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Meiosis at Microsporogenesis in Siberian Fir (*Abies sibirica* Ledeb.) in Natural Populations and in an Arboretum

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Abstract

Meiosis in Siberian fir is characterized by asynchrony (prophase I and tetrads in the same microsporangium simultaneously). Up to 4 nucleoli (in natural stands) and 5 (in an arboretum) are observed at prophase I. The shortest meiotic stages are telophase 1 and 2. Each of them is observed in 0.2% (in an arboretum) and 0.05% (in natural stands) of cells. In natural stands meiosis elapses quickly (within 2 days). In an arboretum it can continue for more than two weeks. Mainly meiosis is regular. Meiotic irregularities are, though, also encountered at different stages (fragments, bridges, etc.). In trees growing in an arboretum the total number of irregular cells can reach 17.9%. In natural populations only 11.7-13.3% of cells are irregular. The spectrum of meiotic irregularities in an arboretum is wider than in natural stands.

Key words: *Abies sibirica* Ledeb., Chromosomes, Microsporogenesis, Meiosis, Meiotic irregularities

Introduction

Meiosis is one of the critical periods in the cycle of plant development. The meiotic stages of tree species have been described by many authors in different climatic and adaptive conditions. The priority of cell and chromosome levels is conditioned by the great sensitivity of these structures to environmental factors (Druskovic 1997, Zoldos *et al.* 1997, Miceta and Murin, 1998, Butorina *et al.* 2003, Fedotov and Kalchenko 2004). It has been shown that the stages of meiosis are sensitive to temperature, light and other factors. (Christiansen 1960, Mergen and Lester 1961, Kantor and Chira 1965, Eriksson 1968, Ekberg *et al.* 1972, Johnsson 1974, Kozubov 1974, Andersson 1980, Luomajoki 1977, 1986, Romanova and Tretyakova 2005). Latitudinal and longitudinal variation in *Picea abies* and *Pinus sylvestris* meiosis correlates with changes in climate (Wright and Bull 1963, Ruby 1967, Chung 1981, Eriksson 1982, Mikola 1982, Luomajoki 1986). A correlation between meiotic irregularities and certain meteorological conditions has been observed (Luomajoki 1986, Zhuchenko *et al.* 1986). The corresponding reactions to environmental factors may differ in their details. For example, in studies of some conifers species on plantations, it has been revealed that on the one hand meiosis can be stabilized and on the other hand it can be heavily disturbed (Muraya *et al.* 1988, Gavrillov and Butorina 2005).

It is well-known that meiotic processes are under genetic control. Certain genes can cause aberrant processes and the formation of pollen with different genomic constitutions is also observed (Fadeeva *et al.* 1980, Golubovskaya 1979, Sosnikhina *et al.* 1994, Bogdanov 2003). Chromosome behavior influenced by

ecological conditions is also conditioned by genotype (Shkootina 1975).

Abies sibirica Ledeb. is one of the environment forming and improving species in the southern Siberian mountains. Yet *Abies sibirica* meiosis has not been studied extensively. A short description of some stages is given by Nekrasova and Ryabinkov (1978). Some meiotic irregularities produced by air pollution have been described by Presnukhina and Kalashnik (2003).

The aim of this study is to analyse *Abies sibirica* Ledeb. meiosis in the natural forest ecosystems of the East Sayan Mountains and at the arboretum of the V. N. Sukachev Institute of Forest.

Materials and Methods

The study was conducted in fir stands growing in the East Sayan Mountains and at the arboretum of the V.N. Sukachev Institute of Forest during the period of 2002-2004. The fir trees were growing in the low mountains (the valley of the Laletina River, latitudes 450-520 m a.s.l.) and in the middle mountains (the Upper Kaltat, latitudes 640-720 m a.s.l.). In the arboretum trees were planted from seedlings from the nursery of the Siberian Institute of Fruit-farming (Barnaul, Altai). The characteristics of the trees are given in Table 1.

In the middle of April to beginning of May male strobili from six trees which were generally healthy but some of them damaged by rust canker (*Melampsorella cerastii* Wint.) growing in the valley of the Laletina River, six healthy and drying trees growing in the Upper Kaltat and six trees growing in the arboretum were examined for meiotic analysis. The male strobili were fixed in a 3:1 ethanol: acid mixture every day in field conditions and from cuttings of branches in the lab.

Table 1. Some characteristics of the trees.

