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Meiotic investigations on embryo sac mother cells of normal and desynaptic Norway spruce

Meiosstudier hos embryosäckmoderceller från normal och desynaptisk gran

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Abstract

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The purpose of the present investigation was to compare the megasporogenesis in a clone of Norway spruce with an ordinary seed set and a clone of a reduced seed set.

The early prophase I stages had the same appearance in both clones. During the last part of diplotene there was a separation of the bivalents (i.e. desynapsis occurred) in the clone with the reduced seed set. During metaphase I—anaphase I 97 per cent of the embryo sac mother cells from the desynaptic clone contained at least one univalent. As a consequence of the occurrence of univalents micronuclei and other irregularities were observed during subsequent stages. Frequently the irregularities caused a degeneration of the cells. Thus, it was proved that the desynapsis was responsible for the poor seed set in the desynaptic clone of Norway spruce.

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1 Introduction

Seed set in Norway spruce is sometimes poor in Sweden. This constitutes a large problem especially for the work in the seed orchards. According to Andersson (1965) the percentage of empty seeds in natural stands of Norway spruce growing in Sweden varies between 37 and 70 per cent. Significant differences in the percentage of empty seeds occur among different localities. Heikinheimo (1937) reported that the average percentage of empty seeds in *Picea abies* growing in Finland amounted to 45.2 per cent whereas Sarvas (1968) reported a percentage of empty seeds of 66.5 per cent. The amount of empty seeds varies considerably from year to year and might be surprisingly great in this species—according to Sarvas (1968) 39—91 per cent.

The occurrence of parthenocarpy in Norway spruce means that the *quantity of the seed crop* is determined by the number of ovules (or by intensity of female flowering). This in turn is at least partly determined by the temperature conditions and the precipitation during the point of time for differentiation of the generative buds (Tirén 1935, Bergman 1960, Brøndbo 1970). In order to avoid frost damage of the female strobili favourable temperature conditions are needed also during later stages of development.

The quality of the seed crop, i.e. the percentage of filled seeds, is dependent upon many factors. The questions related to various factors influencing the quality of the seed crop in Norway spruce have been treated in a series of papers (*e.g.* Sarvas 1955, 1957, 1968 and Andersson 1965). According to Andersson (1965) there are two main reasons for the formation of empty seeds:

(I) no fertilization
(II) embryonic lethality

(I) Based upon the papers published it is possible to distinguish different factors which are involved in the first group:

1. Insufficient pollination
2. Occurrence of incompletely developed ovules
3. Gametic sterility
4. Unfavourable weather conditions between pollination and fertilization
5. Insect damage to the tissues of the ovules before fertilization

(II) Belonging to the second group are:

1. Presence of recessive lethal factors in homozygous condition (mostly caused by self-fertilization)
2. Unfavourable weather conditions subsequent to the fertilization
3. Insect damage

Since this investigation can be referred to point (I) the most interesting factors of group (I) will be treated in some detail.

The main reason for the formation of empty seeds in Norway spruce is according to Sarvas (1955, 1957) *insufficient pollination*. This might be due to

a) sparse male flowering
b) partial or complete destruction of male strobili by frost
c) unfavourable weather or wind conditions during pollen dispersal (*e.g.* the constancy in wind direction means that the pollen does not reach the ovules on the leeward side of the female strobili—Sarvas 1962)
d) frost damage of the pollination drop system causing an inhibition of the fertilization in spite of sufficient pollen supply (Sarvas 1968)
e) The occurrence of metandry in Norway

spruce can in some cases cause the female strobili to be too old for pollination and fertilization at the time for pollen dispersal (Andersson 1965)

Another possibility for the insufficient pollination has been discussed in larch by Eriksson *et al.* (1969). The pollen dispersal takes place to its greatest extent during a limited time, whereas the receptivity in the ovules within a female strobilus is of long duration compared to the time for pollen dispersal and to the receptivity of the individual ovules. This increases the probability that at least some of the ovules are receptive during the time for pollen dissemination, but it also means that the possibility of obtaining a high percentage of filled seeds following open pollination will decrease. Whether or not similar conditions are prevailing in Norway spruce are not known.

Investigations carried out by Sarvas (1955) suggest that all ovules in the central part of a female strobilus in Norway spruce were abundantly pollinated—on an average 19 pollen grains per micropyle. In spite of the abundant pollination the percentage of empty seeds amounted to 24 per cent. This phenomenon was partly explained by the data from a study of the female flowers of spruce. Between the sterile scales growing in the base and in the apex of the strobilus and the fertile scales growing in the central part of the strobilus there is a zone with scales which carry *incompletely developed ovules*. The majority of these ovules only form seed wings whereas some of those which are growing close to the scales with normal ovules can form empty seeds. This explains about one third of the empty seed formation in Norway spruce (Sarvas 1955).

Sterility of the gametes might be:

a) genetically conditioned
b) caused by unfavourable temperature conditions during the gametophyte formation.

A summary of the works which have been carried out within this area was recently presented by Andersson *et al.* (1969).

Meiotic irregularities which might cause both male and female sterility and in this way considerably influence the seed set thus constitute a great potential hazard for formation of empty seeds in conifers. The occurrence of irregularities during the meiosis in the embryo sac mother cells (EMC) has probably more serious consequences for the seed set than meiotic irregularities in pollen mother cells (PMC). (The number of pollen grains is much larger than the number of ovules, which means that sterile pollen grains can, to a certain extent, be substituted for fertile ones.) Therefore, it is of great interest to get information about meiosis not only in the PMC but also in the EMC. In spite of this the information concerning meiosis in the EMC in conifers is very limited. No investigation of the meiosis in the EMC in Norway spruce has been carried out as far as I know.

