PROCEEDINGS
of the
FIRST IUFRO CYTOGENETICS
WORKING PARTY S2.04-08 SYMPOSIUM
September 8-11, 1993
BRIJUNI NATIONAL PARK
CROATIA

REPRINTED FROM

Cytogenetic studies of forest trees and shrub species

EDITED BY

Želimir Borzan and Scott E. Schlarbaum

ISBN 953-6253 15-1
ISBN 953-6307-28-6
UDC 630*1:575.13(063)

CROATIAN FORESTS, Inc., Zagreb
FACULTY OF FORESTRY, UNIVERSITY OF ZAGREB

1997

Studies on nucleolar chromosomes in representatives of *Pinaceae* Lindl.

Elena N. Muratova
V N. Sukachev Institute of Forest
Russian Academy of Sciences, Siberian Branch
Krasnoyarsk, 660036, Russia

Received December 1993 Accepted March 1994

Karyotype analyses of eight *Pinus* L. species, five *Larix* Mill, species, two *Picea* A. Dietr species, and one *Abies* Mill, species were conducted. All species were found to have the same chromosome number (2n=2x=24), and a similar chromosome morphology within each genus. The number and localization patterns of nucleolar regions differed among the investigated species. Variability in the numbers of the secondary constrictions in metaphase chromosomes and nucleoli in the interphase nuclei suggest the existence of a definite protein biosynthetic level in the different populations in relation with their adaptive ability.

KEYWORDS: karyotype, chromosome, secondary constriction, *Pinus, Picea, Larix, Abies,* adaptability, nucleolar organization, nucleoli

Pinaceae Lindl. is the largest and the most economically important family in the gymnosperms. The family *Pinaceae* includes 10-11 genera and about 250 - 260 species growing in temperate to boreal zones of the northern hemisphere (KOZUBOV and MURATOVA 1986). The genera with the largest number of species are: *Pinus* L. - pine, (100 ± species); *Picea* A. Dietr. - spruce (35 - 50 species); *Larix* Mill. - larch (approximately 20 species); and *Abies* Mill. - true fir (45 - 60 species).

The absence of polyploid species, a constant chromosome number and similar chromosome morphology are characteristic to most coniferous genera. Nucleolar polymorphism, however, has been shown in this group of plants. Early cytogenetic studies demonstrated that nucleolus formation is due to the function of loci localized in particular chromosome regions, i.e., the nucleolar organizing regions (NAVASHIN 1912, DERMEN 1933). The occurrence of the secondary constrictions often can be related to nucleolus organization in the cell. Localization and number of the secondary constrictions and satellites in the chromosomes are distinct features of karyotypes.

Nucleolar chromosomes are of great importance in the cell protein metabolism. Direct methods of molecular DNA-rRNA hybridization have established that ribosomal RNA genes, as well as ribosome formation genes, are lo-

calized in the nucleolar organization region (RITOSSA and SPIEGELMANN 1965, BIANCHE *et at.* 1980). In the last two decades, cytogenetic investigations have shown a wide variability of nucleolar regions among different plant species (DERYAGIN and IORDANSKY 1971, BOUGOURD and PARKER 1976, ANASTASSOVA-KRISTEVA and NICOLOFF 1978, MALAKHOVA 1979, DUBROVA and MALAKHOVA 1980, BONDAR *et al.* 1980). Differences in the number and positions of secondary constrictions indicate karyotypic variability within populations. This phenomenon can be especially useful when determining cytotaxonomic relationships among species with constant chromosome number and morphology, such *Pinaceae.* This paper addresses karyotypic and nucleolar chromosome variability of selected *Pinus, Picea, Larix* and *Abies* species.

Material and methods

Experimental materials

Seeds of eight pine species *(Pinus sylvestris* L., *P. sibirica* Du Tour, *P. pumila* Regel, *P. koraiensis* Siebold et Zucc, *P. thunbergii* Parl., *P. funebris* Kom., *P. densiflora* Siebold et Zucc, *P. eldarica* Medw.), five larch species *(Larix sibirica* Ledeb., *L. sukaczewii* Dylis, *L. gmelinii* Rupr., *L. cajanderi* Mayr, *L. ochontensis* Kolesn.), two spruce species *(Picea obovata* Ledeb., *P. ajanensis* Fisch. ex Carr.), one fir species *(Abies sibirica* Ledeb.) from different populations were used for karyological investigations. The seed source for each taxon is shown in Table 1.

The taxonomic system published by LITTLE and CRITCHFIELD (1969), was accepted for classifying the pine species in the study. These pines belong to two subsections of the genus *Pinus: Cembrae* (subgenus *Strobus = Haploxylori)* and *Sylvestres* (subgenus *Pinus = Diploxylon).* The two spruce species are classified (cf. BOBROV 1978) in two sections of the genus *Picea: P. obovata* in section *Eupicea* (series *Obovatae)* and *P. ajanensis* in section *Casicta* (series *Ajanenses).* The five *Larix* species in the present study belong to two series in the section *Pauciseriales* (cf. BOBROV 1978). *Larix sibirica, L. ochotensis,* and *L. sukaczewii* belong to the series *Eurasiaticae,* and *L. gmelinii* and *L. cajanderi* belong to the series *Paucisquamatae.* The taxonomic position of *L. ochotensis* is controversial; this species is closely related to *L. gmelinii* and *L. cajanderi.* The only representative of the genus *Abies* in this study, *A. sibirica,* belongs to the series *Sibiricae* of the section *Piceaster* (MATSENKO **1964).**

Chromosome studies

Seeds from each species were germinated on moist filter paper under laboratory conditions. The root tips were excised and pretreated in 0.5 percent colchicine solution for 6 - 8 hours at room temperature (about 22 °C), fixed in a

3 : 1 ethanol: acetic acid mixture and then refrigerated. Prior to slide preparation, the materials were stained with acetohematoxylin. The slides were prepared by the squash technique using root tip meristems.

Table 1 Origin of species examined.

Species	Origin
Pinus sibirica Du Tour	Western Sayans, low mountains in Krasnoyarsk Territory
	Western Sayans, high mountains in Krasnoyarsk Territory
	Krasnoyarsk Territory, Turukhansk
	Tyumen district, Khanty-Mansyisk
	Tyumen district, Urmannii
	Tyumen district, Berezovo
	Ekaterinburg
	Ekaterinburg district, Karpinsk
Pinus pumila (Pall.) Regel	Buryatia, Barguzin region, Ulyun
	North-Western Yakutia, Zhigansk
	Magadan district, Tauisk
	Magadan district, Orotukan
	Central Kamchatka
	Northern Kamchatka
Pinus koraiensis Siebold et Zucc.	Khabarovsk Territory, Obluchje
	Primorski Territory, Chuguevka
Pinus sylvestris L.	Buryatia, Kyakhta
	Buryatia, Novoselenginsk
	Krasnoyarsk Territory, Minusinsk
	Central Yakutia, Yakutsk
	Tomsk district, Bakchar, oligotrophic swamp
	Tomsk district, Timirjazevka, eutrophic swamp
	Tomsk district, Golovinka, dry valley
Pinus densiflora Siebold et Zucc.	Barnaul, Institute of Horticulture of Siberia, Arboretum
Pinus funebris Kom.	Primorski Territory, Turii Rog.

Table 1 cont.

Species	Origin
Pinus thunbergii Parl.	Korea, Pyongyang, Botanical Garden
Pinus eldarica Medw.	Georgia, Saguram National Park
Picea obovata Ledeb.	Western Yakutia, Lensk
	Western Yakutia, Suntari
	Western Yakutia, Nyurba
	South-Western Yakutia, Kochegarovo
	Central Yakutia, Yakutsk
	Central Yakutia, Sangari
	Central Yakutia, Handiga
	Southern Yakutia, Aldan
	Krasnoyarsk Territory, Turukhansk
Picea ajanensis (Lindl. et Grod.) Fisch. ex Carr.	South-Eastern Yakutia, Chagda Primorski Territory, Dalnegorsk
Larix sibirica Ledeb.	Eastern Kazakhstan, Markakol
	Eastern Kazakhstan, Zaisan
	Khakasia, Sonski
	Tuva, Shagonar
	Tuva, Balgazin
	Tuva, Chagitai
	Buryatia, Zakamensk
	Mongolia, Tosontsengel
	Mongolia, Chuluut
	Mongolia, Solgotoin-Daba
Larix sukaczewii Dylis	Arkhangelsk district, Pinezhski reserve
	Ivanov district, Volzhski
	Udmurtia, Grakhov
	Bashkiria, Uchali
	Bashkiria, Tirlyanski
	Perm district, Krasnovishersk
	Ekaterinburg district, Revda
	Ekaterinburg district, Bulanash
	Ekaterinburg district, Novaya Lyalya

Table 1 cont.

Species	Origin
Larix gmelinii (Rupr.) Rupr.	Chita district, Karimskoe Mongolia, Bayan-Ula Evenkia, Vivi
Larix cajanderi Mayr	Central Yakutia, Kobyai Southern Yakutia, Ust-Maya Central Kamchatka
Larix ochotensis Kolesn.	Magadan district, Tauisk
Abies sibirica Ledeb.	Tomsk district, Chainski Krasnoyarsk Territory, Emelyanovo Buryatia, Babushkin

Karyotype analyses

The karyotypes were analyzed according to methods published by PRAVDIN *et al.* (1972) with some modifications. The length of long and short arms of each chromosome were measured on photomicrographs. The following parameters were determined: absolute length of the chromosome (L^a, in micrometers); the total diploid complement chromosome length (SL^a, in micrometers); relative chromosome length (I/, the ratio of absolute length to the total chromosome length, in percent); centromeric index (I^c, the ratio of the short arm length to absolute chromosome length, in percent); and the localization of secondary constriction (sc, in percent). The chromosomes were classified according to recommendations by GRIF and AGAPOVA (1986). Only localization and frequency of secondary constrictions were determined for *Pinus thunbergii*.

