Cytogenetical study on Scots pine (*Pinus sylvestris* L.) in the Central Yakutia

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Scots pine (*Pinus sylvestris* L.) has a diploid complement of 24 chromosomes (2n=2x=24). The Central Yakutain population includes eleven pairs of metacentric (I - X, XII) and one pair of submetacentric chromosomes (XI). Seven pairs of metacentric chromosomes have secondary constrictions; two pairs of chromosomes have more than one constriction. Genome and chromosomal mutations were found in this population. Chromosome irregularities were rare but some individuals have aberrations or disturbances in more than 1 percent of the cells. Mitotic anomalies were also present in the same trees. Analysis of meiosis in pollen mother cells has shown irregularities in the generative sphere development. Some of these anomalies suggest the mutant nature of trees.

**KEY WORDS:** *Pinus sylvestris* L, karyotype, chromosome, secondary constriction, mitosis, meiosis, mutation

Scots pine (*Pinus sylvestris* L.) occupies a vast range throughout Eurasia from 37° to 70° northern latitude and from 7° western longitude to 125° eastern longitude (PRAVDIN 1964). The Central Yakutia region is at the northern border of the species’ range. This region has relatively severe climatic conditions including a permafrost zone, cool continental climate, low annual moisture, severe long winters, and hot short summers are characteristic of this region.

Populations of Scots pine growing in marginal environments differ in genotypic structure (IROSHNIKOV 1978, MININA et al. 1988, TRETYAKOVA 1990). Highly variable forms of this species are observed along the borders of its natural range. Typical trees, trees with new growths on the stems and branches, unusual crown shaped forms, semi-dwarfed individuals, and trees with other anomalies occur in these populations.

As shown in previous studies (SUNTZOV 1982, MURATOVA and SUNTZOV 1988, MURATOVA 1991a), populations of Scots pine near the southern border of area are characterized by cytogenetic instability. The purpose of this study was to analyze the karyotype, mitosis, and meiosis of Scots pine at the northern border of species range.
Material and methods

Experimental material

Seeds of Scots pine trees from the Central Yakutia were used for cytogenetic investigations. Unusual phenotypes were investigated in detail. They are: semi-dwarfed trees (#35), trees with tumors (#1 op, #11 op, #17 op), and weeping crown shaped trees (#1 p, #2 p, #3 p, #4 p). Studies on typical trees (#1 t, #2 t, #3 t, #4 t and #33 t) were made for contrast.

Nine adult plants were used for meiotic studies: typical trees (#4 t and #33 t), tree with a tumor (#32 op), semi-dwarfed tree (#31), trees with wide (#5 and #6) and weeping shaped crown (#1 p and #29 p). At the end of May microsporocytes (pollen mother cells) were observed for meiotic analysis.

Cytogenetic analysis

Mitotic chromosomes were studied in root tip cells. Analysis of the chromosome aberrations was conducted in metaphase or for some plants, by anaphase according to the methodology of Bochkov et al. (1972). The materials were prepared and analyzed according to generally accepted techniques for coniferous plants (Pravdin et al., 1972) with some modifications. An improved hematoxylin squash technique was used to study the somatic and meiotic chromosomes. The root tips were pretreated in 0.5 percent colchicine solution for 6-8 hours, fixed in 3:1 ethanol : acetic acid mixture and stained with aceto-hematoxylin. The slide were prepared using the squash technique. Suitable cells were selected for analysis and photographed.

A 50 percent solution of silver nitrate was used to stain nucleoli. Male strobili from trees were used for meiosis study. During the time for the meiotic divisions fixations of the strobili were conducted every day or two days. Materials were fixed in 3:1 ethanol: acetic acid mixture and stained with aceto-hematoxylin. From each male strobilus, PMC (pollen mother cells) were analyzed with respect to stage of development and the occurrence of different types of irregularity.

