------------------------|-------|---|-------|--------------------------------|-------|--|-------|
|                    | Mean $\pm$ SE                      | CV, % | Mean $\pm$ SE                           | CV, % | Mean $\pm$ SE                  | CV, % | Mean $\pm$ SE                                  | CV, % |
| I-II               | $14.7 \pm 0.14$                    | 8.5   | $4.6 \pm 0.03$                          | 6.1   | $47.0 \pm 0.24$                | 4.4   | –  |       |
| III-IV             | $14.7 \pm 0.14$                    | 8.5   | $4.6 \pm 0.03$                          | 6.1   | $47.0 \pm 0.24$                | 4.4   | $62.8 \pm 0.41$                                | 5.1   |
| V-VI               | $14.7 \pm 0.14$                    | 8.5   | $4.6 \pm 0.03$                          | 6.1   | $47.0 \pm 0.24$                | 4.4   | –  |       |
| VII-XII            | $10.1 \pm 0.08$                    | 9.8   | $3.1 \pm 0.02$                          | 7.2   | $32.3 \pm 0.26$                | 9.9   | $41.6 \pm 0.75$                                | 12.7  |

Total  $297.6 \pm 2.56 \mu m$

the distal regions of the arms. The chromosome parameters of *L. gmelinii* karyotype were: I – VI  $L^a = 15.1 \pm 0.21 \mu\text{m}$ ,  $L^r = 4.8 \pm 0.05\%$ ,  $I^c = 46.5 \pm 0.37\%$ ; VII  $L^a = 12.1 \pm 0.29 \mu\text{m}$ ,  $L^r = 3.9 \pm 0.07\%$ ,  $I^c = 30.6 \pm 1.14\%$ ; VIII – XII  $L^a = 10.1 \pm 0.10 \mu\text{m}$ ,  $L^r = 3.3 \pm 0.03\%$ ,  $I^c = 33.0 \pm 0.27\%$  (Table 2). Some preparations showed one B-chromosome of meta- or submetacentric types ( $B_1$   $L^a = 5.3 \pm 0.23 \mu\text{m}$ ,  $L^r = 1.5 \pm 0.01\%$ ,  $I^c = 47.4 \pm 1.60\%$ ;  $B_2$   $L^a = 4.8 \pm 1.13 \mu\text{m}$ ,  $L^r = 1.4 \pm 0.01\%$ ,  $I^c = 39.9 \pm 1.05\%$ ).

In *L. sibirica* and *L. gmelinii* from Mongolia and adjacent regions in Russia (Tuva, Buryatia, Khakasia), and Kazakhstan's Altai genome and chromosome mutations were found (Table 3). Among genome mutations, there were aneuploids, mixoploids and in some cases polyploids; among the chromosome mutation rings and polycentric chromosomes occurred. Pericentric inversion in *L. gmelinii* was found for the first time for conifers (Muratova, 1994). Examples of

chromosome anomalies and B-chromosomes of these species are given in Fig. 2.

Two populations of larch species in Mongolia and Southern Siberia were found to have some similar karyological features. Mixoploidy and aneuploidy were found in samples of *L. sibirica* from Mongolia and Tuva. Three Mongolian populations of *L. sibirica* were similar to populations from Southern Zabaikalje (Zakamensk) close to Mongolia in the number of nucleolar regions in chromosomes and nucleoli in interphase nuclei. The karyotype of *L. gmelinii* in Bajan-Ula was similar to the karyotype of this species in Eastern Zabaikalje. It can be explained by close proximity of the two areas.

The karyotype of *P. sylvestris* included 10 pairs of long symmetric (metacentric) chromosomes. Only I and X pairs of chromosomes could be identified among them. Chromosomes of XI and XII pairs were shorter and asymmetric (submetacentric or almost

Table 2. Morphometric characteristics of *Larix gmelinii* (Rupr.) Rupr. Chromosomes.