Nucleolus studies

The slides for nucleolus counts were prepared according to methods described by RATTENBURY (1952). Excised root tips from germinating seeds were fixed in alcohol-formaldehyde (2:1) mixture, hydrolysed in IN HCl during 2 hours at 60 °C and stained with acetohematoxylin. In some cases, the slides were treated with a 50 percent solution of $AgNO_3$ for 1 - 2 hours at 60 °C (HIZUME *et al.* 1980). To stain the nucleolar organization regions (NOR) of chromosomes, a modification of the same method was employed. Concentrations of silver nitrate, time of treatment and temperature were different and determined experimentally for each species.

Results

Pinaceae family representatives have well stained large chromosomes that ai clearly differentiated into two arms. *Pinaceae* species do not produce chrome somes with satellites of the »classical« form as a small oval body with well d(fined satellite thread. There are secondary constrictions that look like unstair ed chromosome sites varying in length.

All examined species were stable diploids, having 24 somatic chromoso mes (2n=2x=24). The results of karyological investigations have shown tha many chromosomes of the pine species have the secondary constrictions.

Karyotype analyses of *Cembrae* species

The karyotypes of three species of the subsection *Cembrae (Pinus sibirica, I pumila,* and *P. koraiensis)* had 11 pairs of metacentric chromosomes. Karyo types of *P. sibirica* and *P. koraiensis* are shown in Figures 1 and 2. Only one chro mosome pair (XII), the shortest and most asymmetric in the complement, wa; distinctly different from the other chromosomes. This chromosome pair hac the following parameters: *P. sibirica* L^a = *9.7* ± 0,12 цт, I/ = 3.2 ± 0.03 %, I = 40.4 ± 0.28 *%; P. pumila* L^a = 9.6 ± 0.13 цт, I/ = 3.2 ± 0.03 %, I^e = 40.9 ± 0.36 *%; P. koraiensis* L^a = 10.3 ± 0.23 цт, I/ = 3.2 ± 0.07 %, I^e = 40,4 ± 0.37 %.

Figure 1 Karyotype *ofPinus sibirica.* 2n=2x=24. The chromosomes are distributed into groups according to results of the karyotype analysis. I - XII indicates the numbers of the chromosomes.

Figure 2 Karyotype of *Pinus koraiensis* 2n=2x=24. The chromosomes are distributed into groups according to results of the karyotype analysis. I - XII indicates the numbers of the chromosomes.

Eleven pairs (I - XI) of *P. sibirica* chromosomes formed one group: L^a = 12.8 ± 0.07 μm, I/ = 4.1 ± 0.02 %, I^e = 46.9 ± 0.08 %. In *P.pumila,* chromosome pairs 1-X composed one group with similar parameters: L^a = 12.8 ± 0.06 μm, I/ = 4.3 ± 0.02 %, I^e = 48.3 ± 0.21 %. The eleventh chromosome pair was distinct in *P. pumila:* L^a = 12.0 ± 0.14 μm, I/ = 4.0 ± 0.11 %, I^e = 46.6 ± 0.16 %.

The I - XI chromosome pairs of *P. koraiensis* formed three distinct groups. The first group consisted of seven chromosome pairs: L^a = 14.1 ± 0.09 μm, L^r = 4.5 ± 0.02 %, I^e = 48.1 ± 0.12 %. The second and the third group included two pairs of chromosomes, respectively: L^a =13.3 ± 0.19 μm, I/ = 4.3 ± 0.05 %, I^e = 46.5 ± 0.18 % and L^a = 11.1 ± 0.09 μm, I/ = 3.6 ± 0.03 %, I^e = 47.8 ± 0.23 %.

The karyotypes of *P. sibirica* and *P. pumila* contained up to seven pairs with secondary constrictions. The karyotype *oiP. koraiensis* contained up to six pairs of chromosomes with secondary constrictions. In both species, one pair of symmetric chromosomes had as many as four secondary constrictions per chromo-

some. Two constrictions were localized in one arm in *P. pumila:* sci = 35.1 ± 0.39 %, sc_2 = 59.6 ± 0.90 %, and two constrictions were localized in the other arm in sc_3 = 41.9 ± 0.59 %, sc_4 = 68.1 ± 1.32 %. In *P. koraiensis,* similar pair of chromosomes had secondary constrictions in one arm: sCi = 36.1 ± 2.07 %, sc_2 = 66.2 ± 1.90 %, and in the other arm: sc_3 = 51.7 ± 1.61 %, sc_4 = 75.2 ± 1.77 %.

Pinus sibirica had one pair of symmetric chromosomes with three secondary constrictions per chromosome. Two constrictions were observed in one arm (sci = 38.1 ± 0.56 %, sc_2 = 66.1 ± 0.64 %) and one constriction was observed in the other arm (sc_3 = 52.1 ± 0.50 %). *Pinus pumila* had one pair of chromosomes with two secondary constrictions per chromosome. These constrictions were localized in two arms; however, they were not observed in all investigated populations (sci = 36.9 ± 0.99 %, sc_2 = 46.8 ± 1.77 %). One pair of *P. sibirica* and *P. koraiensis* chromosomes had a secondary constriction in the proximal part of the arm: *P. sibirica* sc = 41.3 ± 0.47 % and *P. koraiensis* sc = 36.2 ± 0.92%).

The three subsection *Cembrae* species had one pair of chromosomes with the secondary constrictions in the medial part of the arm: *P. sibirica* sc = 53.4 ± 0.20 %, *P. pumila* sc = 58.3 ± 0.30 %, and *P. koraiensis* sc = 53.6 ± 0.63 %. All species had some chromosomes with secondary constrictions in the distal part of the arm. The karyotype of *P. koraiensis* had two chromosome pairs with distal secondary constrictions (sc = 67.6 ± 1.33 % for both pairs). *Pinus pumila's* karyotype contained three pairs (sc = 62.4 ± 0.31 % for one pair and sc = 73.3 ± 0.62 % for two pairs). Distal secondary constrictions were present in three *P. sibirica* chromosome pairs: (sc = 62.0 ± 0.22 % for one pair and sc = 71.9 ± 0.46 % for two pairs). In *P. sibirica,* one of these chromosomes had the secondary constriction at a similar location in the second arm.

In addition, secondary constrictions were observed in the asymmetric chromosome pair (XII) of these species. *Pinus sibirica* had two secondary constrictions: in the short arm sc! = 61.4 ± 1.52 % and in the long arm sc_2 = 70.3 ± 0.61 %. The XII chromosome in *P. pumila* and *P. koraiensis* had three secondary constrictions. The XII short arm had secondary constrictions in different locations in *P. pumila* and *P. koraiensis,* sc_a = 52.0 ± 0.98 % and sc! = 54.9 ± 5.32 %, respectively. The XII long arm had two secondary constrictions, again at different locations in each species: sc_2 = **71.3** ± 0.80 % *(P. pumila)* and sc_21 67.0 ± 1.61 % *(P. koraiensis),* and sc_3 = 42.6 ± 1.87 % *(.P. pumila)* and sc_3 = 43.1 ± 1.50 % *(P. koraiensis).* The secondary constrictions in the short arm and in the distal portion of the long arm occurred infrequently in both species and were not present in all investigated populations.

Interphase nuclei of *P. pumila* and *P. koraiensis* contained 4 - 1 2 nucleoli, and the interphase nuclei of *P. sibirica* contained 4 - 1 4 nucleoli. Figure 3 shows nucleoli of *P. pumila.*

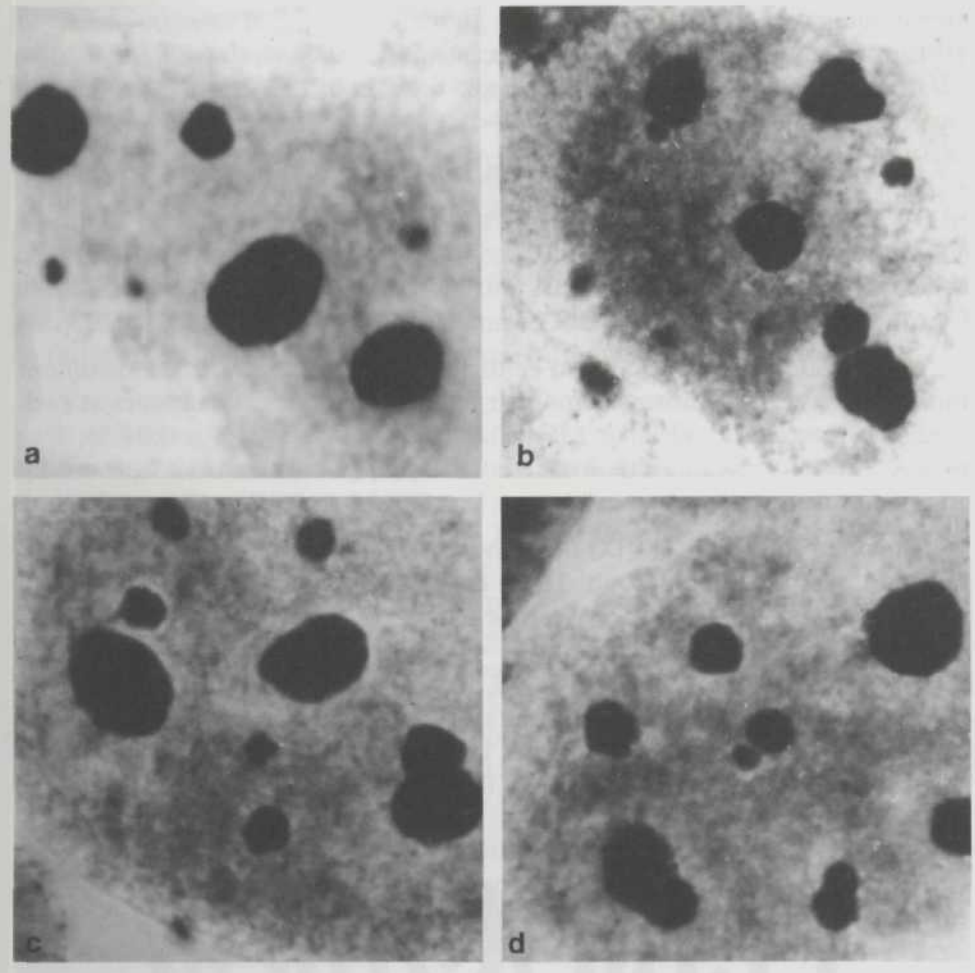


Figure 3 a - d Interphase nuclei of *Pinus pumila* with different numbers of nucleoli.