The chromosomes were measured on photomicrographs. The following parameters were determined: absolute length of the chromosome (L, in micrometers); the total diploid complement chromosome length (ZL, in micrometers); relative chromosomes length (L, the ratio of absolute length to the total chromosome length, in percent); centromeric index (I, the ratio of the short arm length, in percent); and localization of secondary constriction (sc, in percent). The chromosomes were classified according to nomenclature developed by Grif and Agapoiva (1986).
Morphometric analysis of the chromosomes

All experimental materials had a diploid complement of 24 chromosomes (2n=2x=24). A metaphase plate of *P. sylvestris* is shown in Figure 1. In 53 metaphase plates, the total length of a diploid chromosome set varied from

![Figure 1 Metaphase plate of Scots pine (*Pinus sylvestris* L.) of root-tip meristem. 2n=24. Material stained with acetohematoxylin. Bar indicates 5 μm.](image_url)

![Figure 2 Variation of the total lengths of the diploid chromosome complements of Scots pine from the Central Yakutia. The abscissa axis is the total lengths of the diploid chromosome complements (SL^a); the ordinate axis is number of the metaphase plates (n). Marked interval shows total lengths selected for statistical analysis.](image_url)
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249.4 to 475.3 μm (Figure 2). Thirty-one plates from interval Sl' = 330.0 - 390.0 μm were used for making of the polykaryogram (Figure 3).

Figure 3 The polykaryogram of Scots pine from the Central Yakutia. The abscissa axis is the centromeric index (I', %), the ordinate axis is the relative length of the chromosomes (I/, %). I-XII are the numbers of the chromosomes.

Two chromosome pairs (XI and XII), the shortest and most asymmetric in the complement, could be readily identified. These chromosomes have the following parameters: XI L' = 12.2 ± 0.15 μm, I/ = 3.3 ± 0.7 %, I' = 39.3 ± 0.37 %; XII L' = 11.5 ± 0.24 μm, I/ = 3.1 ± 0.07 %, I' = 42.4 ± 0.24 %. It was also possible to distinguish chromosome pairXL' = 13.0 ± 0.18 μm, L' = 3.6 ± 0.04 %, I' = 47.6 - 0.29 %. The other ten pairs of chromosomes (I - X) form one group with similar parameters: L' = 16.6 ± 0.07 μm, I/ = 4.5 ± 0.02%, I' = 47.6 - 0.15 %.

Many chromosomes had the secondary constrictions. Two constrictions could be observed in both arms of chromosome pair V (sci = 44.2 ± 0.70 %, sc2 = 61.1 ± 0.66 %) and three constrictions with different frequencies were observed in chromosome pair VIII: two constriction are in one arm (sci = 37.7
± 0.63 %, \( sc_2 = 63.8 \pm 0.78 \% \) ) and one constriction is in the other arm (\( sc_3 = 62.7 \pm 1.51 \% \)). Two pairs of the chromosomes (VI - VII) had the secondary constrictions in the distal part of the arm (\( sc = 62.9 \pm 0.48 \% \)) and one pair of the chromosomes (IX) had a constriction in the proximal part of the arm (\( sc = 40.9 \pm 0.64\% \)).

Chromosome pair X was characterized by the secondary constriction in the medial portion of the arm (\( sc = 59.9 \pm 0.88 \% \)). The asymmetric chromosomes pair XI also had a constriction in the proximal part of the long arm (\( sc = 41.0 \pm 2.06 \% \)). Both constrictions occurred infrequently. The interphase nuclei contained 1-11 nucleoli (Figure 4). Coefficient or correlation between the numbers of interphase nuclei nucleoli and the numbers of the secondary con-

Figure 4 a - f Different numbers of nucleoli in the interphase nuclei of Pinus sylvestris.
Material stained with silver nitrate.
strictrions in the metaphase chromosomes was \( r = 0.15 \pm 0.05 \). The connection between the nucleoli and the secondary constrictions is shown in Figure 5.

![Figure 5](image_url)

**Figure 5 a - d** Connection between the regions of the secondary constrictions and the nucleoli. Material stained with acetohematoxylin.