The purpose of the present investigation was to study the meiosis in the EMC in:

a) one clone of Norway spruce with normal seed set
b) one clone of Norway spruce with a poor seed set

a) The problems which will be treated are: the normal procedure of the meiosis—the point of time for the start of the meiosis, the appearance and the duration of various meiotic stages as well as the tetrad formation.

b) It was of particular interest to study the meiotic development in EMC from one Norway spruce clone with a poor seed set (Figure 1). According to Andersson (1947 a) only two per cent of the seeds from the mother tree of this clone were of normal size and as much as 98 per cent of the remaining small seeds were empty. The cytological investigation of the PMC from this particular Norway spruce (Andersson 1947 a) showed that the pairing of the chromosomes was disturbed and as a consequence of this the entire meiotic division was irregular and delayed. The morphologically determined pollen fertility

amounted to 2.6 per cent (Andersson 1947 b). In the reports by Andersson (1947 a and b) this Norway spruce was classified as asyndetic. As Norway spruce is a cross-fertilizing species the asyndesis in the PMC can not explain the poor seed set in this particular tree. Therefore Andersson (1947 a) assumed that asyndesis might also occur to the same extent in the EMC.

2 Megasporogenesis in conifers in literature

In spite of its importance for seed set the meiosis in the EMC in conifers has been studied to a lesser extent than meiosis in the PMC. The investigations on the female strobili have mostly been concentrated on the development after fertilization. The questions concerning megasporogenesis have sometimes been treated as the introduction to embryological studies. Frequently the authors just reported number of the EMC and the megaspores. The meiosis has not been studied at all or it has been studied only partially. The material investigated was often too limited to draw any conclusions.

2.1 The point of time for differentiation of the embryo sac mother cells

One of the questions that have been discussed is the point of time for differentiation of the EMC. In most cases it takes place in the spring but a considerable variation occurs. The connection with latitude is discernible. In some conifers the differentiation of the EMC takes place in the autumn (*e.g. Juniperus communis,* Norén 1907; *Larix europaea,* Saxton 1930; *Larix sibirica,* Juel 1900; *Taxodium,* Coker 1903; *Taxus baccata,* Strasburger 1904). Sometimes contrary statements for one species can occur in literature—*e.g.* Ottley (1909) has in contrast to Norén (1907) never observed the differentiation of the EMC in *Juniperus communis* until the spring. Dupler (1917) reported in *Taxus canadensis* not only the differentiation of EMC but also the occurrence of female prothallium with free nuclei before the winter.

2.2 The number of the embryo sac mother cells in the ovule

Another question of interest is the number of EMC differentiated in the nucellus (Table 1). In most cases one EMC is reported but even higher counts have been obtained (*Callitris verrucosa,* Saxton 1910 a; *Cryptomeria japonica,* Lawson 1904 b; one case in *Juniperus communis,* Nichols 1910; *Libocedrus decurrens,* Lawson 1907; *Sequoia sempervirens,* Shaw 1896 and Lawson 1904 a; *Taxus baccata,* Strasburger 1904; *Taxus canadensis,* Dupler 1917; *Thuja orientalis,* Coker 1904 and Lawson 1907; *Widdringtonia cupressoides,* Saxton 1909 and 1910 b).

In some species with several differentiated EMC only one of them functions as a mother cell (*Taxus baccata,* Strasburger 1904; *Taxus canadensis,* Dupler 1917; *Widdringtonia cupressoides,* Saxton 1909 and 1910 b) in other species two or more EMC may undergo a reduction division (*Cryptomeria japonica,* Lawson 1904 b; one case in *Juniperus communis,* Nichols 1910; *Libocedrus decurrens,* Lawson 1907; *Sequoia sempervirens,* Shaw 1896 and Lawson 1904 a; rare cases with meiotic development in two EMC in *Taxus baccata* and *Taxus canadensis,* Strasburger 1904 respectively Dupler 1917; *Thuja orientalis,* Lawson 1907).

2.3 The number of megaspores formed

The EMC undergoes a reduction division which usually results in four megaspores but other figures have also been reported (Table 1). Lawson (1910) suggested that no cell wall is laid down in *Sciadopitys* after the first meiotic division. In both daughter nuclei the second division takes place followed by the cell wall formation. In such a way a row of three cells will be formed with two nuclei in the most central of them.

Ottley (1909) and Saxton (1910 b) could not with certainty determine whether there

were three or four megaspores in *Juniperus communis* and *Widdringtonia cupressoides* respectively. According to Norén (1907) and Nichols (1910) usually only three megaspores were formed in *Juniperus communis* since only the basal dyad cell underwent the second division. However, both authors reported a few cases with four megaspores.

Similar results have been obtained by Saxton (1930) for *Larix europaea*. The apical dyad cell may but must not undergo the second meiotic division which causes the number of megaspores to vary between three and four. According to Singh and Chatterjee (1963) only three megaspores have been formed in *Cryptomeria japonica*.

The lack of the second division in the apical daughter cell is often associated with unequal size of daughter cells. If the spindle during the first division is localized to the apical part of the EMC the apical daughter cell will be smaller than the basal one. The second division takes place only in the latter and the meiosis results in three cells (*Cryptomeria japonica,* Kurihara 1936; *Cunninghamia sinensis,* Miyake 1910; *Pinus strobus* and *P. rigida,* Ferguson 1904; *Taxodium,* Coker 1903 and 1904.

In other species even the smaller daughter cell has been reported to undergo the second division which results in four megaspores (*Larix sibirica,* Juel 1900; *Taxus baccata,* Coker 1904; *Torreya californica* Robertson 1904). In such cases the second division may be nonsynchronous *i.e.* it starts first in the larger basal daughter cell (*Larix sibirica,* Juel 1900; *Torreya californica,* Robertson 1904).

The only case of nonsynchronous second division with reverse situation I found in literature has been observed by Dupler (1917) in *Taxus canadensis.* The division of the apical daughter cell was completed before that of the basal one. However the second meiotic division was in most cases synchronous in this species. The same situation has also been reported for *Taxus baccata* (Coker 1904), *Pinus austriaca* (Ferguson 1904) and *Sciadopitys* (Lawson 1910).