Karyotype analyses of *Sylvestres* species

The five investigated species belong to the subsection *Sylvestres*. They are: *Pinus sylvestris, P. densiflora, Pfunebris, P. thunbergii* and *P. eldarica*. In comparison with subsection *Cembrae* pines, subsection *Sylvestres* pines had two pairs of short and asymmetric chromosomes. These chromosomes were metacentric or submetacentric and had the following parameters:

P. sylvestris

Chromosome XI L^a = 9.8 * 0.10 urn, L^r = 3.5 ± 0.04 %, I^e = 42.4 ± 0.32 %

Chromosome XII L^a = 8.8 ± 0.10 urn, I/ = 3.1 ± 0.04 %, I^e = 43.1 ± 0.29 %

P densiflora

Chromosome XI L^a = 11.3 ± 0.20 μm, L^r = 3.4 ± 0.04 %, I^e = 43.7 ± 0.30 %
Chromosome XII L^a = 9.9 ± 0.20 μm, I/ = 3.0 ± 0.10 %, I^e = 42.3 ± 0.50 %

Pfunebris

Chromosome XI L^a = 11.9 ± 0.15 μm, I/ = 3.5 ± 0.04 %, I^e = 40.6 ± 0.44 %
Chromosome XII L^a = 9.9 ± 0.14 μm, L^r = 2.9 ± 0.05 %, I^e = 41.1 ± 0.30 %

£ *eldarica*

Chromosome XI L^a = 14.2 ± 0.21 μm, I/ = 3.6 ± 0.03 %, I^e = 39.7 ± 0.28 %
Chromosome XII L^a = 12.6 ± 0.24 μm, I/ = 3.1 ± 0.03, I^e • 36.5 ± 0.26 %

Chromosome pair X also could be distinguished in *P. sylvestris, P. densiflora* and *Pfunebris.* The X chromosome pair had the following parameters in each respective species: *P. sylvestris* L^a = 10.8 * 0.04 μm, I/ = 3.8 ± 0.04 %, I^e = 46.7 ± 0.26 %; *P. densiflora* L^a = 12.8 ± 0.30 μm, I/ = 3.9 ± 0.10 %, I^e = 44.4 ± 0.40 %; *Pfunebris* L^a = 12.4 ± 0.15 μm, L^r = 3.6 ± 0.04 %, I^e = 47.3 ± 0.38 %.

The other nine pairs (I - IX) of *P. sylvestris, P. densiflora* and *Pfunebris* chromosomes comprised one group. These chromosome pairs were characterized by the following parameters: *P. sylvestris* L^a = 14.0 ± 0.14 μm, L^r = 4.1 ± 0.12 %, I^e = 48.1 ± 0.11 %; *P. densiflora* L^a = 14.8 ± 0.32 μm, L^r = 4.4 ± 0.09 %, I^e = 47.5 ± 0.39 %; *Pfunebris* L^a - 15.3 ± 0.20 μm, *V* = 4.5 ± 0.05 %, I^e = 47.5 ± 0.29 %. In *P. eldarica,* ten pairs of chromosomes (I - X) formed one group with the following parameters: L^a = 17.2 ± 0.19 μm, L^r = 4.0 ± 0.02 %, I^e = 48.0 ± 0.15 %. A karyotype of *P. sylvestris* is shown in Figure 4.

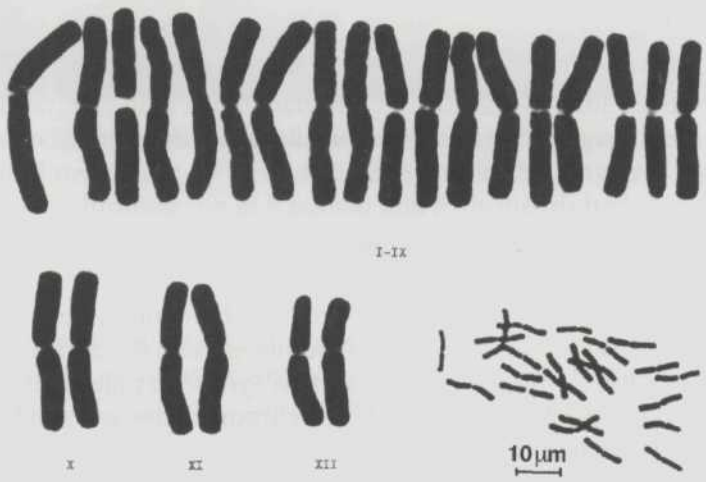


Figure 4 Karyotype of *Pinus sylvestris.* 2n=2x=24. The chromosomes are distributed into groups according to results of the karyotype analysis. I - XII indicates the numbers of the chromosomes.

A karyotype of *P. sylvestris,* growing in optimum conditions, contained eight pairs of the chromosomes with the secondary constrictions. One pair of chromosomes from the I - IX group had two secondary constrictions located in the one arm (sci = 36.7 ± 1.36 %, sc_2 = 64.3 ± 1.43 %), two pairs had constrictions in the distal part of the arm (sc = 63.3 ± 0.71 %), one pair had constrictions in the medial part of the arm (sc = 54.6 ± 0.83 %) and one pair of chromosomes had constrictions in the proximal part (sc = 39.2 ± 1.07 %). The chromosomes of the X pair were characterized by the secondary constriction in the medial portion of the arm sc = 57.7 ± 1.36 %. Asymetric chromosomes XI and XII had secondary constrictions sc = 37.9 ± 3.26 % and sc = 60.5 ± 1.43 % respectively, but the constrictions were infrequent in occurrence.

Karyotypes of *P. densiflora* and *P. funebris* contained seven chromosome pairs with secondary constrictions. One pair of symmetric chromosomes of these species had three secondary constrictions, two constrictions were located in the one arm, sc! = 36.9 ± 1.15 % and sc_2 = 65.3 ± 1.54 % *(P. densiflora)* and sci = 38.1 ± 0.98 % and sc_2 = 67.1 ± 1.38 % *(P. funebris)*, and one constriction was located in the other arm, sc = 42.4 ± 1.30 % and 41.2 ± 1.39 % (both species). Each species had one pair of chromosomes with a secondary constriction proximal to the centromere sc = 39.8 ± 0.71 % *(P. densiflora)* and sc = 40.6 ± 1.00 % *(P. funebris),* one pair of chromosomes with the secondary constriction in the medial region sc = 54.5 ± 0.84 % and sc = 53.6 ± 0.52 % (in both species), and two pairs of chromosomes with the secondary constrictions in the distal region sc = 62.1 ± 0.57 % and sc = 63.6 ± 0.48 % (in both species).

In *P. funebris,* secondary constrictions were observed in the X chromosome pair, sc = 67.5 ± 1.08 % and in the short arm of the XII chromosome pair, sc = 54.5 ± 3.86 %. The XI chromosome pair in *P. densiflora* had a secondary constriction in the short arm.

The karyotype of *P. thunbergii* had six pairs of chromosomes with the secondary constrictions. Two constrictions were observed in an arm of one long symmetric chromosome (sci = 39.3 ± 2.04 %, sc_2 = 65.9 ± 1.64 %). Two pairs of symmetric chromosomes had secondary constrictions in the proximal part an arm (sc = 40.2 ± 0.76 %), one pair had a secondary constriction in a medial region (sc = 54.1 ± 2.71 %), and one pair had a secondary constriction in the distal region (sc = 67.6 ± 2.28 %). A secondary constriction was observed in the long arm of the XI chromosomes pair (sc = 58.8 ± 1.85 %).

The karyotype of *Я eldarica* included seven pairs of chromosomes with secondary constrictions. One pair of symmetric chromosomes had two constrictions in one arm (sc_a = 37.5 ± 1.01 %, sc_2 = 64.7 ± 0.83 %), another pair had constrictions in both arms (sci = 38.9 ± 0.56 %, sc_2 = 46.5 ± 1.13 %). Secondary constrictions were observed in the distal regions of two pairs (sc = 60.6 ± 0.45 %) and in region proximal to the centromere of one pair of chro-

mosomes (sc = 40.9 ± 0.88 %). The asymmetric chromosomes had secondary constrictions in the long arms, sc = 41.7 ± 1.31 % and 63.4 ± 2.37 %, respectively.

Karyotype analyses of *Picea* species

The two investigated spruce species had 10 pairs of metacentric and 2 pairs of submetacentric chromosomes. Four chromosome pairs (IX, X, XI, XII) could be identified in the complement of *P. obovata*. They have the following parameters: chromosome IX L^a = 12.3 ± 0.13 urn, I/ = 3.5 ± 0.01 %, I^e = 36.8 ± 0.25 %; chromosome X L^a = 11.9 ± 0.20 um, L^r = 3.3 ± 0.04 %, I^e = 42.2 ± 0.49 %; chromosome XI L^a = 10.7 ± 0.11 um, I/ = 3.1 ± 0.02 %, I^e = 44.0 ± 0.29 % and chromosome XII L^a = 9.5 ± 0.13 um, I/ = 2.6 ± 0.01 %, I^e = 33.7 ± 0.30 %. The other eight chromosome pairs (I - VIII) comprised one group: L^a – 15.0 ± 0.24 um, I/ = 4.4 ± 0.13 %, I^e = 46.9 ± 0.63 %. The karyotype of *P. obovata* is shown in Figure 5.

