ISSN 1062-3590, Biology Bulletin, 2010, Vol. 37, No. 6, pp. 574–578. © Pleiades Publishing, Inc., 2010.
Original Russian Text © E.A. Vasutkina, L.S. Lauve, G.D. Reunova, Yu.N. Zhuravlev, 2010, published in Izvestiya Akademii Nauk, Seriya Biologicheskaya, 2010, No. 6, pp. 670–675.

BOTANY

Larch Chromosomal Mosaicism

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Received January 18, 2010

Abstract—Karyological study of larch population members from different parts of the *Larix olgensis* L. Henry areal in the Primorskii krai has been carried out. The main amount of chromosomes for larch as $n = 12$ ($2n = 24$) has been confirmed. Mixoploidy was observed in all studied populations. The difference of individuals from the larch areal based on the cell amount with a different level of ploidity has been found in the population of *L. olgensis* locus classicus and both *L. sibirica* and *L. gmelinii*, which is probably a consequence of their hybrid nature.

DOI: 10.1134/S106235901006004X

Olgan larch *Larix olgensis* L. Henry as The Red Book species is a Pliocene relic belonging to the most ancient larches (Bondarenko, 2007). The larch covers the small areal along the Japanese sea coast and the eastern part of the Sikhote-Alin in the southeastern part of the Primorskii krai on the territory of the Russian Far East. The larch is represented by small isolated regions within its areal (Gudkov, 1976). The larches of the Primorskii krai characterize a high variability of morphological features of generative organs, which makes difficult the determination of species status of some populations from the areal of the Olgan larch.

Earlier, we studied the genetic variability of populations of the Olgan larch using RAPD-markers. It was shown that their greater part is removed from population collected from the described place for this species (Vasutkina et al., 2007; Vasutkina, 2009).

The data of karyology are used in order to solve the point at issue in taxonomy. Sudy of internal and interspecies polymorphism due to the karyotype is the primary important diagnostic feature of a species. The karyotypic diversity and a wide specter of chromosome abnormalities have been found in studies of Siberian and Far Eastern species of conifers, i.e., representatives of genus *Pinus, Larix, Picea*, and *Abies* (Muratova, 1991, 1994, 2000; Muratova et al., 2005).

Larches of the Primorskii krai have a diploid chromosome set typical for the genus *Larix* ($2n = 24$) (Il'chenko, 1973). There are data on changes in this set for larches in the Primorskii krai. According to Il'chenko's studies carried out in 1973 and 1978, both primitive (one pair of chromosomes with secondary chromosomal strangulation) and more organized types of chromosome apparatuses (secondary strangulations in two pairs of metacentric or in two pairs of metacentric and one pair of submetacentric chromosomes) can be found within the populations of larches in the Primorskii krai, and interspecies and interpopulation polymorphism expresses in various frequency of appearance of these karyotypes. In general, the larches of the Primorskii krai differ with a high level of hybridization and formation (Bobrov, 1972). It is known that hybrid plant forms can be characterized by mixoploidy, which is the presence of cells with different levels of ploidity in one somatic tissue (Kunakh, 1980; Butorina, 1989; Butorina, Gavrilov, 2001). The event of polyploidy of cells in somatic tissues during the ontogenesis process is widespread within the plant world (Kunakh, 1980). There are published data on the influence of unfavorable and extreme factors, and alteration of growing conditions can cause physiological breaks resulting in abnormal mitosis and appearance of cells with different levels of ploidity (Kunakh, 1980; Butorina, 1989; Muratova, 1994). It is shown that populations of conifers at the areal borders in extreme habitat conditions and anthropogenically broken ecosystems have morphological alterations of chromosomes and deviations of their amount (Muratova, 2004; Muratova et al., 2005; Kalashnik, 2008; Sedel'nikova, Pimenov, 2007).

This study is aimed at observation of the variability of the amount of chromosomes in somatic tissues of the Olgan larch along the whole areal in the Primorskii krai and data comparison with Siberian and Dahurian larches.