| Chromosome numbers | Absolute length ( $L^a$ ), $\mu\text{m}$ |       | Relative chromosome length ( $L^r$ ), % |       | Centromeric index ( $I^c$ ), % |       | Localization of secondary constriction (sc), % |       |
|--------------------|--|-------|---|-------|--------------------------------|-------|--|-------|
|                    | Mean $\pm$ SE                            | CV, % | Mean $\pm$ SE                           | CV, % | Mean $\pm$ SE                  | CV, % | Mean $\pm$ SE                                  | CV, % |
| I-II               | 15.1 $\pm$ 0.21                          | 13.7  | 4.8 $\pm$ 0.05                          | 10.5  | 46.5 $\pm$ 0.37                | 6.9   | –  |       |
| III-IV             | 15.1 $\pm$ 0.21                          | 13.7  | 4.8 $\pm$ 0.05                          | 10.5  | 46.5 $\pm$ 0.37                | 6.9   | 60.0 $\pm$ 0.71                                | 10.5  |
| V-VI               | 15.1 $\pm$ 0.21                          | 13.7  | 4.8 $\pm$ 0.05                          | 10.5  | 46.5 $\pm$ 0.37                | 6.9   | –  |       |
| VII                | 12.1 $\pm$ 0.29                          | 9.5   | 3.9 $\pm$ 0.07                          | 7.2   | 30.6 $\pm$ 1.14                | 14.3  | 62.0 $\pm$ 0.88                                | 7.5   |
| VIII-XII           | 10.1 $\pm$ 0.10                          | 15.8  | 3.3 $\pm$ 0.03                          | 15.2  | 33.0 $\pm$ 0.27                | 12.8  | –  |       |
| $B_1$              | 5.3 $\pm$ 0.23                           | 7.5   | 1.5 $\pm$ 0.01                          | 3.2   | 47.4 $\pm$ 1.60                | 5.9   | –  |       |
| $B_2$              | 4.8 $\pm$ 1.13                           | 13.5  | 1.4 $\pm$ 0.01                          | 3.5   | 39.9 $\pm$ 1.05                | 5.3   | –  |       |

Total 311.4  $\pm$  3.52  $\mu\text{m}$

Table 3. Chromosome mutations in seedlings of *Larix sibirica* and *L. gmelinii*.

| Location   | Number of observed seedlings / cells | Cells with mutations Number / percent |
|--|--------------------------------------|---------------------------------------|
| <i>L. sibirica</i> , Kazakhstan's Altai (Markakol) | 24 / 103                             | 4 / 3.9                               |
| <i>L. sibirica</i> , Kazakhstan's Altai (Zaysan)   | 35 / 306                             | 23 / 7.6                              |
| <i>L. sibirica</i> , Khakasia (Sonsky)             | 48 / 521                             | 1 / 0.2                               |
| <i>L. sibirica</i> , Tuva (Shagonar)               | 43 / 526                             | 12 / 2.3                              |
| <i>L. sibirica</i> , Tuva (Balgazin)               | 35 / 400                             | 4 / 1.0                               |
| <i>L. sibirica</i> , Tuva (Chagitai)               | 30 / 593                             | 2 / 0.3                               |
| <i>L. sibirica</i> , Buryatia (Zakamensk)          | 42 / 351                             | 2 / 0.6                               |
| <i>L. sibirica</i> , Mongolia (Tosontsengel)       | 38 / 352                             | 6 / 1.7                               |
| <i>L. sibirica</i> , Mongolia (Tchuluut)           | 22 / 145                             | 9 / 6.2                               |
| <i>L. sibirica</i> , Mongolia (Solgotoin-Daba)     | 31 / 120                             | 9 / 0.9                               |
| <i>L. gmelinii</i> , Chita region (Karymskoye)     | 63 / 1088                            | 3 / 0.3                               |
| <i>L. gmelinii</i> , Mongolia (Bayan-Ula)          | 20 / 150                             | 3 / 2.0                               |