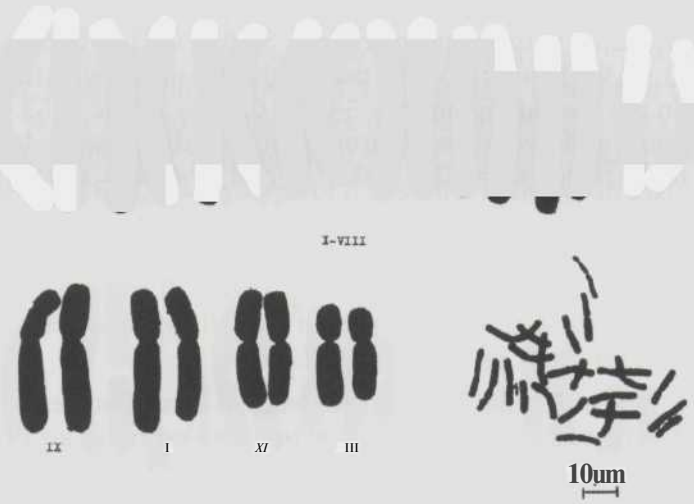


Figure 5 Karyotype of *Picea obovata*. 2n=2x=24. The chromosomes are distributed into groups according to results of the karyotype analysis. I - XII indicates the numbers of the chromosomes.

Only two chromosome pairs, IX and XII, had distinct morphology in the complement of *P. ajanensis*. The parameters of these chromosomes were: chromosome IX L^a = 12.8 ± 0.21 um, I/ = 3.9 ± 0.06 %, I^e = 37.8 ± 0.67 % and chromosome XII L^a = 9.4 ± 0.16 um, I/ = 2.8 ± 0.04 %, I^e = 33.0 ± 0.43 %. Eight pairs of chromosomes (I - VIII) formed one group, (L^a = 15.5 ± 0.37

urn, I/ = 4.6 ± 0.08 %, I^e = 47.2 ± 0.37 %) and two pairs (X - XI) formed the second group, (L^a = 11.1 ± 0.15 urn, I/ = 3.4 ± 0.05 %, I^e = 42.0 ± 0.46 %).

Many chromosomes in both species had secondary constrictions. In the *P. obovata* karyotype, two pairs from chromosome group I—VIII had secondary constrictions in the medial region of an arm, sc = 57.1 ± 0.38 %. Two other pairs of the chromosomes had secondary constrictions in the distal region, sc = 67.1 ± 0.57 % and sc = 76.7 ± 0.53 %. Furthermore, secondary constrictions were found near the centromeric region for two chromosome pairs, sc = 48.3 ± 0.20 % and sc = 34.1 ± 0.37 %. In *P. ajanensis,* two chromosome pairs from group I -VIII had two secondary constrictions located in both arms, SC! = 38.5 ± 0.94 %, sc_2 = 43.5 ± 2.90 % and sci = 53.8 ± 0.43 %, sc_2 = 63.5 ± 0.75 %). Secondary constrictions in the medial region were observed for two chromosomes pairs, sc = 50.3 ± 0.46 %, and distal secondary constrictions in the distal part were observed for one chromosome pair, sc = 63.5 ± 0.75 %.

The two spruce species had secondary constrictions in the IX and X chromosome pairs. Two secondary constrictions, located in both arms, were observed in the IX pair of *P. obovata,* sc_a for the long arm = 41.2 ± 0.39 and sc_2 for the short arm = 55.0 ± 0.45 %. One constriction was found in the long arm in the IX chromosome pair in *P. ajanensis.* In both species, the X chromosome pair of chromosomes was with one secondary constriction located in the short arm, sc = 57.5 ± 0.74 % *(P obovata)* and sc = 52.6 ± 1.71 % *(P. ajanensis).*

Supernumerary chromosomes were detected among the populations of these two species. Metacentric B-chromosomes was observed in *P. obovata,* and two meta- and submetacentric B-chromosomes were found in certain *P. ajanensis* seedlings. Figure 6 shows the B-chromosome of *P. ajanensis.*

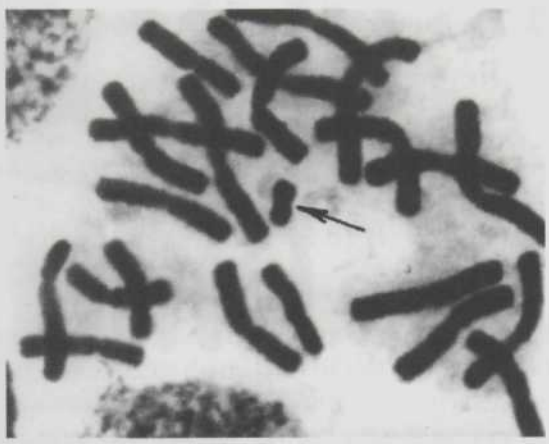


Figure 6 The chromosome complement of *Picea ajanensis* with a supernumerary chromosome. Arrow poiajs to the B-chromosome.

Karyotype analyses of *Larix* species

All of the examined larch species had six pairs of symmetric (metacentric) and six pairs of asymmetric (submeta- or intercentric) chromosomes. Two chromosome groups could be distinguished in the karyotypes of I. *sibirica, L. gmelinii, L. cajanderi* and *L. ochotensis.* A third chromosome group could be identified in these species by due consistent secondary constrictions.

The chromosome complement of *L. sibirica* had the following parameters: I - VI L^a = 14.7 ± 0.14 цт, I/ = 4.6 ± 0.03 %, I^e = 47.0 ± 0.24 %; VII - XII L^a = 10.1 ± 0.08 цт, I/ = 3.1 ± 0.02 %, I^e = 32.3 ± 0.26 %.

Chromosome parameters of *L. sukaczewii* were: I - VI L^a = 11.3 ± 0.12 цт, I/ = 5.0 ± 0.04 %, I^e = 46.9 ± 0.24 %; VII - IX L^a = 7.6 ± 0.10 цт, L^r = 3.3 ± 0.05 %, I^e = 27.1 ± 0.24 %; X - XII L^a = 7.3 ± 0.09 цт, I/ = 3.1 ± 0.03 %, I^e = 33.1 ± 1.17%.

The karyotype of *L. sukaczewii* is shown in Figure 7. Two pairs of metacentric chromosomes in these species had secondary constrictions located in the distal region of an arm (I. *sibirica* sc = 62.8 ± 0.41 %; *L. sukaczewii* sc = 63.3 ± 0.55 %).

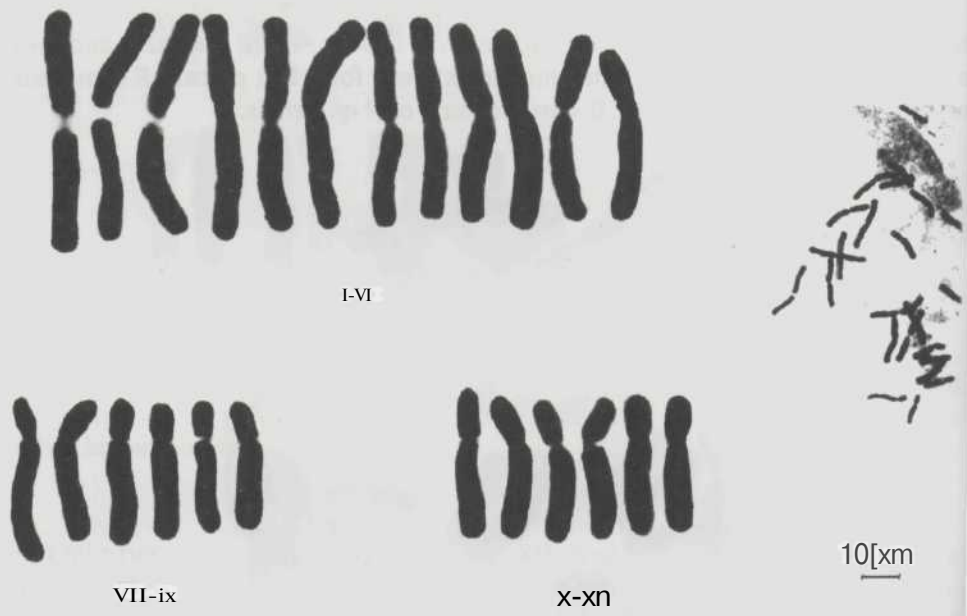


Figure 7 Karyotype of *Larix sukaczewii.* 2n=2x=24. The chromosomes are distributed into groups according to results of the karyotype analysis. I - XII indicates the numbers of the chromosomes.

The chromosome parameters of *L. gmelinii* and *L. ochotensis* karyotypes were: I - VI L^a = 15.1 ± 0.21 urn, *V* = 4.8 ± 0.05 %, I^e = 46.5 ± 0.37 % and L^a = 13.3 ± 0.14 urn, I/ = 4.5 ± 0.03 %, I^e = 47.0 ± 0.22 %; VII L^a = 12.1 ± 0.29 urn, L^r = 3.9 ± 0.07 %, I^e = 30.6 ± 1.14 % and L^a = 9.7 ± 0.16 um, *V* = 3.7 ± 0.07 %, I^e = 28.9 ± 0.83 %; VII - XII L^a = 10.1 ± 0.10 um, L^r = 3.3 ± 0.03 %, I^e = 33.0 ± 0.27 % and L^a = 8.8 ± 0.07 ит, L^r = 3.2 ± 0.02 %, I^e = 31.7 ± 0.21%.

The karyotype of L. *gmelinii* is shown in Figure 8. Some preparations of *L. gmelinii* showed a meta- or submetacentric B-chromosome (Figure 9).