MATERIALS AND METHODS

The growing needles of larch from 11 natural populations of *L. olgensis* and populations of *L. olgensis* locus classicus (from Ol'ga Bay) including AP3 (Arzamazovka River head), CHER1 (Cheremukhovaya River), CHER2 (Cheremukhovaya River),

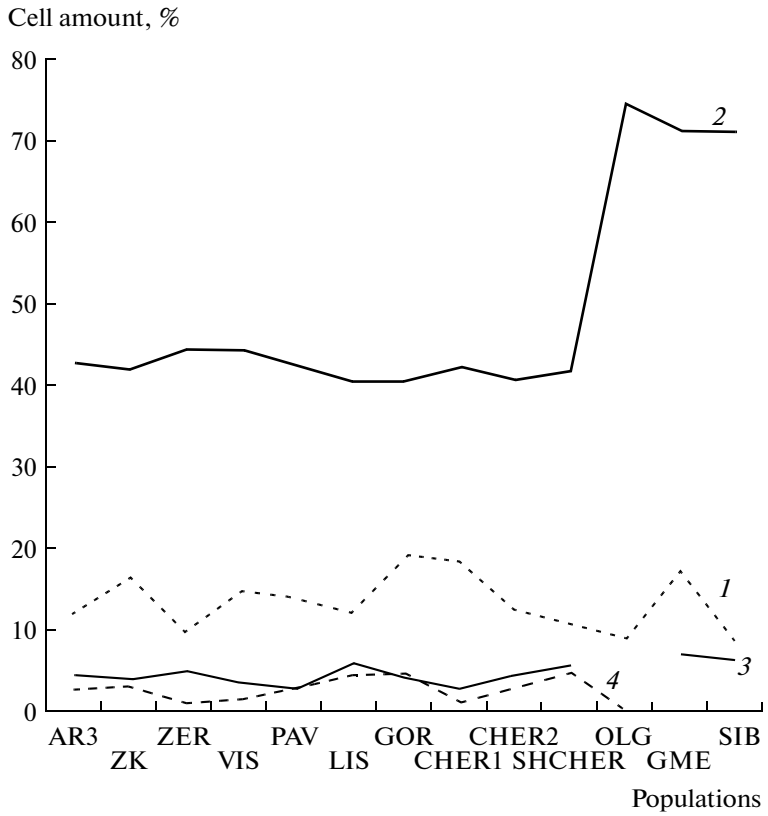

Fig. 1. Amount of haploid (*1*), diploid (*2*), triploid (*3*), and tetraploid (*4*) cells in studied populations of larch (see Materials and Methods for Figs. 1, 3, 4).

GOR (Gorbushka river (Rudnaya River inflow)), LIS (middle flow of Listevennaya River (Margaritovka River inflow)), OLG (Ol'ga Bay), PAV (upper Pavlovka River (Ussuri river inflow)), SHCHER (Shcherbakovka settlement neighborhood), VIS (Visokogorskaya River head), ZER (Zerkal'naya River head), ZK (Zmeinii kluch (Bolshaya Ussurka River inflow)) served as the study material. The needles of Dahurian larch *Larix gmelinii* (Rupr.) Rupr. (GME, Tura settlement neighborhood, Evenk autonomic district) and the Siberian larch *Larix sibirica* Ledeb. (SIB, Lopatka settlement neighborhood, Krasnoyarsk krai) have been used for comparison. Karyological analysis was carried out by the generally accepted method for conifers (Pravdin et al., 1972) with some modifications. The material was fixed in an alcohol–acetic mixture (3 : 1) with preliminary freezing and poisoned in 4% solution of iron alum, stained by aceto-iron-hematoxylin and prepared in pressured preparations by the standard method. Chromosomes were calculated on metaphase plates viewed under an Axioskop-40 microscope (Carl Zeiss, Germany). In all 2894 metaphase cells from 37 trees of Olgan larch, 95 cells from 3 trees of Dahurian larch, and 98 cells from 5 trees of Siberian larch were analyzed. Two hundred eighty-two metaphase cells from 3 trees from ARZ, 231 cells from 3 trees of ZK, 81 cells from one tree in ZER, 260 cells from 3 trees of VIS, 257 cells from 3 trees of PAV, 459 cells from 5 trees of LIST, 172 cells from 2 trees of GOR, 180 cells from 2 trees of CHER1, 247 cells from 3 trees of CHER2, 418 cells from 5 trees of SHCHER, and 307 cells from 7 trees of OLG were studied within the populations of the Olgan larch. Plotting and consecration of the studied populations was performed by the PCA method (Beals, 1984) using the programs Excel (2003) and Statistica 8.0 (StatSoft Inc., 2007), respectively.