2.4 **The arrangement of the megaspores**

The appearance of the tetrads is settled by the orientation of the spindles during the first and the second meiotic divisions. Mostly the spindles of both divisions agree more or less with the longitudinal axis of the nucellus which, as a rule, results in a row of four megaspores. Several investigators have observed a considerable variation in the orientation of the spindle in the apical daughter cell *i.e.* besides the above mentioned even oblique or perpendicular orientation of the spindle to the longitudinal axis of the nucellus occurred (*Larix sibirica,* Juel 1900; *Sequoia sempervirens,* Shaw 1896; *Taxus baccata,* Coker 1904 and Strasburger 1904). The deviating orientation of the spindle in the basal dyad cell is rare but not unknown. Nichols (1910) suggested that the two spindles of the second division may lie either side by side or in a row in *Juniperus communis.* Coker (1904) has never found a linear tetrad in *Thuja orientalis.* The arrangement of the four megaspores was more or less tetrahedral. All megaspores were of similar appearance and it was impossible to distinguish which one would become functional.

2.5 **The number of megaspores which becomes functional**

In most cases only one megaspore of a tetrad develops into a female gametophyte (Table 1). In *Taxus baccata* (Strasburger 1904) and *Taxus canadensis* (Dupler 1917) individual cases have been observed in which several megaspores of the same tetrad began to develop into embryo sacs.

Usually, it is the innermost megaspore of a tetrad which continues to develop but other alternatives have been reported (for *Callitris verrucosa* Saxton 1910 a, for *Taxus canadensis* Dupler 1917).

When several megaspores have arisen as a result of the reduction division in several EMC two or more of the megaspores develop into a female gametophyte (*Sequoia sempervirens,* Shaw 1896 and Lawson 1904 a). In other cases only one of the EMC con-

tinues the further development (*Cryptomeria japonica,* Lawson 1904 b; *Libocedrus decurrens,* Lawson 1907).

The instances with several gametophytes have been observed by many investigators in different species (Table 1). The development in the gametophytes, however, was often already so advanced that it was impossible to determine whether they have arisen from one or several EMC.

3 Material and methods

In order to study the normal procedure of the meiosis of the Norway spruce one graft of clone 83 from Åkersberga was selected. For the investigation of the meiosis in the Norway spruce clone with the poor seed set two grafts originating from Andersson's (1947 a, b) asyndetic tree (No 181 in Gröttvål, Gunnarskog) were selected. The grafts are growing at Röskär, Bogesund.

It was assumed that the meiosis in the EMC of Norway spruce starts at about the same time as in the PMC. Therefore, I tried to estimate the most convenient point of time for the commencement of the fixation of the female strobili by the aid of orientative analysis of PMC in which the stage of development could easily and quickly be determined. Based upon the results of this the fixations of female strobili during 1968 were carried out on April 19th, 22nd and 25th. During 1969 the development was started somewhat later. The fixations were carried out on May 7th and May 10th. The number of female strobili fixed at the different occasions of fixation varied according to the number of female strobili available. Usually 2—6 strobili could be fixed simultaneously.

The female strobili were directly placed in the fixing fluids—two different fluids were used:

1. Acetic acid—alcohol, 1:3
2. Formalin—acetic acid—alcohol, FAA (50 ml 70 per cent alcohol, 5 ml 100 per cent acetic acid, 10 ml 40 per cent formalin and 35 ml H_2O)

After about two days the acetic acid—alcohol was substituted by 70 per cent alcohol. The fixed material was kept in a refrigerator at a temperature of + 4° C. The female strobili were dissected and the individual scales were embedded in paraffin. The strobili, which should be investigated with regard to the distribution of meiotic stages within a strobilus, were divided into four equal pieces and each part was investigated separately.

The thickness of the sections amounted to 12 μ (1968) or 10 μ (1969). The material was stained according to the Feulgen method (10 minutes hydrolysis with HCl at 60° C, 25 minutes Schiff reagent and 5 minutes fast green).

Since the analysis had to be carried out on sectioned material it was sometimes necessary to analyse 2—5 sections of each EMC to get information on the meiotic procedure in an individual EMC. This means that in certain cases it was completely impossible (*cf.* the columns above *x* in Figure 21) to determine the meiotic stage. All evaluation of the number and the size had to be carried out with great care. The occurrence of such disturbances as for instance fragmentation, was impossible to establish. It would have been possible to cut thicker sections in order to approach the ideal situation with the entire specimen within an individual section. Owing to the difficulties in obtaining microphotographs from thick sections this was not a satisfactory solution to the problem.

In order to be able to illustrate the conditions in the given EMC in the best way it was possible to obtain microphotographs in some of the following ways:

I. microphotographing of the EMC at different sections which leads to a series of microphotographs (Figures 11 A, D)
II. microphotographing of the EMC in the same section at different focusing levels which leads to:
1. Several negatives

a) all negatives can be copied; a result of which will be a series of microphotographs (Figures 11 A, B)

b) at the photographing of EMC containing few objects which are not possible to focus simultaneously (certain meiotic stages with two groups of chromosomes *e.g.* anaphase I, telophase I, interkinesis, prophase II) it might be recommended to make one copy from two different negatives (Figure 5 C)

2. One negative—in EMC in which there are many objects (*e.g.* chromosomes, micronuclei) which can not be focused simultaneously it is in certain cases possible to use a long exposure time and continuously change focusing level during the exposure (Figure 11 C).

The explanation of the denotations used in the text: *E.g.*: prophase II/metaphase II means prophase II in the apical daughter cell, metaphase II in the basal daughter cell, whereas prophase II—metaphase II means a transition from prophase II to metaphase II.