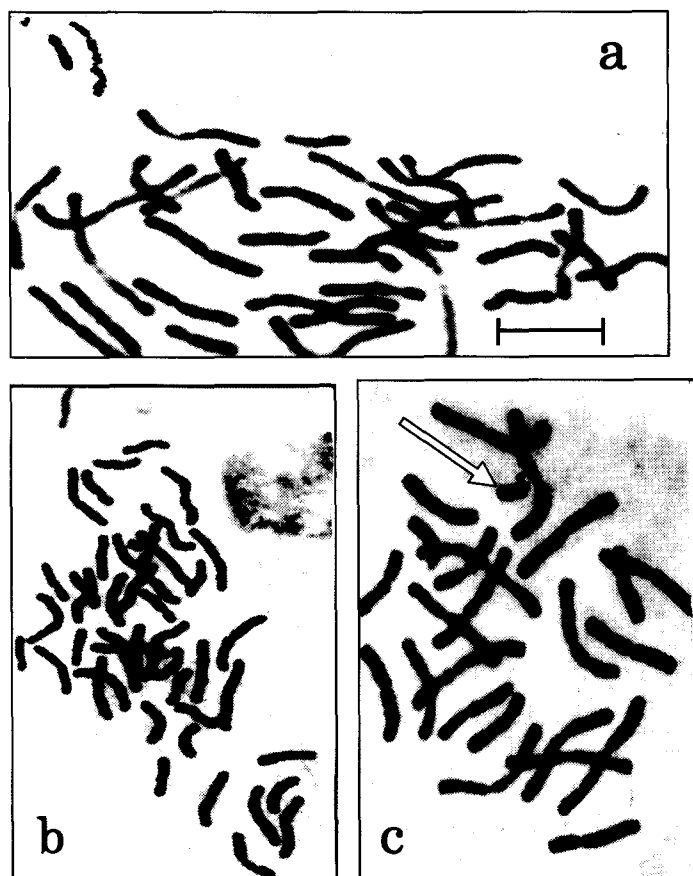


Fig. 2. Chromosome anomalies in *Larix* in Southern Zabaikalje and Mongolia:  
 a – triploid cell ( $2n=3x=36$ ) in *Larix gmelinii*; bar indicates 5  $\mu\text{m}$ ;  
 b – tetraploid cell ( $2n=4x=48$ ) in *L. sibirica*;  
 c – B-chromosome in *L. gmelinii*; arrow points to B-chromosome.

Table 4. Morphometric characteristics of *Pinus sylvestris* L. chromosomes

| Chromosome numbers | Absolute length ( $L^a$ ), $\mu\text{m}$ |       | Relative chromosome length ( $L^b$ ), % |       | Centromeric index ( $I^c$ ), % |       | Localization of secondary constriction (sc), %        |                     |
|--------------------|--|-------|---|-------|--------------------------------|-------|---|---------------------|
|                    | Mean $\pm$ SE                            | CV, % | Mean $\pm$ SE                           | CV, % | Mean $\pm$ SE                  | CV, % | Mean $\pm$ SE   | CV, %               |
| I                  | 14.0 $\pm$ 0.14                          | 6.0   | 4.1 $\pm$ 0.12                          | 6.3   | 48.1 $\pm$ 0.11                | 3.3   | 35.6 $\pm$ 0.84<br>50.5 $\pm$ 0.87<br>55.3 $\pm$ 0.99 | 14.6<br>10.5<br>8.4 |
| II – III           | 14.0 $\pm$ 0.14                          | 6.0   | 4.1 $\pm$ 0.12                          | 6.3   | 48.1 $\pm$ 0.11                | 3.3   | 62.8 $\pm$ 0.53                                       | 6.7                 |
| IV – VII           | 14.0 $\pm$ 0.14                          | 6.0   | 4.1 $\pm$ 0.12                          | 6.3   | 48.1 $\pm$ 0.11                | 3.3   | –   |                     |
| VIII               | 14.0 $\pm$ 0.14                          | 6.0   | 4.1 $\pm$ 0.12                          | 6.3   | 48.1 $\pm$ 0.11                | 3.3   | 41.6 $\pm$ 0.75                                       | 12.7                |
| IX                 | 14.0 $\pm$ 0.14                          | 6.0   | 4.1 $\pm$ 0.12                          | 6.3   | 48.1 $\pm$ 0.11                | 3.3   | 55.1 $\pm$ 1.59                                       | 10.2                |
| X                  | 12.1 $\pm$ 0.15                          | 8.1   | 3.6 $\pm$ 0.04                          | 7.6   | 45.7 $\pm$ 0.17                | 3.2   | 55.3 $\pm$ 1.19                                       | 10.1                |
| XI                 | 10.4 $\pm$ 0.15                          | 8.3   | 3.1 $\pm$ 0.03                          | 6.8   | 41.0 $\pm$ 0.30                | 3.9   | 41.9 $\pm$ 1.43                                       | 12.9                |
| XII                | 10.1 $\pm$ 0.20                          | 10.9  | 3.0 $\pm$ 0.03                          | 8.1   | 43.0 $\pm$ 0.25                | 3.6   | 68.9 $\pm$ 2.73                                       | 11.9                |

submetacentric). In A. V. Suntsov's data (Milyutin *et al.*, 1988) the chromosomes of *P. sylvestris* in Bogdo-Ula had the following parameters:

I  $L^a = 16.4 \pm 0.08 \mu\text{m}$ ,  $L^r = 5.0 \pm 0.02\%$ ,  $I^c = 48.2 \pm 0.16\%$ ; II – IX  $L^a = 15.2 \pm 0.13 \mu\text{m}$ ,  $L^r = 4.5 \pm 0.03\%$ ,  $I^c = 47.6 \pm 0.19\%$ ; X  $L^a = 12.2 \pm 0.12 \mu\text{m}$ ,

$L^r = 3.8 \pm 0.03\%$ ,  $I^c = 46.5 \pm 0.23\%$ ; XI  $L^a = 11.6 \pm 0.11 \mu\text{m}$ ,  $L^r = 3.4 \pm 0.02\%$ ,  $I^c = 42.6 \pm 0.24\%$ ; XII  $L^a = 10.1 \pm 0.13 \mu\text{m}$ ,  $L^r = 2.9 \pm 0.02\%$ ,  $I^c = 40.6 \pm 0.30\%$ .