RESULTS AND DISCUSSION

The cytology study has confirmed that the diploid chromosome set is equal to 24 for the members of all populations of the Olgan larch. The amount of these cells for individuals of populations from the areal of *L. olgensis* varied from 40.5% (LIS) to 44.4% (ZER), while these cells from the population in Ol'ga Bay (OLG) are 74.7% of the total analyzed amount (Fig. 1). In comparison with the previous larch, the Dahurian and Siberian larch contain more than 71% of diploid cells.

Mixoploidy was observed in all studied preparations besides the main amount of chromosomes (Fig. 2), diploid, aneuploid, haploid, tri-, and tetraploid. The members of population from different parts of the

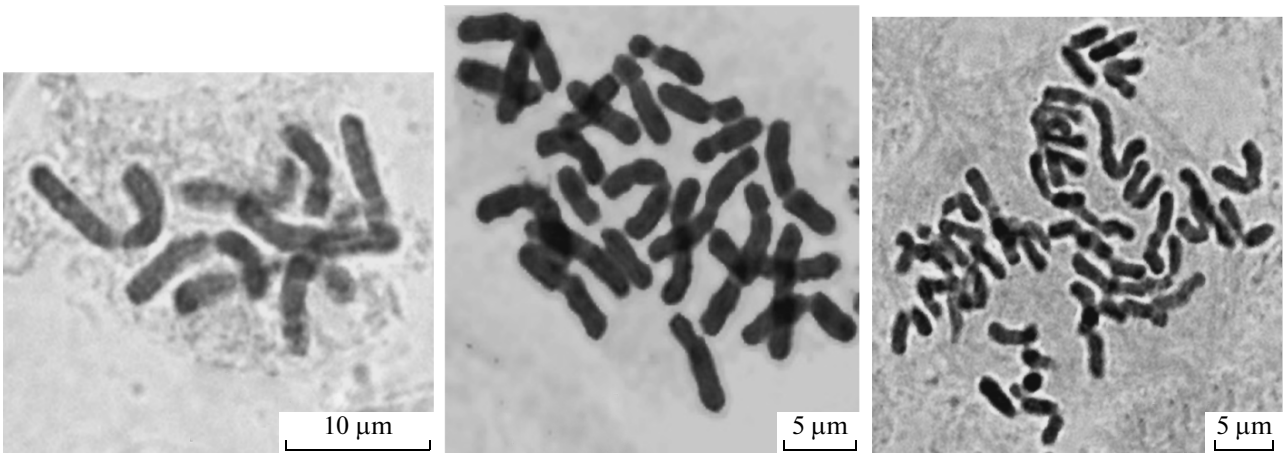

Fig. 2. Cells with different level of ploidy in the Olgan larch: $2n = 8, 24, 42$ (from left). Magnification: × 100, × 10.