In Figure 6, the idiogram of Scots pine at the northern border of its range is illustrated. According to the GRIF and AGAPOVAS (1986) nomenclature, chromosome pairs I-X and XII were metacentric and chromosome pair XI was submetacentric. The karyotypic pattern of the Central Yakutian Scots pine was similar to patterns for this species from the other parts of its range (SAYLOR 1964, MIHAILESCU and DALU 1972, KORMUTAK 1975, ABATUROVA 1978, ILCHENKO 1978, BARANEC 1979, ABATUROVA and VISHNYAKOV 1980, ABATUROVA and SHERSHUKOVA 1981, BORZAN, 1981, 1988, MURATOVA 1991a, MURATOVA and SEDELNIKOVA 1993). However, the northern populations differ from the central and southern ones by increases in chromosome lengths and in the number and position of secondary constrictions.
Figure 6 Idiogram of Scots pine (*Pinus sylvestris* L.) from the Central Yakutia. I — XII — the numbers of the chromosomes. Bar indicates 5 μm.

**Genome and chromosome mutations**

Seedlings of the unusual phenotypes exhibited mixoploidy: they have triploid (2n=3x=36) and tetraploid (2n=4x=48) cells, but the majority of the cells possessed of the diploid chromosome number (2n=2x=24). The typical trees had cells with 2n=2x=24 chromosomes clones.

The mitotic studies revealed a number of chromosomal anomalies. Some anomalies were similar to the chromosome irregularities that were found by SUNTZOV (1982) in the Central Tuva region and by MURATOVA (1991a) in the Southern Zabaikalje region. The anomalies were: small or the large acentric rings, ring chromosomes (one or two), a ring chromosome of 8-shaped configuration, rings with fragments, a rod chromosome surrounded by the ring chromosome, polycentric chromosomes (di- or tricentric), dicentric together with ring chromosomes, one or two fragments and other anomalies.

The ring and polycentric chromosomes were not additions to the genomes. They were one or two out of the diploid set chromosomes. Ring and polycentric chromosomes have been observed in several conifer species including *Pseudotsuga menziesii* (REH Feld *et al.* 1983), *Picea obovata* (MEDWEDEWA and MURATOVA 1987), some species of *Larix* (MURATOVA and CHUBUKINA 1985, MURATOVA 1991b). In addition rare type of anomalies have been found in the Central Yakutian population. In some cases, the karyotype contained one asymmetric chromosome (submeta-, subacro- or acrocentric), while the second homologue was metacentric. Occurrence of this asymmetric chromosome with very short arm in *P. sylvestris* karyotype shows a heterozygous deletion or deficiency.

Chromosomal aberrations occurred only in separate cells. The frequency of anomalies is low, from 0.2 to 2.7 percent. It is significantly lower than fre-
frequency of aberrations in Scots pine from the southern Zabaikalje region. Only
the progenies of three trees had more than 1 percent cells with irregularities. They are variants #2 t (typical) and #2 p and #3 p (with weeping shape of
crown). Figure 7 shows some of the chromosomal anomalies.

Rings are the results of chromosome and chromatic structural changes. Ring chromosomes are due to deletion of the telomere parts and conjunction of the »sticky« ends of a middle fragment (NEMTSEVA 1970, DUBININA 1978). During this process acentric fragments arise also. The acentric rings have not a
centromere, while the polycentric chromosomes have more than one centromere. Polycentric chromosomes are the result of asymmetric translocations
(NEMTSEVA 1970).

**Analysis of mitosis**

Mitosis has been studied in four trees: thypical (#4 t), tumorous tree (#2 op)
and weeping-crown trees (#1 p and #3 p). The number of anaphases with anomalies varies from 1.6 - 4.0 percent. A high level of mitotic irregularities (4.0 percent) were observed in the #1 op tree. Single and double fragments, chromosome and chromatid bridges with and without fragments, bridges, precocuous separtation and chaotic distribution of chromosomes were detected
(Figure 8). At anaphase, a ring chromosome of 8-shaped configuration also
was observed.