Research showed that all chromosomes in the karyotype were metacentric but centromeric indexes of XI and XII pairs were close to submetacentric. Secondary constrictions occurred in six pairs of chromosomes.

In *P. sylvestris* from Southern Zabaikalje I – X chromosomes were metacentric and XI – XII pairs were close to submetacentric, too. Only the chromosome pairs X, XI, XII could be distinguished. Secondary

constrictions located in I (three constrictions), II–III, VIII, IX, X, XI, XII chromosome pairs (Table 4). Therefore the chromosomes of *P. sylvestris* from Southern Zabaikalje differed from populations of Bogdo-Ula in size of some chromosomes and also in occurrence and localization of secondary constrictions. However these differences were insignificant.

Chromosome mutations were detected in *P. sylvestris* populations from Bogdo-Ula and Zabaikalje. Mainly they occurred in ring and polycentric chromosomes. In comparison to populations from the central portions of the area, frequency of chromosome anomalies near the southern border of the ranges was higher. The extreme environmental conditions are a possible explanation for this phenomenon in these populations. Examples of chromosome mutations in *P. sylvestris* are presented in Fig. 3.

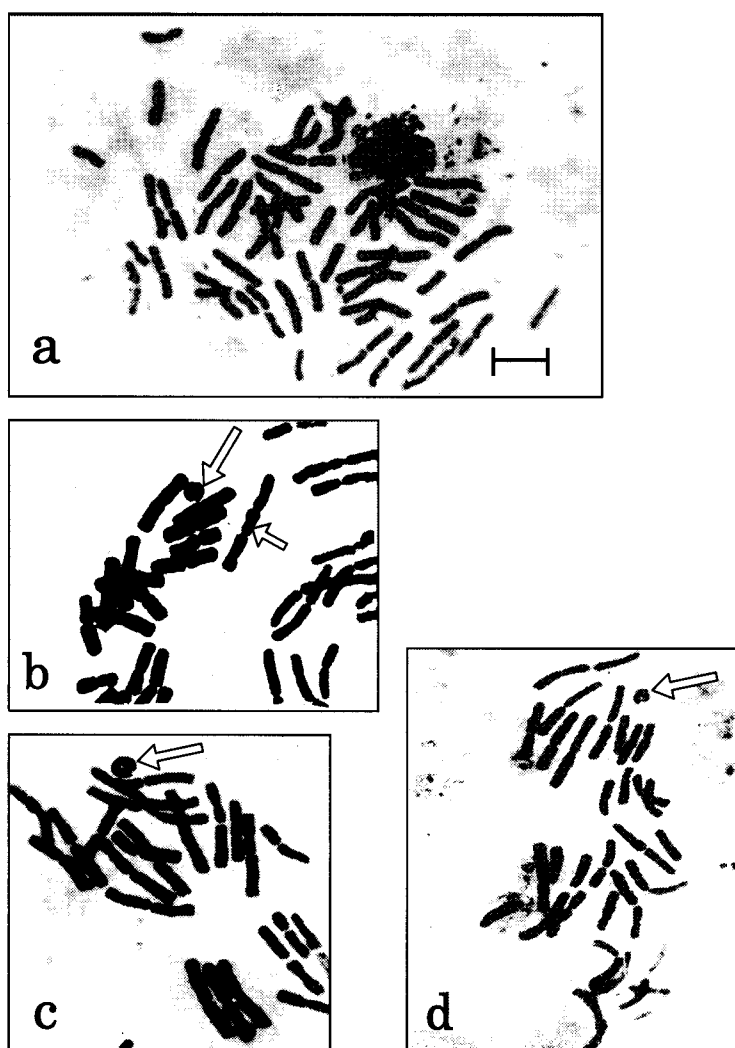


Fig. 3. Chromosome anomalies in *P. sylvestris* in Southern Zabaikalje:

a – tetraploid cell ( $2n=4x=48$ ); bar indicates  $5 \mu\text{m}$ ;

b – chromosome plate with ring and dicentric chromosomes (arrows).

c, d – chromosome plates with ring chromosomes (arrows).