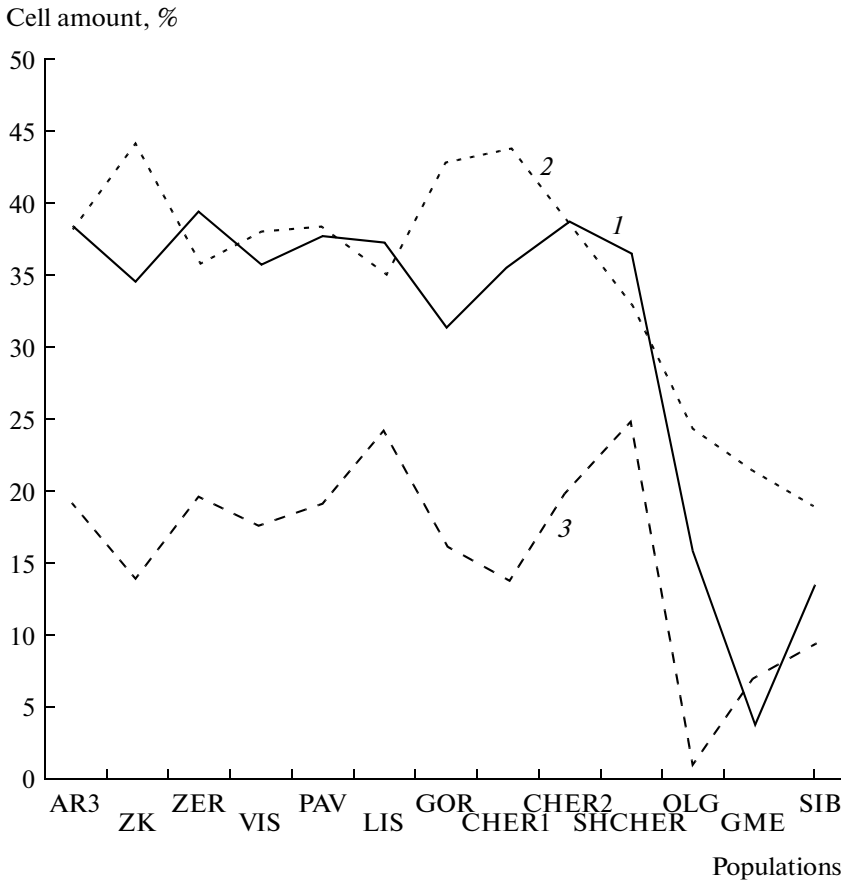

Fig. 3. Amount of aneuploid (*1*), hypodiploid (*2*), and hyperdiploid (*3*) cells in studied larch populations.

Olgan larch areal excluding OLG population are characterized by a similar ratio of cells with various level of ploidity in somatic tissue (Figs. 1, 3). The share of aneuploid cells ($2n = 14, 16, 18, 30, 32, 42$) ranged from 31.4 (GOR) to 39.5 % (ZER) and haploid from 9.9 (ZER) to 19.2% (GOR). The amount of tri- and tetraploid cells in all samples was less 6.0% (Fig. 1).

For individuals of the OLG population from Ol'ga Bay, the amount of aneuploid cells ($2n = 6, 8, 14, 16, 18, 20, 30$) is 16% (Fig. 3), and haploid and tetraploid

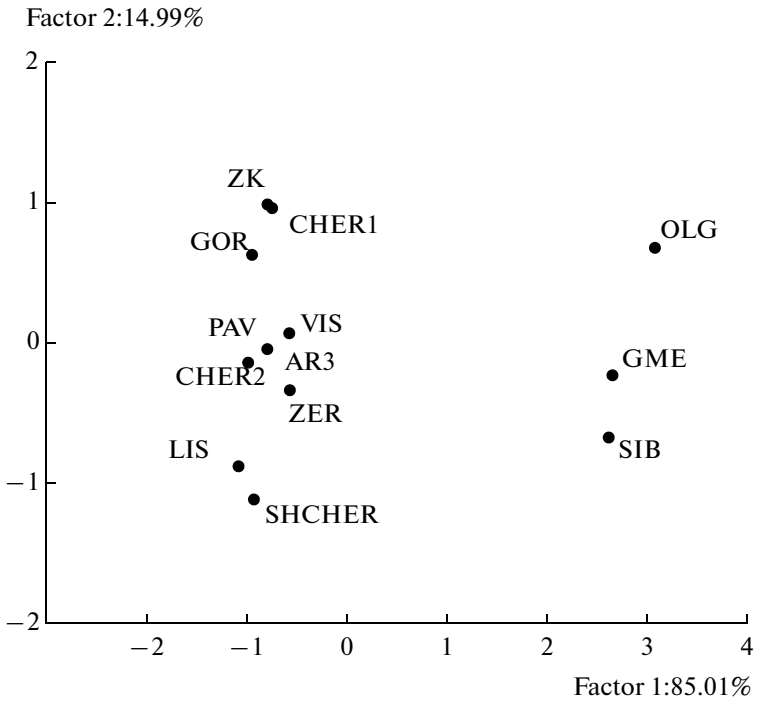

Fig. 4. Ordination of studied larch populations built using multivariate scaling.

are 9.1 and 0.3% respectively (Fig. 1). Cells with a triploid chromosome set were not found in this population.