4 Results and discussion

4.1 The point of time for the meiosis and duration of this phase of development

The point of time for the onset of meiosis in the EMC varies from year to year depending upon the temperature conditions. In the Stockholm area the meiosis starts during the second part of April or the first part of May, that means simultaneously to, or somewhat later than, the meiosis in the PMC.

The results obtained concerning the duration of the meiosis agree with the results published by Andersson (1947 a). According to these results the entire meiosis lasts for 2—3 days in the PMC of Norway spruce.

The first division (especially pachytene and diplotene) seems to be of long duration in the EMC. Diakinesis and anaphase I are of short duration. This is also true for the interkinesis which agrees with the data of Andersson (1947 a) concerning the duration of the interkinesis in PMC of Norway spruce. The second meiotic division seems to be passed relatively rapidly.

4.2 The meiosis in the embryo sac mother cells from the normal Norway spruce

The EMC which is somewhat elongated at the onset of the meiosis is situated relatively close to the apex of the nucellus and is oriented in such a way that the longitudinal axis agrees more or less with the longitudinal axis of the nucellus (Figure 2). The same orientation of the EMC has been reported for other conifers as well. The only reported exception I found has been observed in *Thuja orientalis* in which the longitudinal axis of the EMC was oriented perpendicularly to the longitudinal axis of the nucellus (Coker 1904).

The procedure of meiosis in EMC of the normal Norway spruce is illustrated in Figures 3—8. During the two earliest prophase stages, leptotene and zygotene the chromosomes are very thin, elongated threads wound into a ball. This means that a detailed study of the individual chromosomes is not possible to carry out. Owing to the spiralization during zygotene (Figure 3 A) it is somewhat easier to recognize individual chromosomes than during leptotene. During zygotene it is possible to observe how the chromosomes start the pairing. The continuing spiralization causes the chromosomes during the pachytene stage (Figure 3 B) to be shorter and thicker than in the preceeding stages. However, it is impossible to discern the individual bivalents during this stage. It is possible to recognize one or more nucleoli during pachytene as well as during the two preceeding stages.

The contraction of the chromosomes continues during diplotene (Figures 3 C—E). The chromosomes are relatively thick and short which means that it is easy to recognize individual bivalents (Figure 3 E). During diplotene the bivalents are distributed throughout the nucleus which still is surrounded by the nuclear membrane. It is possible to recognize large as well as small nucleoli.

Diakinesis (Figure 3 F) was rarely observed which suggests that this stage is of short duration. The chromosomes are heavily contracted in this phase. It is still possible to identify nucleoli and the nuclear membrane. However, both are dissolved at the end of the diakinesis.

During metaphase I (Figures 4 A—C) the bivalents are oriented in the equatorial plane. Both ring and rod bivalents were observed. Any quantitative determination of the different types of bivalents was not possible to carry out. The spindle appears clearly during metaphase I. In PMC of

Picea abies and *Larix* species individual bivalents had been observed outside the equatorial plane (Andersson 1947 a; Chandler and Mavrodineau 1965 and Ekberg *et. al.* 1968). This type of metaphase I has not been observed in the EMC of Norway spruce.

In some reports (*cf.* page 9) it was noted that the nuclear spindle was formed during metaphase I in the apical part of the EMC which means that the daughter cells formed were of a different size. My data suggest that the nuclear spindle is situated approximately in the central part of the EMC. However, these data have to be taken with great care as I have used sectioned material.

During anaphase I (Figures 4 D, E) the homologous chromosomes separate and move from the equatorial plane to the poles. It is possible to observe that the chromatids have separated from each other (Figure 4 E). In the course of telophase I (Figure 4 F) the chromosomes despiralize. This process is completed during the interkinesis (Figures 5 A, B), when two daughter nuclei have been formed. During this stage it is possible to recognize nucleoli. In the course of the late interkinesis a wall is usually formed between the two daughter nuclei in such a way that two daughter cells arise (Figure 5 B).

The second meiotic division can proceed in two different ways. It can start and continue synchronously in both of the daughter nuclei or it may start at different times in the two daughter nuclei, which means that the second division is nonsynchronous.

If the division is synchronous the chromosomes in both of the daughter nuclei start a contraction simultaneously during prophase II (Figure 5 C). The nuclear membrane and the nucleoli dissolve. Furthermore two metaphase plates are formed (Figure 5 D) and two spindles oriented after each other with the common longitudinal axis can be seen. This axis frequently agrees with the longitudinal axis of the nucellus.

The chromosomes during anaphase II are very tiny and elongated (Figures 5 E, F). They move towards the opposite poles of the daughter cells where they form four telophase nuclei (Figures 6 A, B) which mostly are oriented in a row.

A nonsynchronous division was observed in 65 per cent of the investigated second meiotic divisions ($n = 77$). In Figure 19 the percentage of the nonsynchronous divisions in four different strobili from the normal Norway spruce is shown. Nonsynchronous second division occurred as far as I could see only in those EMC where a wall formation had taken place subsequently to the first meiotic division. The second division always started in the basal daughter cell, in which later stages were observed than in the apical one. Usually prophase II in the apical daughter cell was combined with metaphase II (Figure 7 A), anaphase II (Figure 7 B), telophase II (Figure 7 C) or two megaspores in the basal daughter cell. Other combinations appeared also, *e.g.* metaphase II/telophase II, metaphase II/two megaspores (Figures 7 D, E), telophase II/two megaspores (Figure 7 F). The position of the spindle in the EMC during metaphase I and the size of both dyad cells suggest that there is a relatively equal separation of the cytoplasm to the two daughter cells after the first meiotic division. In spite of this a conspicuous difference could be recognized concerning the size of the daughter cells with a nonsynchronous second meiotic division. Frequently the basal daughter cell was 2—3 times larger than the apical one. In the EMC with a synchronous division no conspicuous differences were observed regarding the size of the two daughter cells.