Site	Age, years	Height, m	Diameter at 1.3 m, cm	Length of crown, m	Projection of crown, m
The Laletina River Valley	110 - 130	11 - 14	20 - 26	9.0 - 12.0	5.0 - 6.0
The Upper Kaltat River	90 - 140	11 - 14	22 - 28	13.0 - 16.0	3.5 - 4.5
The Arboretum	45	9 - 11	20 - 24	7.0 - 9.0	3.0 - 4.5

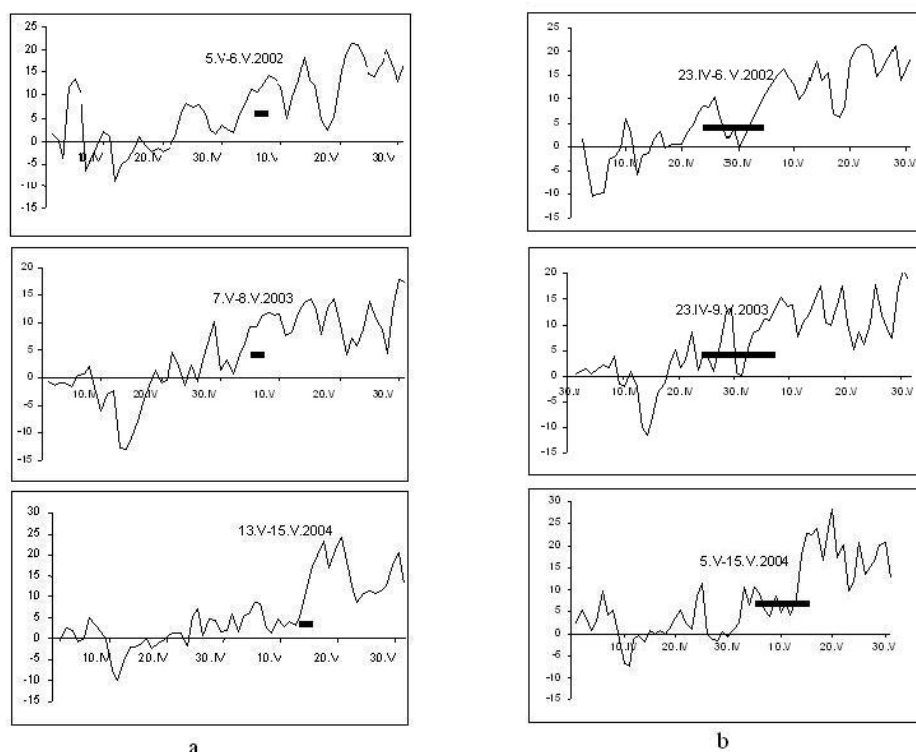


Fig.1. Dates of *Abies sibirica* meiosis in relation to air temperature in 2002-2004:
a – in natural populations (Laletina River valley), b – in the arboretum.
On the X-axis – air temperature, °C. Bars – dates of meiosis.

Pollen buds were stained with 1% acetohematoxylin for making a squash culture. Temporary squash slides of the separate anthers were viewed with a microscope (MBI-6) using the line survey method with respect to the stage of development and the occurrence and frequency of different types of irregularities. To simplify the microscopic work, the zygotene phase was not separately identified but included in the leptotene stage which in this investigation was associated with the moment when the chromosome strands first became visible. The results were statistically analyzed using standard statistical methods.

Results and Discussion

The pattern of meiosis in *Abies sibirica* trees was found to be largely the same as in other conifer species. The beginning point and rate of progress in meiosis is highly temperature dependent. In the East Sayan Mountains pollen mother cells (PMCs) usually start meiotic division in the first to second ten-day periods in May (Fig. 1a). In 2002 and 2003 in the low mountains the first divisions were noticed on May 5th and 7th and lasted one to two days. In 2004 meiosis started only on

May, 13th. Apparently the reason for this was a cold spring. In the middle mountains meiosis usually started some days later (usually one or two days in different years). This phenomenon was due to the temperature which decreases in the East Sayan Mountains 0.50 (May) to 0.55 (April) degrees per hundred meters (calculated using the Reference Book on the USSR Climate, 1967).

In the arboretum meiotic divisions of PMCs usually started earlier than in the natural populations. However, the duration (the interval from the beginning of leptotene to the end of telophase II) was 18-21 days. In 2002-2003 the first meiotic divisions were observed on April 23rd, while in 2004 they were observed only on May 5th. In 2002-2003 microsporogenesis continued up to May 11th through the 13th, and in 2004 on May 13th there was still meiosis in PMCs. This could be due to the temperature decreasing during this period (Fig.1b). The duration of meiosis in the trees growing in the same place varied not more than one day. As a rule, in microsporangia situated in the lower part of strobile meiosis proceeded quicker than in the upper parts.

The initial stages of meiotic prophase showed chromosomal threads that were tangled and interwoven (Fig. 2b). The PMCs were relatively large cells ($1.3\text{--}1.5$ times larger than tapetal cells – 57.3 ± 1.12 and 43.3 ± 0.81 μm , respectively) and were characterized by distinctive nucleoli. At the initial stages of meiotic prophase from 1 to 4 nucleoli were present in PMCs in natural stands and from 1 to 5 nucleoli in the arboretum. One of these nucleoli was markedly larger in most cells. The study revealed that in all trees nucleoli disappeared in diplotene (Fig. 2c) although earlier (Nekrasova and Ryabinkov, 1978) it was believed that they were present up to the end of diakinesis.