The two approaches to investigation (metaphase and anaphase) have reve-
aled almost the same number of chromosome anomalies from a typical tree (#4 t). For the other trees, the anaphase method has shown considerable gre-
er number of mitotic irregularities. The occurrence of fragments at the ana-
phase of mitosis is characteristic of deletions. Formation of bridges with frag-
ments is ascribed to asymmetrical translocations. Chromosome bridges are indicative of dicentric chromosomes, and their number in anaphase and meta-
phase was nearly equal. Other irregularities (lagging chromosomes, centric
misdivisions etc.) are caused by anomalies of the centromeric region (ALOV 1978).

**Analysis of meiosis**

Nine adult plants were used for meiotic studies: typical trees (#41 and #33), a
tumorous tree (#2 op), a semi-dwarf tree (#31), trees with wide crowns (#5
and #6) and weeping-crowned trees (#1p and #29 p). At the end of May micro-
sporocytes (pollen mother cells) were observed for meiotic analysis. After
prophase I, the chromosomes were observed to shorten and appear like »lamp-
brush« chromosomes during diplotene. This phenomenon is associated with
the return of the chromosomes to an active transcription state (GOLUBOVSKAYA
1975, KLASTERSKA 1976). During this period, nucleoli were not observed.
Figure 7 Chromosomal mutations in Scots pine in the Central Yakutia (arrows point to irregularities): a part of metaphase plate with single fragment in tree #1 p (weeping crown shaped), b, c part of metaphase plate with ring (b) and ring chromosome (c) in tree #1 p, d part of metaphase plate with dicentric chromosome in tree #1 p, e part of metaphase plate with dicentric chromosome and one fragment in tree #1 p, f part of metaphase plate with two rings and one long submetacentric chromosome in tree #1 p, g metaphase plate with acentric ring in tree #2 p (weeping crown shaped), h metaphase plate with ring chromosome in tree #2 p, ...
Figure 7 cont.  i metaphase plate with ring chromosome in tree #1 op (with tumor)  
j metaphase plate with dicentric chromosome and two fragments in tree #3 p (weeping crown shaped),  
k part of metaphase plate with ring and acrocentric chromosomes in tree #11 op (with tumor),  
l metaphase plate with one subacrocentric chromosome in tree #4 p (weeping crown shaped),  
m metaphase plate with one subacrocentric and one ring chromosomes in tree #35 (typical).
Figure 8 Chromosomal anomalies in anaphase cells in Scots pine in the Central Yakutia: a two breaks in a single bridge in tree #3 p (weeping crown shaped), b two breaks in double bridges in tree #1 p (weeping crown shaped), c lagging chromosome fragments in tree #3 p, d lagging chromosome in tree #3 p, e going ahead chromosome in tree #3 p, f chaotic distribution of the chromosomes in tree #2 op.

At diakinesis, when the contraction reached its' maximum, the configuration of bivalents could be observed and chiasmata were visible. Scots pine has up to 4 chiasmata per bivalent. At metaphase I, the chromosomes were oriented in the center of the cell. Separation of homologous chromosomes occurs almost immediately during anaphase of the first meiotic division. Diakinesis, metaphase I, and anaphase I stages were of short duration.
Figure 9  The first division of meiosis in Scots pine in the Central Yakutia: a - d early prophase I (leptotene - pachytene), e - g prophase I (diakinesis), ...
At telophase, daughter nuclei were formed and then interphase could be observed. Four microspores are produced as a result of the second meiotic division. In Figures 9 and 10, the meiotic stages from leptotene to tetrads are illustrated. The appearance of the chromosomes during the different stages did not differ from the ordinary pattern of meiosis in conifers, except in the diplotene stage where they appeared similar to lamp-brush chromosomes.

Asynchronous meiosis was observed not only within one tree, but within one microsporangium. This was one karyotypic characteristic of Scots pine growing in northern marginal locations. The meiosis occurred quickly and concluded in three or five days between the first meiotic divisions and the formation of tetrads. This was another karyotypic characteristic of trees from this region. A third meiotic characteristic was a delay at the early prophase I stage in many cells.