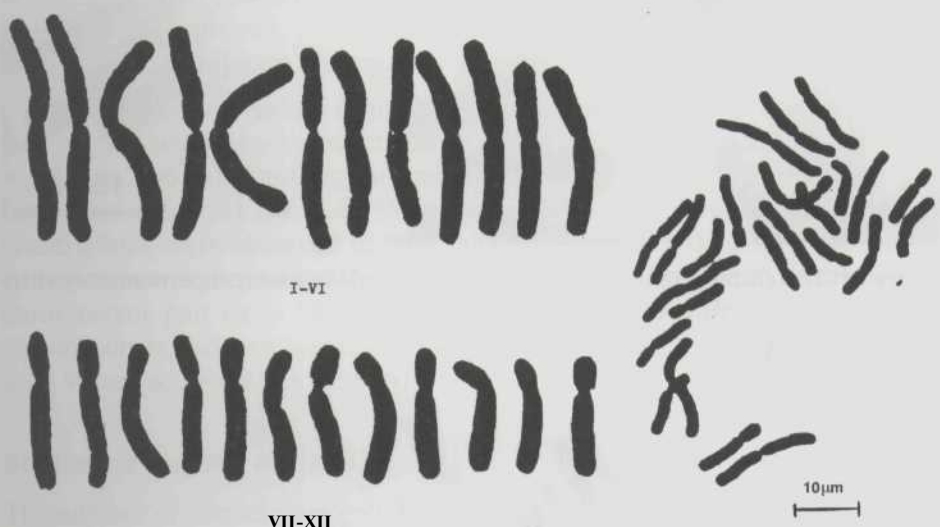


Figure 8 Karyotype of *Larix gmelinii*. 2n=2x=24. The chromosomes are distributed into groups according to results of the karyotype analysis. I - XII indicates the numbers of the chromosomes.

The chromosomes I - VI of *L. cajanderi* had following parameters: L^a = 12.0 ± 0.13 urn, I/ = 4.4 ± 0.02 %, I^e = 47.5 ± 0.31 %; VII -VIII L^a = 9.2 ± 0.18 urn, L^r = 3.8 ± 0.06 %, I^e = 28.5 ± 0.61 %; IX-XII L^a = 9.1 ± 0.10 urn, I/ =3.7 ± 0.02 %, I^e =32.9 ± 0.33 %.

In I. *gmelinii* and L. *ochotensis,* two pairs of metacentric chromosomes had a secondary constrictions in the distal part of the arm: sc = 60.9 ± 0.71 % and sc = 64 ±0.64 %. Additionally, one pair of asymmetric chromosomes in these species contained a secondary constriction in the long arm (sc = 62.0 ± 0.88 % and sc = 66.4 ± 1.26 %). *Larix cajanderi* had two pairs of metacentric chromosomes with one secondary constriction (sc = 61.6 ± 0.67 %), and one chro-

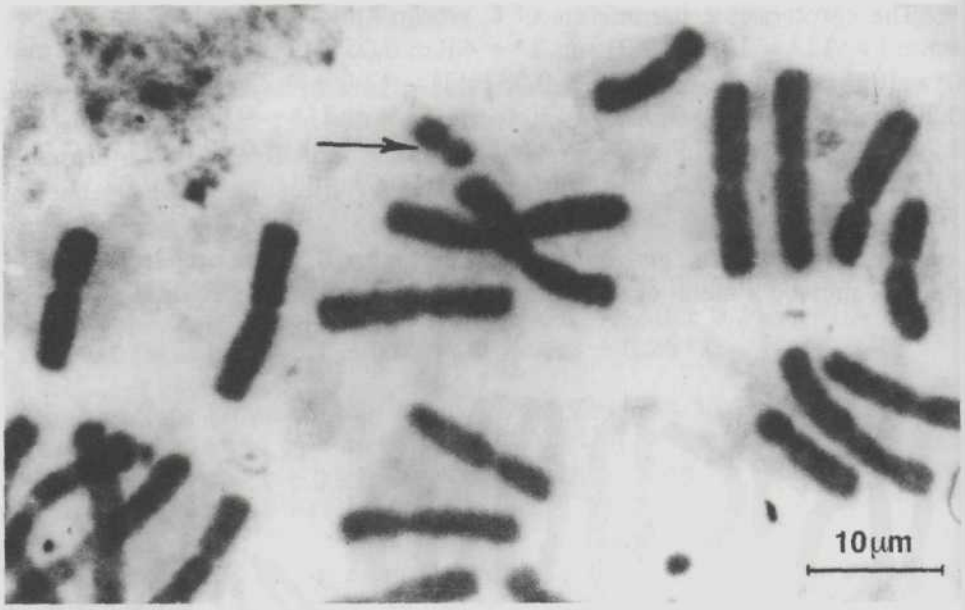


Figure 9 The chromosome complement of *Larix gmelinii* with supernumerary chromosome. Arrow points to B-chromosome.

Figure 10 Metaphase chromosomes from acetohematoxylin preparations of root tip meristem of *Abies sibirica.* 2n=2x=24.

mosome with two constrictions (sci = 44.7 ± 1.57 %, sc_2 = 61.6 ± 0.67 %). A secondary constriction was observed in this species in one of the asymmetric chromosomes (sc = 63.5 ± 0.58 %).

Karyotype analysis of *Abies sibirica*

The karyotype of Siberian fir had seven pairs (I - VII) of metacentric chromosomes: L^a = 13.7 ± 0.21 um, U = 4.6 ± 0.09 %, I^e = 46.3 ± 0.56 %; four pairs (VIII, IX-XI) of submetacentric chromosomes: L^a = 10.2 ± 0.15 um, L^r = 3.2 ± 0.05 %, I^e = 37.9 ± 0.33 % (VIII) and IX-XI pairs: L^a = 9.5 ± 0.30 um, I/ = 3.1 ± 0.07 %, I^e = 33.8 ± 0.45 %. One pair (XII) of intercentric chromosomes had parameters: L^a = 9.5 ± 0.11 цт, L^r = 3.1 ± 0.04 %, I^e = 30.0 ± 0.42 %. The metaphase chromosomes of *A. sibirica* are shown in Figure 10.

A pair of chromosomes from the chromosome group I - VII was characterized by two secondary constrictions in one arm (sci = 36.1 ± 1.61 % and sc_2 = 68.9 ± 1.06 %). Another chromosome pair had secondary constrictions in both arms (sc_a = 51.6 ± 1.67 % and sc_2 = 64.6 ± 2.69 %). Distal secondary constrictions were observed in two pairs, (sc = 68.9 ± 0.50 %), and another constriction was inconsistently observed in the medial region in the arm of one chromosome pair (sc = 58.4 ± 0.48 %). In addition, a pair of submetacentric chromosomes had two secondary constrictions in the long arm (sci = 51.4 ± 2.24 % and sc_2 = 66.2 ± 1.95 %).

Studies on somatic nucleoli

The number of somatic nucleoli in the *Pinus* species of the subsection *Sylvestres* ranged from 1 to 12 per cell at interphase. The number of nucleoli observed in each species was as follows: 3 to 10 in *P. sylvestris,* 2 to 8 in *P.funebris,* 3 to 12 in *P. densiflora,* 1 to 8 in *P. thunbergii* and 3 to 12 nucleoli in *P. eldarica.* The interphase nuclei the two spruce species had a wide range of somatic nucleoli. *Picea obovata* cells contained 1 - 14 nucleoli (Figure 11), and cells *of P. ajanensis* contained 1 - 12 nucleoli. The interphase nuclei of the *Larix* species generally contained the lowest numbers of somatic nucleoli in the study. *Larbc sibirica* and *L. sukaczewii* cells contained 1 - 4 nucleoli. Interphase nuclei of *L. gmelinii, L. cajanderi* and *L. ochotensis* contained 1 - 6 nucleoli (Figure 12). The interphase nuclei of *Abies sibirica* was found to contain 1 - 8 nucleoli (Figure 13).

Nucleolar organization region staining in *Larix*

The metaphase chromosomes of five *Larix* species were specifically stained to show the nucleolar organization regions (NORs). The species had similar patterns of NORs localization (Figures 14 - 16). The NORs were localized at the secondary constrictions and at the telomere regions in some chromosomes.

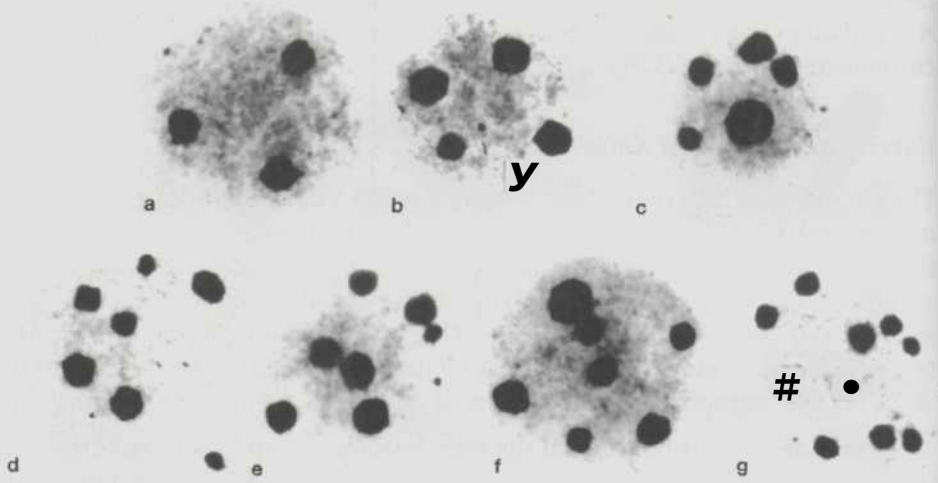


Figure 11 a - g Interphase nuclei of *Picea obovata* with different numbers of nucleoli. Material stained with silver nitrate.