After the diplotene stage was completed, microsporogenesis developed rapidly and took some days for the advance from diakinesis to an immature microspore. At the stage of diakinesis when the contraction reached its maximum, the configuration of bivalents could be observed and 1-4 points of contact chiasmata were visible. In *A. sibirica* there were 12 bivalents, the morphology of which is determined by the localization and the number of chiasmata (Fig. 2d). On average the frequency of chiasmata was 2.6 ± 0.07 per bivalent and 31.2 ± 0.86 per metaphase plate. According to Sax and Sax (1933), in *A. cephalonica* and *A. nordmanniana* the quantity of chiasmata was on average 2.4 per bivalent.

Our research has shown that *A. sibirica* meiosis has some peculiarities as compared with other conifers:

1. PMC development occurs synchronously till diakinesis. At the beginning of this stage the cells develop asynchronously. In populations in the same sporangium there are PMCs at different stages of the first or second divisions, while in the arboretum there were PMCs with prophase I and immature pollen grains (Fig. 2e-l).
2. Telophase I and II occur very quickly. They were noticed only in 0.05% of the cells in the general populations and 0.2% in those of the arboretum.
3. A distinct prophase II is one of the peculiarities of *Abies sibirica* and possibly genus *Abies* (Mergen and Lester 1961) on the whole (Fig. 2h). In other Siberian conifers, for example in *Pinus sylvestris*, it is absent (Butorina *et al.* 1982, Muratova 1995).
4. In the general populations a few PMCs (less than 1%) and in the arboretum 0.3 – 20.5% have an abnormal (parallel) orientation of spindles. According to Butorina and co-authors (1985) the parallel orientation of the spindle could result in the formation of a dyad consisting of two $2n$ microspores or a triad of one $2n$ and two n microspores. However the character of the fusion significantly depends on environmental factors.