Analysis of meiosis in pollen mother cells revealed anomalies in the generative sphere development. Meiotic aberrations were shown at different stages. Univalents at the stage prophase I, lagging chromosomes, spindle anomalies at the metaphase I, bridges, fragments, bridges with fragments, formation of three poles instead of four at anaphase I, bridges and fragments at anaphase II and telophase II, triads, sterility of pollen and other irregularities were observed (Figure 11).
Meteorological factors probably influenced the processes of generative organ formation, microsporogenesis, pollination and fertilization. Many stages of meiosis in conifers are sensitive to low and high temperatures. Investigations of different authors have shown that meiotic irregularities are due to the effects of temperatures (BARNER and CHRISTIANSEN 1960, CHANDLER and MAVROD NEANU 1965, CHIRA 1973, EKBERG et al. 1968, LUOMAJOKI 1977. Furthermore
anomalies of meiosis could be a result of a hybrid state, the presence of chromosomal aberrations or mutant genes controlling the meiotic process. It is known that these processes are under genetic control (GOLUBOVSKAYA 1979).

Some irregularities suggest the mutant nature of trees. For example, occurrence of univalents during the prophase I (Figure 11a) can be related to mutant genes for desynapsis (KODURU and RAO 1981). Univalents were revealed in tree #1 p with a weeping-crown. Anomalies in anaphase I are due to mutations affecting the spindle (GOLUBOVSKAYA 1979, 1985). Cytological display of these mutations (unequal distribution of the chromosomes between two or three poles, lagging chromosomes, small fragments, stickiness of the chromosomes) was observed in a typical tree (#41), the tumorous tree (#2 op) and a weeping-crown tree (#1 p) (Figure 11 b - e).

The appearance of chromatid bridges with fragments during anaphase I and anaphase II suggests the presence of heterozygous paracentric inversions or homozygous duplications due to inversions (KHOSTOVA and YACHEVSKAYA 1975). The chromosome inversions are widely distributed in plant and animal populations (BURNHAM 1956, STEBBINS 1971). Pericentric inversions have been recently revealed in Larix gmelinii (MURATOVA 1994). Different types of bridges, in-
Figure 11 Irregularities of meiosis in Scots pine in the Central Yakutia: a desynapsis of the chromosomes at metaphase I in tree #1 p (weeping crown shaped), b disorderly distribution of the chromosomes at anaphase I in tree #1 p, c disorderly distribution of the chromosomes at anaphase I in tree #4 t (typical), d sticky chromosomes presumable in metaphase I in tree #4 t, e splitting of the spindle at anaphase I in tree #4 t, I bridges at anaphase I in tree #2 op (with tumor), g bridge and fragment at anaphase I in tree #2 op, ...
eluding inversive, have been found in some conifer species (Saylor and Smith 1966, Ekberg et al. 1968, Krukliš 1974, Butorina et al. 1982). These different lines of evidence suggest the possibility of the presence of paracentric inversions and deletions in some Scots pine trees in Central Yakutia region. However, ring and polycentric chromosomes were not observed in meiosis of trees from this region.

The low number of dyads and triads together with tetrads could have originated from a gene mutation controlling parallel spindle formation (cf. Mok and Pelquin 1975, Butorina et al. 1982). The parallel arrangement of spindles in the second division of meiosis has been observed in a tree with a tumor (#2 op) (Figures 10 c, e - f). Appearance of highly spiral chromosomes in some
trees can be due to mutant gene »ameiotic« (cf. RHOADES 1956, BUTORINA et al. 1988). This anomaly is illustrated by Figure 12 and 13. However, the gene action is characterized by not a full penetrance and shows only in the separate cells.

**Figure 12** Presumed phenotypic appearance of mutant gene »ameiotic« at the first division in tree #2 op (with tumor): a metaphase, b anaphase with break bridge and fragment, c anaphase with chromatid bridge.

**Figure 13** Presumable phenotype appearance of mutant gene »ameiotic« at the first division in tree 1 p: a prophase, b metaphase.
In summary, the cytogenetical studies on Scots pine in the Central Yakutia region has shown the occurrence of mixoploidy and mitotic and meiotic genetic changes such as chromosomal anomalies.

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