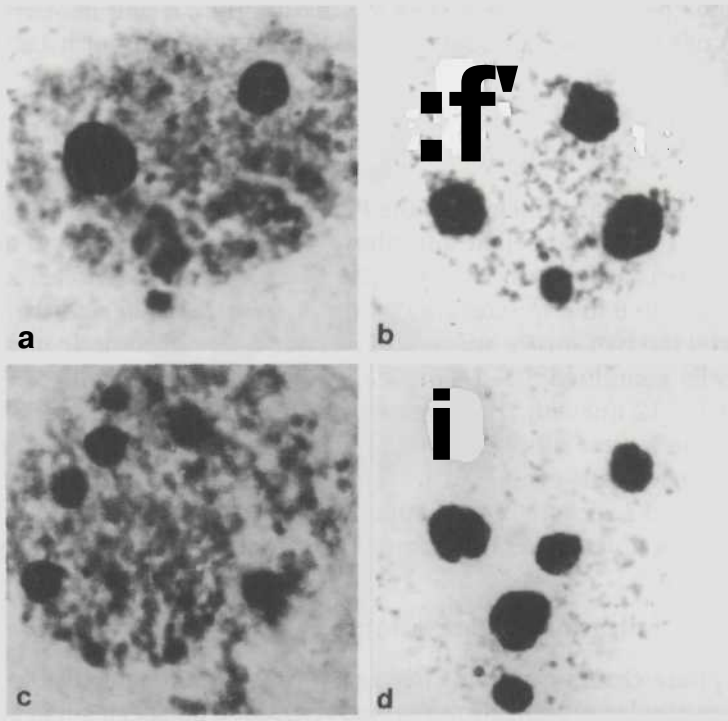


Figure 12 a - d Interphase nuclei of *Larix gmelinii* with different number of nucleoli. Material stained with silver nitrate.

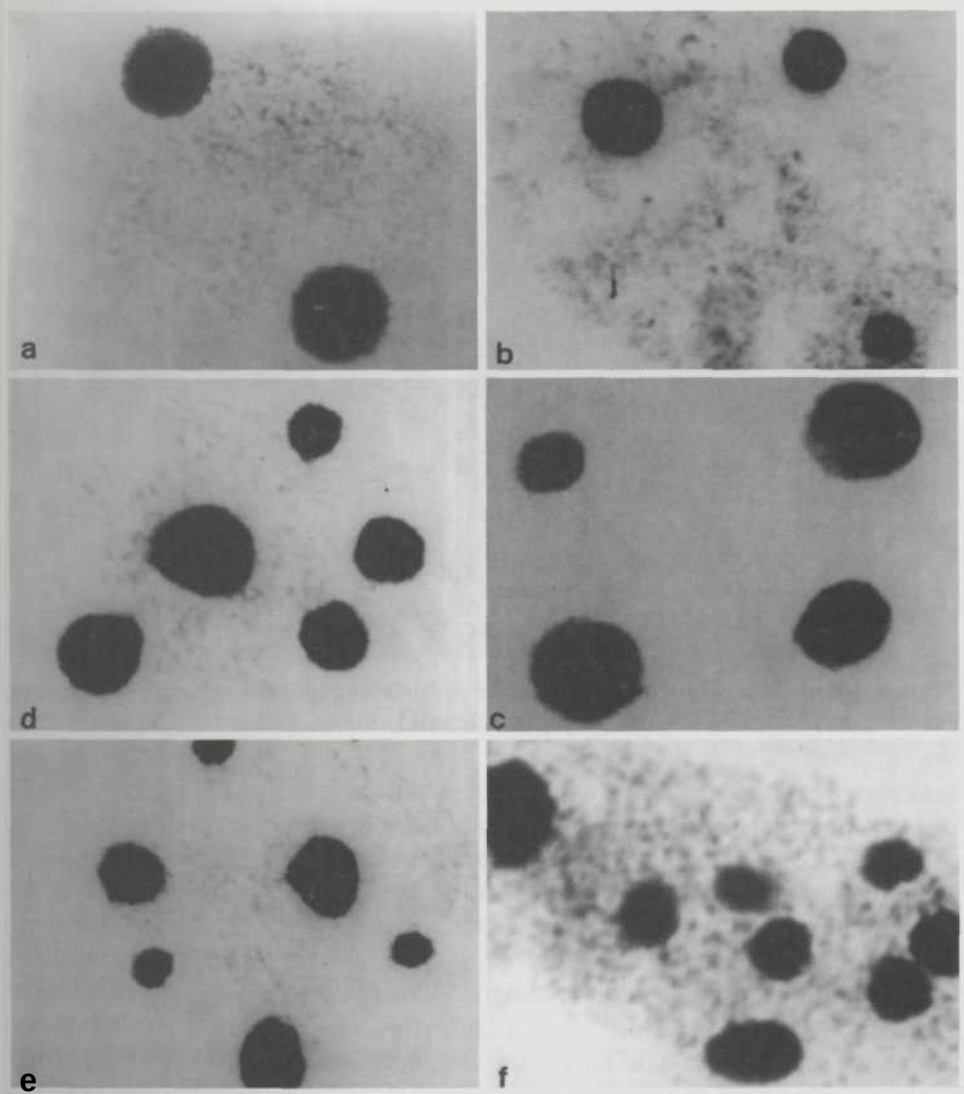


Figure 13 a - f Interphase nuclei of *Abies sibirica* with different numbers of nucleoli. Material stained with silver nitrate.

There was a NOR in the same region in the chromosomes of *L. gmelinii, L. cajanderi* and *L. ochotensis.* In the asymmetric chromosomes of L. *sibirica* and *L. sukaczewii,* the secondary constrictions occurred infrequently and an NOR was observed at this location. Silver-staining revealed a nucleolar organizer region at the telomere of the short arm of asymmetric chromosome in all species.

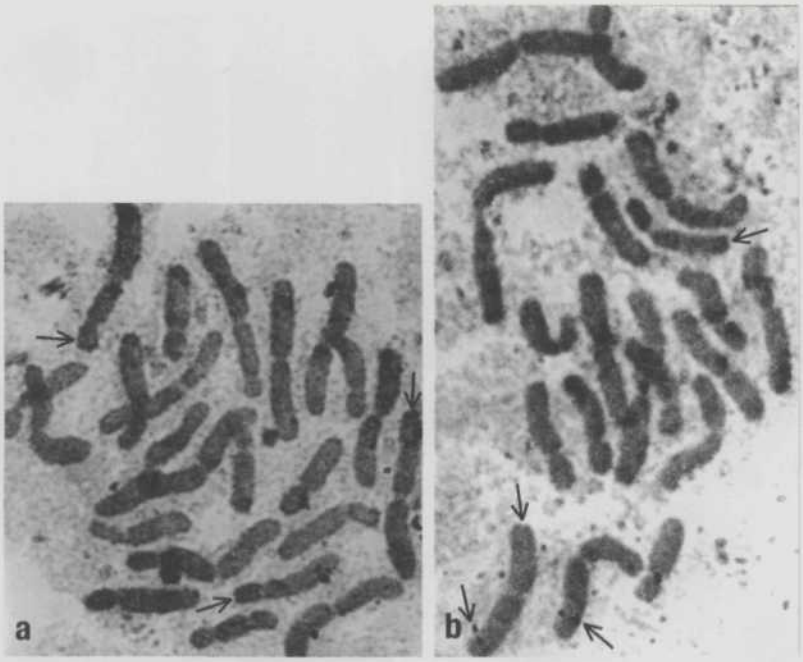


Figure 14 a - b Metaphase chromosomes of *Larix sibirica* stained with silver nitrate. Arrows point to the NORs.

Figure 15 Nucleolar organizer regions of *Larix sibirica* stained with silver nitrate staining: a metacentric chromosomes with silver-stained NORs at the telomere regions, b metacentric chromosomes with NORs at the secondary constrictions, c submetacentirc chromosomes with NORs at the secondary constrictions, d, **e** submetacentric chromosomes with NORs at the telomere regions.

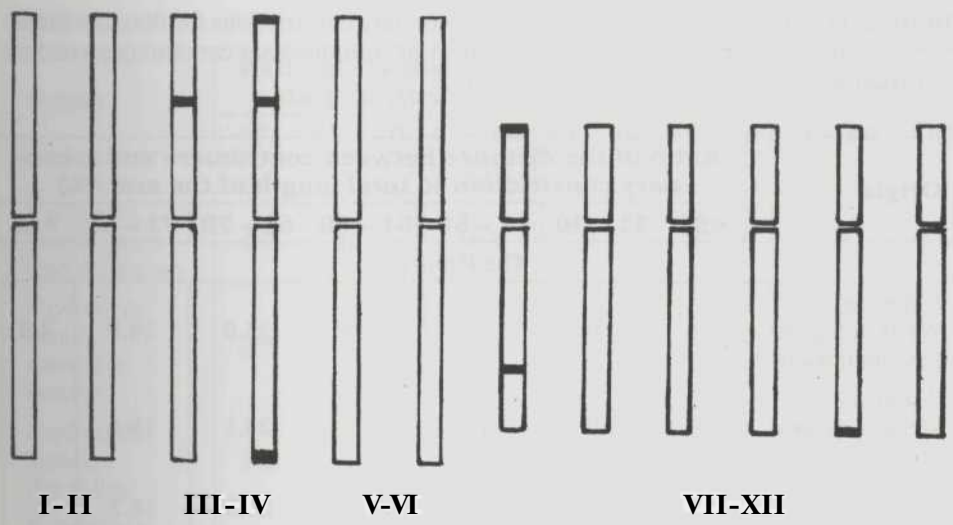


Figure 16 Idiogram of *Larix sibirica.*

Discussion

The above results have shown that secondary constrictions can be located on virtually any chromosome arm throughout the genome of the studied species. These results concur with previous studies of *Pinaceae* species (SAYLOR 1964, 1972, BORZAN 1977, HIZUME 1988). The secondary constrictions appeared as acromatic regions, and the resulting satellite's morphology generally was the same as the adjoining chromosome arm. Similar secondary constrictions and satellites have been observed in some angiospermous genera including *Polygonatum, Lilium, Fritillaria,* and individual species of *Allium* (TERMAN-SUOMALAINEN 1949, KUDRIASHOVA 1969, VERMA and MITTAL 1978, BARANOVA and ZAKHARYEVA 1981). Infrequently, «classical» satellites were observed in the chromosome complements of *P. funebris, P. sylvestris, L. sukaczewii* and *L. sibirica,* but these instances are atypical for conifers.