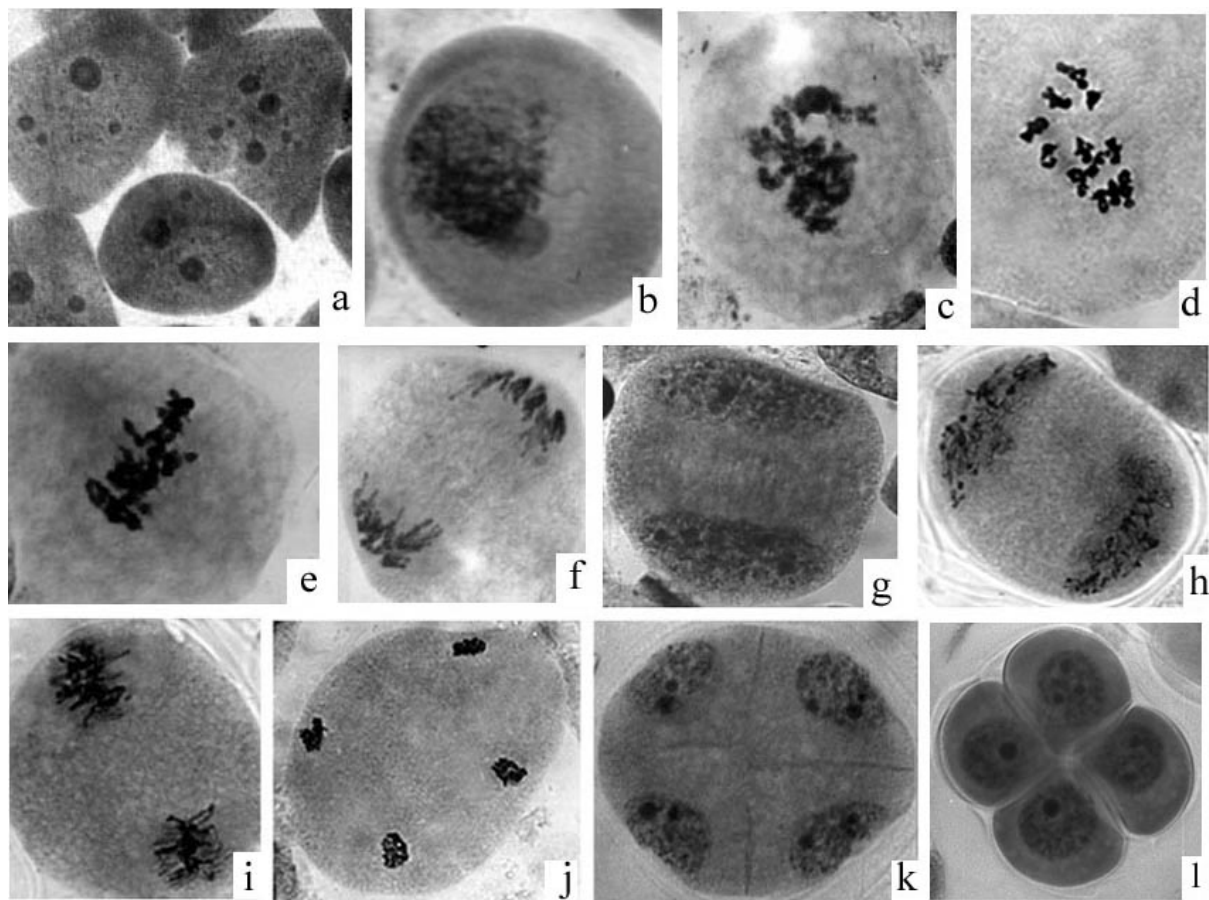


Fig. 2. Meiosis in *Abies sibirica*:

a – primitive archesporium; b – d – prophase I; b – leptotene, c – diplotene, d – diakinesis; e – metaphase I; f – anaphase I; g – dyad; h – prophase II; i – metaphase II; j – anaphase II; k, l – tetrads.

In most meiocytes meiosis was regular but among all trees the different stages throughout microsporogenesis and pollen formation revealed irregularities: 11.7% in the lower mountains, 13.3% in the middle mountains and 17.9% in the arboretum (Table 2). In the arboretum the frequency of irregularities increased in all meiotic stages and reached 48.2% at anaphase I. (Fig. 3). In addition, a high frequency of irregularities in all meiotic stages and pollen in the Upper Kaltat was observed with a higher frequency being observed in drying trees than in healthy ones.

Our study has shown that trees damaged by rust canker growing in the Laletina River valley have the same frequency of abnormal cells as healthy ones. There were differences at the tetrad stage only (up to 8.7% in healthy and up to 21.5% in damaged trees). We surmise that a part of the specific disturbance in damaged trees was not eliminated.

The frequency of irregularities varied in different years and among trees. It was highest in anaphase I and decreased at subsequent stages (Fig. 3). The decreasing frequency of irregularities in second meiotic divisions has been noted in a number of plants (Zhidkova 1989,

Gavrilov and Butorina 2005). As a rule, they do not appear even before the tetrad stage and as a practical matter do not affect the quality of the developing pollen grains. Only some characteristics such as chromatin agglutination may be reflected in pollen heterogeneity by the size and shapes of pollen grains and their decrease in fertility (Pozhidaeva *et al.*, 1985). Irregularities in first division were generally not observed at the dyad stage and the frequency of abnormal cells at the tetrad stage exhibited a similar tendency with second division metaphase and anaphase. This suggests that irregularities of second division result mainly in irregularities at the tetrad stage. At the same time, the frequency of irregularities in pollen was on the whole different from that observed at the tetrad stage. It can be thought that there are different mechanisms of irregularities at these stages. We surmise that the few pollen irregularities due to meiosis are mainly eliminated at the tetrad stage. It is possible that pollen irregularities chiefly result from mitosis when male gametophytes are forming (Bazhina *et al.*, 2006).

Table 2. Average frequency of meiotic irregularities, %.

Site	the Laletina River valley			the Upper Kaltat River	the Arboretum
	healthy trees	Trees damaged by canker	total		
Frequency of irregularities	12.3 ± 0.72	11.2 ± 0.51	11.7 ± 0.43	13.3 ± 1.21	17.9 ± 1.46
Size of samples	141	171	312	58	57