The transition from telophase II to tetrads is completed through despiralization of the chromosomes, the formation of nuclear membrane and the wall formation between the telophase nuclei (Figures 6 C—F). The appearance of the tetrad reveals the orientation of the spindles during the second meiotic division. The spindle in the basal daughter nucleus always agreed with a longitudinal axis of the nucellus. The spindle in the apical daughter cell was oriented in

about 60 per cent of the tetrads investigated (n = 68) in the same way. In the rest of the tetrads it was oriented obliquely (38 per cent) or perpendicularly (2 per cent) to the longitudinal axis of the nucellus.

The normal procedure of the meiosis resulted in the formation of four megaspores. The basal one started to develop to the embryo sac—it grew rapidly, its cytoplasm became vacuolized and the chromosomes were shortly afterwards prepared for the first mitosis subsequent to the meiosis (Figure 8 A). The spindle in this mitosis was frequently oriented obliquely to the longitudinal axis of the nucellus (Figure 8 B). The rest of the megaspores degenerated and were compressed by the growing embryo sac (Figures 8 C—F).

In order to investigate the procedure of the meiosis in different parts of a strobilus some of the strobili investigated were divided into four equal parts. In Figure 20 a distribution of the meiotic stages in such a strobilus is demonstrated. There was a variation but the difference in development among the four different parts of the strobilus was not great. The meiosis was most advanced in the central part of the strobilus.

4.3 **The meiosis in the embryo sac mother cells from the Norway spruce with the poor seed set**

The procedure of the meiosis in EMC from the Norway spruce with the poor seed set is illustrated in Figures 9—18.

The early stages of prophase I do not differ from those I could find in the EMC of the spruce with a normal seed set. From the microphotographs (Figure 9 A) it is evident that during the zygotene the chromosome pairing proceeded in the same manner as in the normal spruce. Also the pachytene stage is of normal appearance (Figure 9 B). During diplotene (Figures 9 C—F) it is possible to recognize bivalents—the homologous chromosomes are paired. During the late phase of the diplotene stage and the transition to diakinesis (Figure 10 A) there is a deviation from what can be seen in the normal Norway spruce. The entire nucleus still surrounded by the nuclear membrane seems to be completely filled by chromosomes. The homologous chromosomes had partly started a separation and both bivalents and univalents could be recognized. Thus, in this Norway spruce the chromosomes had once formed bivalents. Some of the bivalents have, during the latest part of diplotene or during the diakinesis, split up and the formation of univalents has taken place. Usually this type of irregularity is called desynapsis. Therefore, this term will from now on be used in this paper.

The appearance of metaphase I was dependent upon the relationship between bivalents and univalents. If the bivalents dominated it also seemed to stimulate the univalents to move to the equatorial plane and the metaphase plate was of relatively normal appearance (Figure 10 B). If there were a higher number of univalents they were mostly distributed throughout the cytoplasm, probably without previous movement to the equatorial plane (Figures 10 C—F, 11 A—D). As many as 22 univalents had been observed in the EMC which were possible to evaluate quantitatively. (The number of chromosomes is 2n = 24 in Norway spruce.) Such a high frequency of univalents severely complicated the delimitation between metaphase I and anaphase I.

During early anaphase I it was sometimes possible to recognize individual rod bivalents (Figure 11 E). Telophase I was in some cases of relatively normal appearance (Figures 11 F, 12 A) but sometimes it was possible to observe lagging chromosomes (Figure 12 B). Occasionally the interkinesis was of normal appearance (Figure 12 C). However, polynuclear interkinesis occurred frequently (Figure 12 D, E), *i.e.* interkinesis in which 2—3 small nuclei were formed instead of one daughter nucleus or more rarely instead of both of them. The nuclei were situated close to each other or were more or less spread. They have probably arisen from groups of univalents which had been surrounded by one nuclear membrane at telophase I.

Also in the desynaptic Norway spruce both synchronous and nonsynchronous second division occurred. The percentage of nonsynchronous second meiotic divisions was higher than in the normal Norway spruce—amounting to 80 per cent (n = 49). During the second division there occurred more or less severe irregularities of the meiosis in 29 per cent of the EMC. In the rest of the EMC a relatively normal appearance of the second division was observed (Figures 12 F, 13 A, B, 15 C, 16 C, D). This does of course not warrant that they actually were normal. Irregularities of a less severe type might be hidden and appear at later stages (*cf.* Eriksson 1968).

As pointed out above polynuclear interkinesis occurred frequently. The second meiotic division in such irregular interkinesis was, as far as could be seen, always nonsynchronous (Figures 14 A—F, 15 A—B, D—F). Thus when 2—3 nuclei of varying size appeared during prophase II of the apical daughter cell, the basal cell had already reached later stages of the second division or had even formed megaspores. The wall formation between the above mentioned prophase II nuclei occurred in some cases (Figures 14 C—F, 15 A—B). It was not possible to determine whether or not the second division took place in those nuclei. There were some indications that those nuclei degenerate (Figures 15 A—B).

If the disturbances in the basal daughter cell were not too serious the second division could proceed and two megaspores were formed. However it is hardly probable that the basal one had got a complete chromosomal complement and could form a functional embryo sac.

In Figures 16 A—B a case is illustrated in which the basal daughter product of the interkinesis contained several nuclei. These nuclei have degenerated. In this particular case it is doubtful whether or not the apical daughter cell is too severely irregular to form megaspores. Furthermore it might be questioned if one of the megaspores formed by a division of the apical cell would be able to form a functional embryo sac. The probability of obtaining a functional embryo sac must be regarded as less when the basal daughter nucleus of an interkinesis is irregular than when the apical one is irregular. The nuclei discussed (Figures 16 A—B) might also be degenerated megaspores. However, this does not change the final result of such a division.

Figures 15 E—F illustrate one case, in which several nuclei have substituted both the apical and the basal daughter nuclei.