### Isoenzyme analysis

Studies of genetic polymorphism in *L. sibirica* from Mongolia and adjacent regions of Russia showed that the level of genetic variability, established on 12 isoenzyme loci, varied in the larch populations studied (Table 5), but the differences between populations were insignificant. The highest values of all the main parameters of genetic variability were revealed in the larch population from Southern Zabaikalje. Larch from Mongolia had lower values of parameters in comparison to other populations of this species studied.

Analysis of gene diversity of *L. sibirica* with the help of Nei's G-statistics showed that 93% of total genetic diversity was within the population and only 6.98% was among populations ( $G_{st} = 0.0698$ ). The estimates obtained indicate a substantial interpopulation differentiation of *L. sibirica* in the regions investigated. Genetic distance coefficient D calculated on allelic frequencies of 12 loci ranges from 0.026 to 0.069 among populations and averaged 0.052 (Table 6).

The most significant differences were observed between the Mongolian population and the Khakasia (Russia) one. Genetic distance (coefficient D) between populations of these regions was equal to 0.069. Genetic differentiation between populations of *L. sibirica* from Mongolia and from Irkutsk Priangarje (basin of Angara river in Irkutsk region) was less expressed ( $D=0.056$ ). The larches with the most similarities to the ones from Mongolia were the larches from Southern Zabaikalje ( $D=0.044$ ).

A study on genetic polymorphism of *P. sylvestris* from Eastern Zabaikalje showed that the isolated steppe

forests of *P. sylvestris* situated in the south-eastern part of its range near Mongolia has significant genetic variability. Over 60% ( $P_{99} = 63.2\%$ ) of the isoenzyme loci assayed were polymorphic at 99% polymorphism criterion. The mean number of alleles per locus was 1.63. The average observed and expected heterozygosities were 0.237 and 0.251, respectively.

The values obtained are comparable with the estimates established for populations of this species from the Southern Urals [Yanbaev *et al.*, 1989], Central Siberia [Goncharenko *et al.*, 1993] and Southern Zabaikalje [Larionova, 1997]. However the level of genetic variability of *P. sylvestris* from the Tsasuchei Forest (Chita region) was significantly lower than that in marginal pine populations from the European part of the former Soviet Union.

### Conclusion

After analyzing the results of these investigations it is obvious that there are similarities in the biodiversity of conifers from Mongolia and Southern Zabaikalje. A possible explanation is the great degree of floristic propinquity between Southern Siberia and Mongolia. They form a common Mongolian-Southern Siberian group (Malyshev, 1965). Results obtained in the research confirm the supposition of a close relationship between *L. sibirica* populations in Zabaikalje and Mongolia, with the exception of its northwest regions bordering Altai and Sayan (Milyutin, 1980).

It is necessary to note that there is insignificant specificity of populations of Mongolian conifers. Besides the materials mentioned above, provenance trials for larch in Siberia showed similar specificity.

Table 5. Genetic variability at 12 isoenzyme loci in populations of *L. sibirica*.

| Populations         | Percentage of polymorphic loci | Average number of |                               |                             |
|---------------------|--------------------------------|-------------------|-------------------------------|-----------------------------|
|                     |                                | alleles per locus | alleles per polymorphic locus | effective alleles per locus |
| Khakasia            | 75.0                           | 2.08              | 2.30                          | 1.52                        |
| Irkutsk Priangarje  | 75.0                           | 2.00              | 2.20                          | 1.54                        |
| Southern Zabaikalje | 83.3                           | 2.25              | 2.50                          | 1.56                        |
| Mongolia            | 75.0                           | 2.08              | 2.30                          | 1.45                        |

Table 6. Estimates of Nei's (1972) genetic distance coefficient based upon data from 12 loci.

| Populations         | Khakasia | Irkutsk Priangarje | Southern Zabaikalje | Mongolia |
|---------------------|----------|--------------------|---------------------|----------|
| Khakasia            | -        | 0.062              | 0.057               | 0.069    |
| Irkutsk Priangarje  |          | -                  | 0.026               | 0.056    |
| Southern Zabaikalje |          |                    | -                   | 0.044    |

Mongolian provenances of *L. sibirica* and *L. gmelinii* differed appreciably from other provenances which exhibited bad growth and low survival rates in the region of the left bank of the Yenisei River (Varaksin, Milyutin, 1996)

**The results of investigations are the first complex generalization of data on biodiversity of forest forming conifer species in Mongolia. This materials gives new evidences on variability of these species near southern borders of their areas. Furthermore these materials can be foundation for selection and plant breeding in Mongolia.**

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