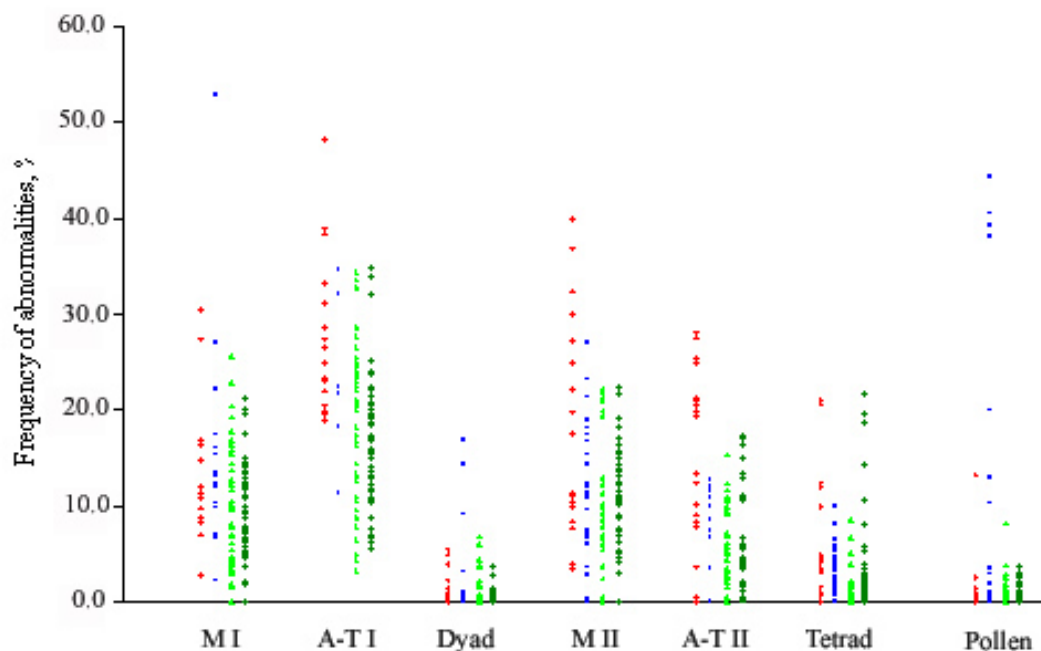


Fig. 3. Frequency of meiotic irregularities in natural populations and in the arboretum, %.

■ – in the arboretum ■ – in the Upper Kaltat ■ – Laletina River valley, healthy trees
■ – Laletina River valley, trees damaged by rust canker.

On the X-axis – stages of meiosis: MI – metaphase I; A-TI – ana-telophase I; dyad – stage of dyads; MII – metaphase II; A-TII – ana-telophase II; tetrad – stage of tetrads; pollen – pollen.

Most irregularities can be classified as a general type. Their spectrum was extensive in the Upper Kaltat and in the arboretum. Together with irregularities of a general type, specific and multiple irregularities were observed. In all trees the first meiotic irregularities could be identified in the metaphase of the first division (metaphase I). There were chromosomes outside spindle divisions, early chromosome separation, and irregular arrangement of bivalents (Fig. 4a). In trees growing in the Upper Kaltat agglutination of bivalents was also observed. In addition, in the arboretum there were fragmentation and agglutination of chromosomes into the ring. In anaphase I, bridges, lagging chromosomes, chromosomes outside spindle divisions,

chaotic chromosome separation, elongated chromosomes and 3-pole anaphases were general (Fig. 4b-d). In the low mountains there were also multiple irregularities: bridges and lagging chromosomes. There also was fragmentation of chromosomes in the Upper Kaltat and unequal chromosomes separation in the arboretum. Some of these irregularities were not eliminated at the interkinesis stage. For example, lagging chromosomes at the anaphase I stage were usually included in a nucleus at the dyad stage. But in some cells they often stood apart in the micronucleus or resulted in the formation of an irregularly shaped nucleus at the interkinesis stage. Bridges at the anaphase I stage which were observed in trees from the