A binucleate prophase II of a particular appearance was in one case observed in the apical daughter cell (Figure 15 D). One of the nuclei showed signs reminiscent of stickiness. In the basal daughter cell the second meiotic division had been completed and two megaspores had been formed.

A considerable number of EMC had reached the tetrad stage. The appearance of the tetrads was frequently relatively normal (Figures 17 B—E). However, the occurrence of micronuclei suggested that the chromosomal complement of the megaspores was irregular. Not even a regular appearance of the megaspores would constitute a complete guarantee for a normal complement of the chromosomes. The tetrad stage is a typical example of a stage during which irregularities might be hidden.

In one case the meiosis in an EMC of the desynaptic clone resulted in the formation of a pentade—*i.e.* the formation of five megaspores of almost the same size (Figure 17 F).

The disturbances were in some cases so serious that the EMC could not complete the meiotic division (Figure 18). The chromosomes were strongly contracted and their structure was invisible. In such cases it was impossible to determine which stage was affected. Therefore, these EMC could not be used in the quantitative determination of irregularities of individual stages.

4.4 **Frequency of irregularities**

The distribution of EMC to different stages of development in the two clones is summarized in Figure 21. The hatched part of the columns is equal to the percentage of irregularities of the individual stages. Since

the analysis comprised sectioned material, it was for technical reasons impossible to determine the stage of development in some EMC. (Such EMC are assembled in the white and black parts of the columns above x in Figure 21.) Some of the EMC in the desynaptic graft could not be classified with respect to stage of development owing to their serious irregularities (the hatched part of the column above x).

Although the material was collected simultaneously there was a conspicuous difference regarding the stage of development in the two clones. In the desynaptic grafts the first meiotic division dominated whereas the tetrad formation had taken place to a large extent in the normal graft. This difference could probably be attributed to the disturbances appearing during the end of the diplotene stage which caused a delay of the rest of the meiotic division in the desynaptic clone. This agrees well with the observations made by Andersson (1947 a) regarding the meiotic development of the PMC of the mother tree of the desynaptic clone. He reported that the first division was twice as long as in the normal spruce trees.

Figure 21 also illustrates that the frequency of irregularities during diplotene—telophase I is greater than during the second meiotic division. It is probable that the EMC which contain a few univalents during the first part of the meiotic division (and thus are classified as irregular) during the second division have a more or less regular appearance in spite of an abnormal chromosomal complement. This partly explains the decrease of the irregularities from 97 per cent during metaphase I—anaphase I to 29 per cent during the second meiotic division. The decrease might also be caused by inclusion of severely irregular EMC which were impossible to classify in the hatched part of the column above x in Figure 21.

The percentage of EMC containing one or more univalents during metaphase I—anaphase I in individual strobili is demonstrated in Figure 22. As may be seen from this diagram the difference among strobili is slight—varying between 94 and 100 per cent.

The percentage of irregularities during all stages is demonstrated separately for the different strobili in Figure 23. The variation was in this case larger than the one shown during metaphase I—anaphase I. The difference in the distribution of developmental stages among the different strobili explains this larger variation. Thus in strobilus No 3, the stages pachytene and interkinesis occurred to a greater extent than in the other strobili. These stages showed a low amount of irregularities. As a consequence of this the percentage of irregular EMC was lower in strobilus No 3 than in the other strobili.

The high frequency of irregularities during the meiosis of the EMC of the desynaptic clone explains the poor seed set of this clone.

5 Summary

From the summary presented of studies on megasporogenesis in conifers it may be seen that the development in the embryo sac mother cells (EMC) has up to now been studied to a relatively small extent. The authors often just determined the number of EMC which were differentiated and the number of megaspores which arose, without studying meiosis in any detail.

Disturbances of the meiotic development can lead to both male and female gamete sterility and belong therefore to the potential reasons for the formation of empty seeds in Norway spruce. Although the frequency of empty seeds in Norway spruce sometimes is very high, no studies on meiosis in EMC in this species have been published to my knowledge.

The purpose of the present investigation was to study meiosis in EMC in one clone of Norway spruce with ordinary seed set as compared to meiosis in one spruce clone with poor seed set, which had earlier turned out to be asyndetic in pollen mother cells (Andersson 1947 a, b).

In Norway spruce growing in the Stockholm region meiosis in EMC takes place in the second part of April or in early May depending on the actual temperature conditions. In both clones investigated the meiosis started at about the same time.

The early prophase I stages had the same appearance in the two clones. It was possible to observe that the homologous chromosomes were paired (Figures 3 A, 9 A) and that they remained paired during almost all diplotene (Figures 3 C, 9 D). During late diplotene and transition to diakinesis the homologous chromosomes started to separate in the EMC in the clone with poor seed set. As the chromosomes once had been paired we can declare this clone to be desynaptic.

Metaphase I had a quite different appearance in the two clones. In the normal clone the bivalents had oriented in the equatorial plane (Figure 4 A). In the desynaptic clone univalents appeared in high frequency (Figure 11 C). During metaphase I—anaphase I 97 per cent of the investigated EMC from the desynaptic clone contained one or more univalents. The univalents scattered in all the cytoplasm caused disturbances during the last part of the first division which led to the rise of polynuclear interkinesis (Figures 12 D, E). In the desynaptic clone the irregularities were observed during the second division too. The entire meiosis was delayed probably due to irregularities which arose in high frequency during the first division.

In the normal spruce clone no irregularities have been observed during the first division and interkinesis (Figure 5 A). In 65 per cent of the EMC the second division proceeded nonsynchronously in the two daughter cells. Both simultaneous and succedaneous type of wall formation occurred. No irregularities have been observed in the second division.

The percentage of tetrads is very low in the desynaptic clone, probably owing to the above mentioned delay of meiosis. The EMC did not reach the tetrad stage during the investigation. Another possible explanation is that the gravely damaged EMC will never reach the tetrad stage.