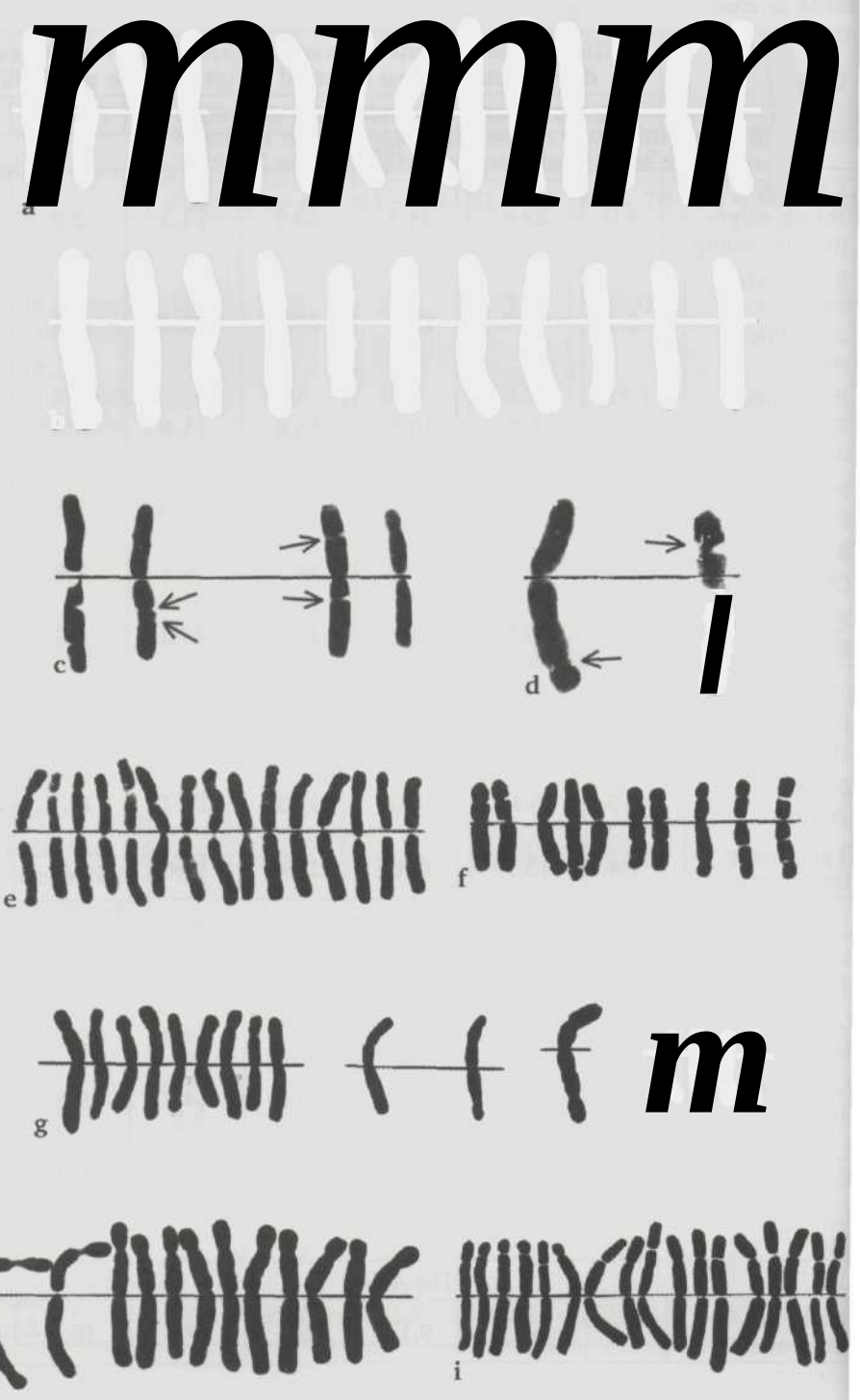
The secondary constrictions of the studied species occurred in a region from 30 to 80 percent of the arm length from the centromere (Table 2). There were differences among the genera in distribution of the secondary constrictions within a chromosome arm. Virtually all *Pinus* and *Picea* species had the majority of secondary constrictions located 50 - 60 percent in distance from the centromere. The majority of secondary constrictions in *Larix* species and *Abies sibirica* occurred further from the centromere (60 - 70 percent) than the previous two genera.

Table 2 Frequency and localization of the secondary constrictions for *Pinaceae* fam representatives as a percent of the total number of chromosomes containing second; constrictions.

Origin	Ratio of the distance between centromere and secoi dary constriction to total length of the arm (%)						
	<31	31-40	41-50	51-60	61-70	71-80	>8
	The *Pinus*						
P. sibirica, Western Sayans, Low mountains	0.6	9.0	15.7	33.3	25.0	14.9	1.
P. sibirca, Western Sayans, high mountains	1.4	9.0	13.8	38.1	24.1	18.6	-
P. sibirica, Turukhansk	2.0	8.8	23.5	24.5	24.5	16.7	-
P. sibirica, Khanty-Mansiyisk	1.5	9.3	12.8	24.1	29.5	21.2	1.
P. sibirica, Ekaterinburg	2.1	6.8	17.3	33.0	22.5	18.3	-
P. sibirica, Karpinsk	-	6.7	14.4	31.7	26.0	20.2	1.
P. pumila, Tauisk	13.1	20.0	26.9	28.3	11.7	-	-
P. pumila, Orotukan	1.8	12.0	18.5	31.5	22.2	13.0	0:
P. pumila, Central Kamchatka	2.0	12.7	25.0	27.5	24.5	7.1	l.i
P. pumila, Northern Kamchatka	0.8	9.4	8.5	34.5	20.0	24.2	2.!
P. koraiensis, Obluchje	3.5	17.6	13.1	19.6	23.6	21.5	1.!
P. koraiensis, Chuguevka	3.4	12.9	9.5	30.2	28.4	13.8	i.:
P. sylvestris, Kyakhta	2.5	16.2	18.2	37.8	22.4	2.5	0.'
P. sylvestris, Novoselenginsk	3.9	10.8	10.1	47.3	25.6	2.3	-
P. sylvestris, Minusinsk	2.1	17.7	20.8	32.3	26.0	1.0	-
P. sylvestris, Yakutsk	1.7	15.7	15.7	38.7	25.9	2.2	-

Table 2 cont.

Origin	Ratio of the distance between centromere and secondary constriction to total length of the arm (%)						
	<31	31-40	41-50	51-60	61-70	71-80	>81
The *Pinus*							
P. sylvestris, Tomsk, oligotrophic swamp	4.0	22.4	24.3	23.7	21.3	3.8	0.5
P. sylvestris, Tomsk, eutrophic swamp	0.7	13.0	21.9	39.7	21.9	2.0	0.7
P. sylvestris, Tomsk, dry valley	1.3	6.5	16.9	55.8	15.6	4.0	—
P. densiflora, Barnaul	1.3	26.6	15.8	32.9	19.6	3.8	-
P.funebris, Chanka	0.6	12.8	17.9	39.7	25.7	2.8	0.6
P. eldarica, Georgia	-	32.5	22.2	22.7	22.2	0.5	-
The *Picea*							
P. obovata, Kochegarovo	1.7	3.4	17.3	44.3	15.2	16.4	1.7
P. obovata, Yakutsk	1.5	6.1	13.0	47.3	13.7	18.7	-
P. ajanensis, Dalnegorsk	1.4	13.9	33.6	31.4	16.8	2.9	-
The *Larix*							
L. sibirica, Zaisan	-	-	4.4	27.9	64.7	2.9	-
L. sukaczewii, Uchali	1.1	-	-	29.3	59.8	9.8	-
L. gmelinii, Karimskoe	-	0.9	9.4	39.6	44.3	5.7	-
L. cajanderi, Kamchatka	1.4	2.8	10.7	30.7	52.1	2.1	-
L. ochotensis, Tauisk							
The *Abies*							
A. sibirica, Emelyanovo	1.6	6.9	9.1	26.7	37.4	18.2	–


mmm
a
b
c
d
l
e
f
g
m
h
i

According to the concept of the chromosome architecture (LIMA-DE-FARIA 1976), genes for 28S and 18S ribosomal RNA in the eukaryotic chromosomes have specific position with respect to the centromere and the telomere. The ribosomal genes occupy definite loci within the chromosome arm. The distribution of these genes can be divided into four main regions. According to LIMA--DE-FARIA (1976), the optimal region represents the zone of maximum frequency with about 80 percent of ribosomal genes. On the both sides of this region, there are zones where ribosomal genes may occur with less frequency. A region exists near the centromere where ribosomal genes rarely occur, having a probability of 0.2 percent. The blocked region is where the probability of occurrence is slightly higher.

For *Pinus* and *Picea,* respectively, zones for ribosomal genes were localized on the left and on the right optimal zone (40 - 50 and 60 - 70 percent). The zones were at 50 - 60 and 70 - 80 percent of the optimal zone in *Abies sibirica.* Species of the genus *Larix* have only one zone located at the distance of 50 - 60 percent (of arm length) from the centromere. In all genera, sites from 25 - 35 percent and from 75 - 85 percent are »blocked« regions. Secondary constrictions never occur less than 25 percent and more than 85 percent from the centromere.