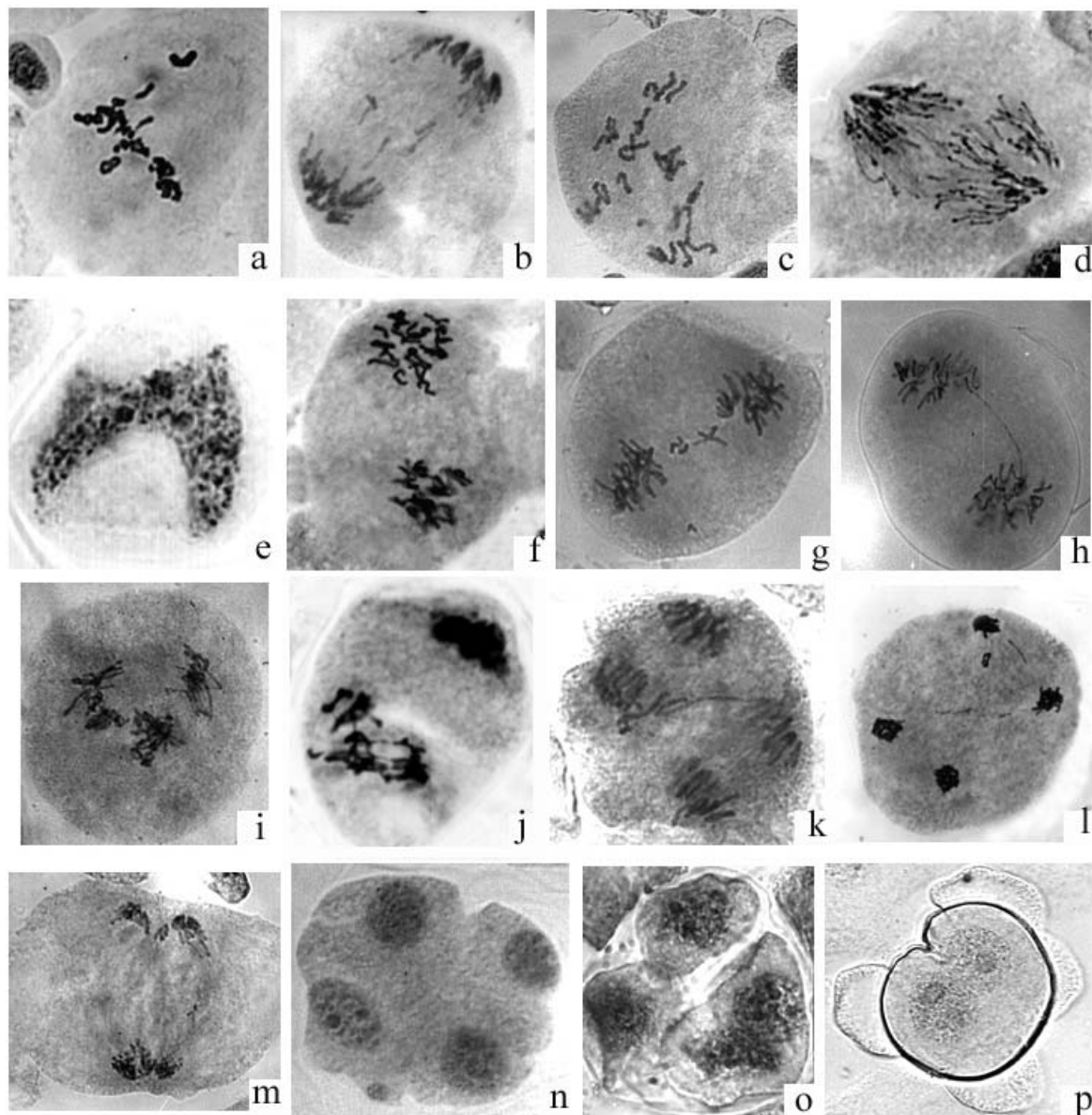


Fig. 4. Irregularities of meiosis: a – chromosomes outside of spindle divisions (metaphase I); b – lagging chromosomes (anaphase I); c – irregular divergence of chromosomes (anaphase I); d – elongated chromosomes (anaphase I); e – partial fusion of nuclei in dyad; f – chaotic chromosome arrangement (metaphase II); g – chromosomes outside spindle divisions (metaphase II); h – bridge (metaphase II); i – three (instead of two) groups of chromosomes (metaphase II); j – agglutination of chromosomes in the second division; k – bridge (anaphase II); l – lagging chromosomes (anaphase II); m – tendency to fusion at parallel orientation of spindles (anaphase II); n – micronucleus (tetrad); o – 2-n microspores in triad; p – 2-n microspores.

low mountains persisted through the dyad stages and sometimes even in the second division. The partial fusion of the nucleolus was observed in this stage in the Upper Kaltat and the arboretum.

In the metaphase of the second division bridges, chaotic chromosome arrangement and chromosomes and chromosome groups outside spindle divisions were general (Fig. 4f-h). In addition, early and abnormal (three groups of chromosomes instead of two) chromosome separations were observed in the trees growing in the Laletina River valley (Fig. 4i). In trees growing in the Upper Kaltat there were also elongated chromosomes in healthy trees and the agglutination of chromosomes in drying trees (Fig. 4j). Elongated chromosomes were also observed in the arboretum.

In anaphase II, chaotic chromosomes separation, bridges, chromosomes outside spindle divisions, and lagging chromosomes were general types of irregularities (Fig. 4k-l). Trees damaged by rust canker, healthy middle mountain trees and trees growing in the arboretum also had 3- and 5-pole anaphases. Multiple irregularities (bridges + 5-pole anaphases or chromosomes outside spindle divisions) were observed in the healthy middle mountain trees too. In drying trees fusion of spindles at their parallel orientation was seldom noticed (Fig. 4m). In the arboretum elongated chromosomes were observed (Fig. 4d).

In 2004 the specific development of some irregularities in the arboretum were noticed. For example, the segregation of lagging chromosomes in the micronucleus was observed at the stage of telophase (Fig. 4n). The bridges from anaphase I were saved in some cases in the second divisions or resulted in the partial or complete fusion of chromosome groups (Fig. 4e). Moreover, in different trees 0.2-2.2% of PMCs stopped their development at early prophase I. In such cells there was the agglutination of chromatin and sometimes the nucleoli came out into the cytoplasm.

Meiosis was normally completed by tetrad formation but we have identified in all trees the formation of not only tetrads, but also dyads and triads. For the first time triads with spores with two nuclei (2-n) were observed (Fig. 4o-p). This type of irregularity was noticed in trees damaged by rust canker and drying trees only. The conditions for the formation of such microspores depend on the orientation of spindle division. In the case of partial separation, there may be a confluence of chromosome groups. Here two alternatives are possible. If the confluence of chromosome groups occurs at both poles, two diploid microspores are formed. Chromosome confluence at only one of the poles (Fig. 4m) produces triads consisting of two haploid and one diploid gamete. The formation of unreduced gametes may be controlled by a meiotic mutation gene as with the potato (Maluta 1980). Trees damaged by canker as well as middle mountain and planted trees had pentads, and a micronucleus (Fig. 4n). In drying trees there were also hexads and the fusion of the nucleus. Divisions in tetrads were noticed in drying trees and in the arboretum.