The average percentage of irregular EMC during all meiotic stages amounted to 67 per cent in the desynaptic clone.

Meiosis in the two investigated clones could lead to completely different products. In the normal spruce clone the ordinary meiotic development resulted in normal tetrads (Figure 6 E). The basal megaspore developed to an embryo sac, the other

three degenerated (Figure 8 C). In the desynaptic spruce clone grave disturbances during meiosis could lead to the degeneration of the EMC (Figure 18). EMC with damages of a more moderate type might complete meiotic division but could probably not give rise to a functional embryo sac (Figure 17 F).

The present investigation proved that abnormalities which occurred during the course of meiosis were responsible for the reduction of the seed set in the desynaptic clone of Norway spruce.

Investigations of the meiotic development may help to reveal clones suffering from disturbances during the meiotic division which is of great practical importance for the work in seed orchards. With the aid of routine controls of the meiotic development it is possible to eliminate clones giving seed of poor quality.

6 Acknowledgements

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7 Sammanfattning

Av den presenterade översikten över arbeten som berör megasporogenesen hos barrträd framgår att utvecklingen i EMC hittills har studerats i relativt liten omfattning. Författarna har ofta endast angivit antal differentierade EMC och antal bildade makrosporer utan att närmare studera meiosförloppet.

Meiosstörningar, som kan leda till gametsterilitet på både han- och honsidan är en av de potentiella orsakerna till tomfröbildning hos gran *(Picea abies)*. Trots att tomfröfrekvensen ofta är hög har det såvitt mig är bekant inte tidigare gjorts några studier av meiosförloppet i EMC hos denna art.

Syftet med föreliggande undersökning var att studera meiosförloppet i EMC hos en granklon med normal frösättning och att jämföra det med meiosförloppet hos en granklon med dålig frösättning som tidigare visat sig vara asyndetisk på hansidan (Andersson 1947 a, b).

Hos gran växande i stockholmstrakten inträffar meiosen i EMC under andra hälften av april eller i början av maj beroende på de rådande temperaturförhållandena. Hos de båda undersökta granklonerna startade meiosen ungefär samtidigt.

Tidiga profas I-stadier hade samma utseende hos båda granklonerna. Man kunde i båda fallen observera att homologa kromosomer parade sig (Figurerna 3 A, 9 A) och att de förblev parade även under större delen av diplotenstadiet (Figurerna 3 C, 9 D). Under sen diploten och övergång till diakines började dock de homologa kromosomerna att separera i EMC hos granklonen med den dåliga frösättningen. Eftersom kromosomerna en gång varit parade kan vi benämna denna granklon som desynaptisk.

Metafas I såg helt olika ut hos de båda klonerna. Hos den normala granklonen hade bivalenterna orienterat sig i ekvatorialplanet (Figur 4 A). Hos den desynaptiska granklonen uppträdde univalenter i hög frekvens (Figur 11 C). Under metafas I—anafas I innehöll 97 % av de undersökta EMC hos den desynaptiska granklonen en eller flera univalenter. Univalenterna, som var utspridda i plasman, orsakade störningar under senare delen av första delningen och ledde till uppkomst av flerkärniga interkineser (Figurerna 12 D, E). Hos den desynaptiska granklonen observerades oregelbundenheter även under andra delningen. Hela meiosen blev fördröjd, antagligen på grund av störningarna som uppkom i hög frekvens under första delningen.

Hos den normala granklonen påträffades inga oregelbundenheter under första delningen och interkinesen (Figur 5 A). I 65 % av EMC förlöpte andra delningen inte samtidigt i de båda dottercellerna. Både simultan och succedan typ av väggbildning har påträffats. Inga oregelbundenheter har observerats.

Det låga procenttalet av tetrader hos den desynaptiska granklonen var betingat dels av den ovannämnda fördröjningen av meiosförloppet (när EMC undersöktes hade de i sin utveckling ännu inte hunnit till tetradstadiet) dels av att allvarligt skadade EMC aldrig nådde tetradstadiet.

Procenttalet oregelbundna EMC under hela meiosförloppet uppgick till 67 % hos den desynaptiska granen.

Meiosen hos de två undersökta granklonerna kunde leda till helt olika resultat. Hos den normala granklonen, där hela meiosen förlöpte normalt, bildades normala tetrader (Figur 6 E). Den basala makrosporen utvecklade sig till en embryosäck, de tre övriga makrosporerna degenererade (Figur 8 C). Hos den desynaptiska gran-

klonen kunde meiosstörningarna vara så allvarliga att de ledde till degenerering av EMC (Figur 18). Om skadorna var av något lindrigare typ kunde meiosen fullföljas men dess produkter var oftast så pass oregelbundna (Figur 17 F) att de inte kunde ge upphov till en funktionsduglig embryosäck.

Föreliggande undersökning har visat att orsaken till den dåliga frösättningen hos den desynaptiska granklonen var oregelbundenheterna under meiosen hos EMC.

Möjlighet att genom undersökning av meiosförloppet avslöja grankloner behäftade med meiosstörningar är av stort praktiskt värde för fröplantageverksamheten. Med hjälp av rutinmässiga kontroller av meiosförloppet kan man eliminera kloner som ger frö av dålig kvalitet.