The amount of aneuploid cells ($2n = 14, 16, 18, 20, 22, 30, 32$) was 4 and 13.6% (Fig. 3), that of haploid cells was 17.3 and 8.5% and that of triploid cells was 7.1 and 6.4% for the Dahurian and Siberian larches, respectively (Fig. 1). Cells with tetraploid chromosome sets were not found in these representatives. However, there exist literature data on the presence of tetraploid cells in somatic tissues of species *L. gmelinii* and *L. sibirica* (Muratova, 1991; Muratova, 1994; Sedel'nikova et al., 2005; Sedel'nikova, Pimenov, 2007; Sizikh et al., 2006).

Figure 3 shows that haploid cells with ploidy less than $2n = 24$ in members of all populations including populations of OLG larches (Siberian and Dahurian) prevail in comparison with hyperdiploid and aneuploid cells.

The main conclusion on the basis of these results is that larches growing in the areal of the Olgan larch are considerably different in the amount of diploid, aneuploid, hyper-, and hypodiploid cells obtained from the OLG larch and species *L. gmelinii* and *L. sibirica* (Figs. 1, 3).

The ordination of population was built on the basis of the frequency of existence of cells with different levels of ploidy by analysis of the main components (Fig. 4). The populations of larch growing in the areal of the Olgan larch except the OLG population form a separate group where they are distributed, except for CHER2 and ZER according to the geographical distribution. These populations are equally distant from the population of *L. olgensis* locus classicus (OLG) and populations of Siberian and Dahurian larches.

Growing in conditions of a fragmented areal and pressure from competition of wood species can promote the studied populations of larch by development of the protective adaptation mechanism as the observed mixoploidy (Butorina, 1989), which resulted in their resistance and viability in extreme conditions. According to Butorina (Butorina, 1989), the level of adaptive value of mixoploids is determined by the ratio of the cells with different ploidy. This raises the question why the representatives of OLG population growing in similar difficult ecological conditions (north stone slope of sea bank) are different in the ratio of cells with different ploidy from other populations of the *L. olgensis* areal. Obviously this population brings the features of a pure species with a similar amount of diploid cells (more than 70%) with other pure species, i.e., *L. gmelinii* and *L. sibirica*. The observed chromosome mosaicism in larches from the *L. olgensis* areal is the result of their hybrid nature. Paleobotanic findings indicate that some parental larch forms with the features of one or two modern species grown in the Pliocene on the territory of the Primorskii krai (Blokhina, 1999; Bondarenko, 2006). The drastic Pliocene–Pleistocene fall of temperature has changed the conditions of forestation and enhanced the hybridization processes between different larch forms (Urusov, 2002).

This supposition is confirmed by molecular-genetic data obtained earlier and shows the genetic similarity of populations from different places of the areal of the Olgan larch with hybrid species of larches growing in the Primorskii krai (Vasutkina et al., 2007). Adrianova et al. (2010) have shown on the basis of morphology of megastrobiles the considerable difference of these populations from populations of *L. olgensis* locus classicus.

Thus, the study results indicate that the Olgan larch in the present occupies a more limited areal in the Primorskii krai than was supposed earlier.

ACKNOWLEDGMENTS

The authors are grateful V.N. Gaponov from the Federal State Organization "Shumninskii leskhoz" in the Primirskii krai for help in material collection and V.Yu. Minkhaidarov from the Institute of Forest and Park Forestry, Primorskaya State Agronomic Academy, Ussuriisk, and Yu.A. Khrolenko for help with photographs (Institute of Biology and Soil Science, Far East Division, Russian Academy of Sciences, Vladivostok).

This study was supported by the Program of the Presidium of the Russian Academy of Sciences, project no. 09-I-P23-06; an integration project with the Siberian Branch, Russian Academy of Sciences, project no. 09-II-SB-06-003; and NSH grant no. 1635-2008.4 of the President of the Russian Federation.

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