For pollen studies about 1000-3000 pollen grains were analysed for each tree (Fig. 5a-e). In all trees small

pollen grains without pollen sacs and those with one, three or four pollen sacs were observed (Fig. 5f-i). In rare cases there were also accreting pollen sacs (Fig. 5j). The greatest frequency and types of irregularities in pollen were found in drying trees growing in the Upper Kaltat, $2.4 \pm 0.84\%$ (for trees growing in the other places, not more than 1%). In addition to common types of irregularities there were gigantic pollen grains with two, three and four pollen sacs and pollen consisting of 5 or 6 cells (Fig. 5k).

Meiotic irregularities were usually eliminated at the tetrad stage. However in the Upper Kaltat pollen had about 5 times more irregularities than in the valley of the River Laletina. It can be surmised that some of the meiotic irregularities could affect the heterogeneity of pollen grains and influence pollen fertility (Pozhidaeva et al. 1985, Berdnikov et al. 2002). Decreasing pollen fertility was noticed in the Upper Kaltat (Bazhina 2001).

Meiosis morphology is determined by the interaction of genotype, chromosome structure and environment (Sosnikhina et al. 1994, 2003). In the *A. sibirica* under observation meiosis did not differ from the ordinary pattern of meiosis in conifers. Latitudinal differences in *A. sibirica* timing as well as that of other conifers were apparent presumably due to changes in climatic conditions. Latitudinal climatic variation in *P. sylvestris* has been frequently reported (Wright and Bull 1963, Chung 1981, Eriksson 1982, Mikola 1982).

Our research revealed that the frequency and spectrum of meiotic irregularities increased in the middle mountains and especially under arboretum conditions. This phenomenon may be due to genetic features of the studied trees, such as mutations, since meiosis is under the genetic control (Khvostova and Yachevskaya 1975, Golubovskaya 1989, Sosnikhina et al. 1994, 1994, Bogdanov 2003). The presence of chromosome mutations in the Siberian fir, including ring chromosomes and fragments, has been previously reported (Muratova and Matveeva, 1996).

The increase in the meiotic period in trees growing in the arboretum could probably be considered as an adaptation to new conditions. It has been shown that for trees planted in arboreta and botanical gardens the new environmental conditions may provoke irregularities in development and growth resulting in increased interspecies differentiation and decreased productivity (Nekrasov 1971, Mamaev and Andreev 1996). A high sensitivity to new conditions is characteristic of juvenile plants and the beginning of the reproductive period when spore- and gametogenesis are coming on line with new temperature and light conditions (Shkootko 1983, Gavrilov and Butorina, 2005).

In *Abies sibirica* meiosis occurs immediately after winter dormancy. Yet only a sufficiently long period of dormancy can protect the meiosis process from damage from cold (Luomajoki 1977). The dormancy responses of plant populations have necessarily evolved for adaptation to a given climate. When moved to an area with a different climate, a plant may show poor