LIMA-DE-FARIA (1976) has analyzed information on the structural organization of the chromosomes for more than 500 plant species from different systematic groups. He concluded that the ribosomal cistrons occur in the short arm in 86.6 percent of the species analyzed. In contrast, many species examined for the family *Pinaceae* have secondary constrictions in the long arm of asymmetric chromosomes that stain positively for NORs. LIMA-DE-FARIA (1976) suggest two explanations for this phenomenon: 1) the kinetochores, the telo-

Figure 17 a - i Morphology of the secondary constrictions of *Pinaceae* family representatives (horizontal lines point to location of the centromere): a the secondary constrictions of symetric chromosomes of *Pinus sibirica,* b the secondary constrictions of asymmetric chromosomes of *Pinus sibirica,* c the chromosomes of *Pinus sibirica* with two secondary constrictions in one arm and with constrictions in two arms (arrows point to the secondary constrictions), d two types of the secondary constrictions in chromosome pair XII of *Pinus pumila* chromosomes, **e** the secondary constrictions of *Pinus koraiensis* chromosomes, f the secondary constrictions of *Pinus sylvestris* chromosomes, **g** the secondary constrictions of *Larix sibirica* chromosomes (metacentric chromosomes with one secondary constriction; metacentric chromosome with two constrictions in one arm; metacentric chromosome with constrictions in two arms; metacentric chromosome with two secondary constrictions in one arm and with one constriction in the other arm; submetacentric chromosomes with secondary constriction in the long arm), h secondary constrictions of *Larix sukaczewii* chromosomes, **i** secondary constrictions *of Picea obovata* chromosomes.

meres or the nucleolar organizers have other properties, or 2) structural rearrangement has recently occurred and the chromosome has not yet attained genetic equilibrium.

The above listed maximum numbers of the secondary constrictions in the conifer chromosomes do not occur in every cell. These data suggest that all ribosomal genes do not function simultaneously. Investigated populations of pine, spruce, larch and fir species differ in the localization patterns and numbers of the secondary constrictions in the metaphase chromosomes and in the numbers of nucleoli in the interphase nuclei. The phenotypic variability of the secondary constrictions, ranging from prominent ones to weak achromatic zones, further support this hypothesis (Figure 17).

The occurrence of the constrictions is greater in populations from areas with severe or marginal climatic conditions. This increase was noticed in populations *of Pinus sibirica* in the north of western and eastern Siberia and in the high mountains of the West Sayans, for *P. sylvestris* in Yakutia and Southern Zabaikalje, for *Picea obovata* in Yakutia, for *Larix sibirica* in Kazakhstan and Tuva. Furthermore, the chromosomes of conifers growing severe or marginal conditions have a wider zone of active nucleolar organizers (for example pine frorc swamp populations).

A great variety in numbers, distributions and frequencies of the secondary constrictions has been described in many other plants both conifers and angiosperms (DERYAGIN and IORDANSKY 1971, KRUKLIS 1974, BOUGOURD and PARKEI 1976, MURAYA *et al.* 1976, ANASTASSOVA-KRISTEVA and NICOLOFF 1978, MALAKHOV/ 1979, DUBROVA and MALAKHOVA 1980, BONDAR *et al.* 1987). The biological significance of the nucleolar chromosomes in natural populations has not beer explained until recently. However, the peculiarities of these regions and variable appearance indicates a definite level of protein metabolism in differen populations in relation with their adaptive ability.

There is not a high correlation between the numbers of secondary constrictions and nucleolus in the interphase nuclei. Several explanations can b< offered for this apparent inconsistency. Some chromosome pairs without secondary constrictions may have nucleolus organization capacity. Alternately, several chromosomes can take part in the organization of one nucleolus. It is als(possible that not all secondary constrictions are related to the nucleolus formation.

The existence of the large numbers of the secondary constrictions and theii variability is a typical feature of conifer chromosome structure, especially fo: pine and spruce chromosomes. This effect is due to both the large variety of th< nucleolar organizing regions in the chromosomes and to the functional state o the chromosomes. Larch chromosomes usually have far fewer of constriction: per metaphase plate and nucleoli in the interphase nuclei.

Staining with silver nitrate reveals a more complex picture of larch chromosomal NORs. The NORs can be selectively stained dark brown by silver solutions, leaving the chromosome arms weak-stained (GOODPASTURE and BLOOM 1975). *In situ* hybridization experiments have demonstrated that the chromosomal locations of genes coding for 18S + 28S ribosomal RNA are identical to sites revealing with silver staining technique (SABANEYEVA 1989).

In conclusion, studies on the karyotypes of *Pinaceae* representatives has shown many nucleolar regions in all investigated species. The large number of NORs may be a feature of the chromosomal evolution in conifers.

Acknowledgments

The author would like to thank V I. Shvetsova for editing the manuscript. This research was supported in part by the Russian Fundamental Science Foundation and the Krasnoyarsk Regional Science Foundation, grant 1F0049.

Literature cited

ANASTASSOVA-KRISTEVA, M. and H. NiCOLOFF: Nucleolus organizing regions and nucleoli formation in *Allium odorum*. Biol. Zentrbl. 97 (1):83 - 89 (1978).

BARANOVA, M. V and O. I. ZAKHARYEVA: On the taxonomical rank of *Korolkovia (.Liliaceae)* (Morphology, ontogenesis, karyotype). Botanicheskyi zhurnal (in Russian) 66 (2): 197 - 207 (1981).

BiANCHE, G., E BASSI, M. J. CARMONA and F. MAGGINI: Contenuto di DNA ribosomale e regioni organizzatrici nucleolari in *Ornithogalum montanum {Liliaceae)*. Atti. Assoc. genet, ital. 25:44-45(1980).

BOBROV, E. G.: Forestforming conifers of USSR. Nauka, Leningrad (in Russian). 188 p. (1978).

BONDAR, L. M., L. V TCHASTOKOLENKO and Y A. BARANOVA: Population analysis of functional activity of the nucleolus organizer in *Vicia cracca h.* plants. Genetika (in Russian) 23 (2):317 -324(1980).

BORZAN, Z.: Contribution to the karyotype analysis of the European black pine *(Pinus nigra* Am.). Annales Forestales 8 (3):29 - 50 (1977).

BOUGOURD, S. M. and J. S. PARKER: Nucleolar-organiser polymorphism in natural populations *of Allium schoenoprasum*. Chromosoma 56 (3):301 - 307 (1976).

DERMEN, H.: Original and behavior of the nucleolus in plants. J. Arnold Arboretum 14 (3): 282-322(1933).

DERYAGIN, G. V and A. B. IORDANSKY: Phenotypic variability of satellited chromosomes. I. *Allium cepa L., Alliumfistulosum* L. *andAllium altaicum* Pall. Genetika (in Russian) 7 (10):13 -22(1971).

DUBROVA, N. A. and L. A. MALAKHOVA: Study of nucleolus-forming chromosome polymorphism in *Actaea* L. species *{Ranunculaceae* Juss.). Cytologija and Genetika (in Russian) 14 (5):3-8(1980).

GRIE V G. and N. D. AGAPOVA: On the methods of description of plant karyotypes. Botanicheskyi zhurnal (in Russian) 71 (4):550 - 553 (1986).

GOODPASTURE, C. and S. E. BLOOM: Visualization of nucleolar organizer regions in mam malian chromosomes using silver staining. Chromosoma 53:37 - 50 (1975).

HIZUME, M.: Karyomorphological studies in the family *Pinaceae*. Memoirs of Fac. Educ. Eh me Univ. Ser. Ill Natural Science, 8 (1):1 - 108 (1988).

HIZUME, M., S. SATO and A. TANAKA: A highly reproductive method of nucleolus organizin regions staining in plants. Stain Technology 55 (2):87 - 90 (1980).

KOZUBOV, G. M. and E. N. MURATOVA: Contemporary gymnosperms (morphologosystematii review and karyology). Nauka, Leningrad (in Russian), 192 p. (1986).

KRUKLIS, M. V: Karyological peculiarities of *Larix czekanowskii* Sz. In: Variation in wood plants in Siberia. Krasnoyarsk (in Russian) p. 11 - 19 (1974).

KUDRIASHOVA, G. A.: A comparative-karyological investigation of Caucasian lilies. Botan cheskyi zhurnal (in Russian) 54 (7):1074- 1082 (1969).

LIMA-DE-FARIA, A.: The chromosome field. I. Prediction of the location of ribosomal cistron Hereditas 83:1 -22 (1976).

LITTLE, E. L. and W. B. CRITCHFIELD: Subdivisions of the genus *Pinus* (pines) (1969).

MALAKHOVA, L. A.: Polymorphism of satellite chromosomes of *Aconitum exselsum (Ranw culaceae)* in mountain populations of West Siberia. Cytologija (in Russian) 21 (9): 1094 1100 (1979).

MATSENKO, A. E.: Firs of Eastern hemisphere. In: Flora and systematics of higher plant Leningrad (in Russian), p. 3 - 102 (1964).

MURAYA, L. S., A. K. BUTORINA and E. M. DUDETSKAYA: Karyotypical characteristic of th« populations of *Pseudotsuga menziesii* (Mirb.) Franco. Lesovedenije (in Russian) (6):66 - 7 (1976).

NAVASHIN, S. G.: On the dimorphism of nuclei in the somatic cells in *Galtonia candican* Proc. Russia Acad. Sci. 6:373 - 385 (1912).

PRAVDIN, L. E, V A. BUDARAGIN, M. V KRUKLIS and O. R SHERSHUKOVA: Methods of karyolog investigation of conifers. Lesovedeniye (in Russian) 2:67 - 75 (1972).

RATTENBURY, J. A.: Specific staining of nucleolar substance with aceto-carmine. Stain Tecl nology 27 (2): 113 - 120 (1952).

RITOSSA, F. M. and S. SPIEGELMANN: Localization of RNA in the nucleolus organizer regioi of *Drosophila melanogaster*. Proc. Nat. Acad. Sci. USA 53 (4):737 - 744 (1965).

SABANEYEVA, E. V: The specificity of silver nitrate staining for the nucleolar organizer reg ons. Cytologija (in Russian) 31 (1):5 - 14 (1989).

SAYLOR, L. C: Karyotype analysis of *Pinus* group *Lariciones*. Silvae Genet: 13 (6):165 - 17 (1964).

SAYLOR, L. C: Karyotype analysis of the genus Pinus - subgenus *Pinus*. Silvae Genet. 2 (5):155-163 (1972).

TERMAN-SUOMALAINEN, E.: Investigations on secondary constrictions in *Polygonatum*. Hen ditas 35:86-108 (1949).

VERMA, S. C and R. K. MITTAL: Chromosome variation in the common garlic, *Allium sativw* L. Cytologia 48 (2):383 - 386 (1978).