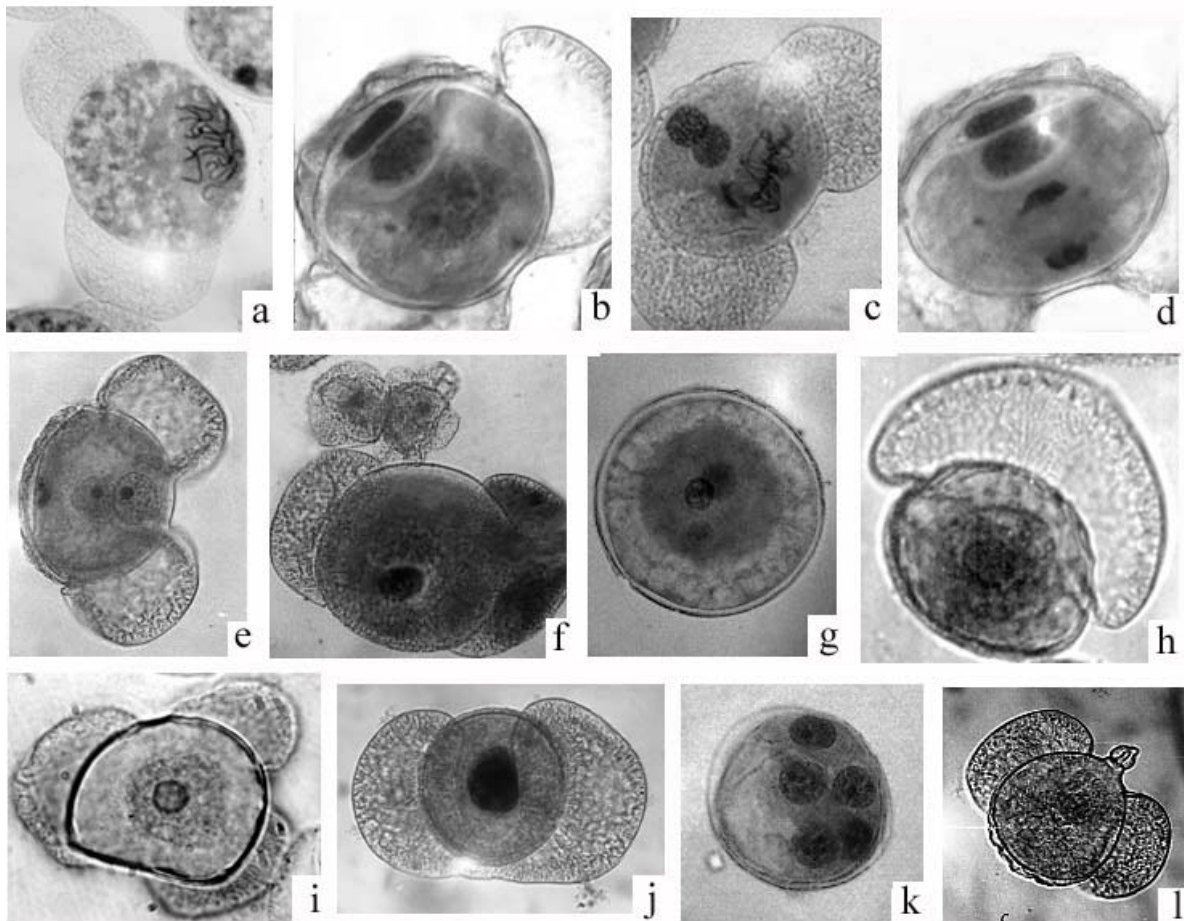


Fig. 5. Pollen development and some irregularities.

a-e – pollen development; f – small pollen grains; g – pollen grains without pollen sacs;
h – one pollen sac; i – three pollen sacs; j – accreting pollen sacs; k – pollen consisting of 5 cells;
l – pollen with abnormal emergence.

adaptation. In this case, the winter period of dormancy ends too early. The probability of unfavorable influences increases due to the early start and lengthened duration of meiosis. Negative temperatures might cause an elevation in the number of abnormal cells and the specific development of certain irregularities in the arboretum.

Meiotic irregularities due to the effect of temperature are especially apparent in areas where trees grow in extreme climatic and soil conditions. Pollen in its initial stages of development is susceptible to temperature damage. Extreme temperature conditions during pollen formation, with meiosis being the most sensitive event, play a significant role in the occurrence of pollen irregularities in several tree species (Christiansen 1960, Chandler and Mavrodineanu 1965, Eriksson 1968, Johnsson 1974, Luomajoki 1977, 1986, Kozubov 1974, Yakovlev 1978, Muratova 1995). Low temperatures have been shown to slow down meiotic progress in several *Larix* species, causing meiotic irregularities, and consequently resulting in irregularities and pollen sterility (Eriksson 1968, Luomajoki 1977). Chira (1964) and Hak and Russell (2004) concluded that temperature extremes, both low and high, have a negative effect on the pattern of meiotic cell division with the consequence of irregularities or pollen sterility.

Our study has shown that the level of meiotic irregularities in the arboretum depends on the incidence of temperature extremes and their timing in relation to the meiotic stage of pollen development. Different levels of disturbances in the same trees during different years may also be connected with differences in absolute and relative variations in temperature and other environmental factors during meiosis.

It is well known that the sensitivity of the individual stages of meiosis to temperature extremes differs (Eriksson 1968, Andersson 1980). The stages from diakinesis to anaphase I and from metaphase II to telophase II have been found to be the most sensitive. In the arboretum some processes of meiosis experienced low temperatures (Fig.1). But in 2002-2003 most PMCs were in interphase – prophase II – at the moment of the extremes. In 2004 the cold spring delayed PMC development with the temperature extremes occurring during the premeiotic phase. It has been established that damage caused in the premeiotic phase becomes visible much later (Luomajoki 1986). We believe that temperature extremes might cause the stoppage of development in some PMCs and elevate the number of irregularities.

Thus, *A. sibirica* meiosis exhibits some peculiarities under different ecological conditions. Prolonged

manifestation of irregularities as well as a general increase in their frequency were observed in the arboretum and to some extent in the Upper Kaltat. Some of meiotic irregularities could result in pollen sterility as was seen in the Upper Kaltat.

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