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Appendix

Table 1 Reports concerning megasporogenesis in conifers
Symbols: ( ) — probably, **2** — most frequently two, 1 x — 1 case, i — the innermost of the row of megaspores, o — the outermost of the row of megaspores

Species	Reference	Number of EMC/number of funct. EMC	Number of megaspores per 1 EMC	Number and position of funct. megaspore	Number of prothallia > 1
Araucaria brasiliensis	Burlingame 1914	(1)		(1)	
Callitris verrucosa	Saxton 1910 a	1—3	4	(1) varying position	2
Cryptomeria japonica	Lawson 1904 b	3—4/3—4	4	1 in the middle of all 12—16 megasp.	
Cryptomeria japonica	Kurihara 1936	1	3	1 i	
Cryptomeria japonica	Singh and Chatterjee 1963	1	3	1 i	
Cunninghamia sinensis	Arnoldi 1900				1—5
Cunninghamia sinensis	Miyake 1908	1		(1 i)	
Cunninghamia sinensis	Miyake 1910	1	(3)	1 i	
Juniperus communis	Norén 1907	1	**3**, 1×4	1 i	
Juniperus communis	Nichols 1910	1, 1×3/ /1×3	**3**—4		
Juniperus communis	Ottley 1909	1	3—4	1 i	
Keteleeria evelyniana	Wang 1948	1	4	1 i	
Larix europaea	Saxton 1930	(1)	3—4		
Larix sibirica	Juel 1900	1	4	1 i	
Libocedrus decurrens	Lawson 1907	1—3, **2**/all	4	1 of all megasp.	
Pinus austriaca	Ferguson 1904	1	**4**	1 i	
Pinus rigida		1	**3**	1 i	
Pinus strobus		1	**3**	1 i	
Pinus banksiana	Chamberlain 1898	(1)			
Pinus laricio	Coulter and Chamberlain 1901	1	4	1 i	
Podocarpus	Coker 1902	1			2
Pseudolarix Kaempferi	Miyake and Yasui 1911	1		1 i	
Pseudotsuga Douglasii	Lawson 1909	(1)	(3)	1	
Sciadopitys	Lawson 1910	1	3	1 i	
Sequoia sempervirens	Arnoldi 1900				several
Sequoia sempervirens	Shaw 1896	5—7/all	4		several 1 domin.
Sequoia sempervirens	Lawson 1904 a	5—6/all			2—3 1 domin.
Taxodium	Coker 1903	1	3	1 i	2

(Table 1 cont)

Species	Reference	Number of EMC/number of funct. EMC	Number of megaspores per 1 EMC	Number and position of funct. megaspore	Number of prothallia > 1
Taxodium	Coker 1904	1	3		
Taxus baccata	Strasburger 1904	3—5/**1**—2	4	1 i, 1×2	1×2
Taxus baccata Taxus canadensis	Coker 1904	1	4	1 i	2
Taxus canadensis	Dupler 1917	sever./**1**—2	4	**1**, several **i**, rarely o	up to 5
Thuja orientalis	Coker 1904	2—3	4		
Thuja orientalis	Lawson 1907	2/2	4		
Torreya californica	Robertson 1904	1	4	1 i	2
Widdringtonia cupressoides	Saxton 1909	sever./1			
Widdringtonia cupressoides	Saxton 1910 b	64/1	(3—4)	1	

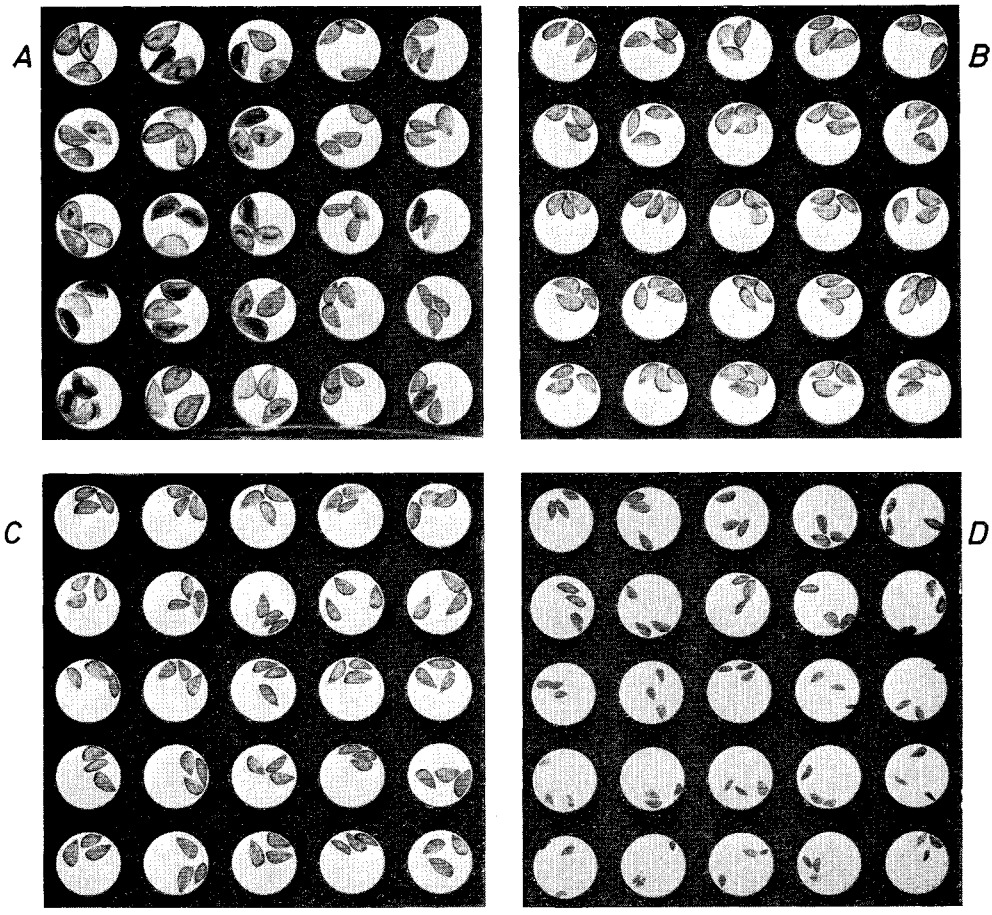

Fig. 1. Radiographs of seed samples from the desynaptic clone of Norway spruce (Sample A contains the largest seeds observed, samples B, C are randomly selected among the seeds of intermediate size, sample D shows the smallest seeds observed). Seed damage caused by insects indicates that some seeds originally were filled.

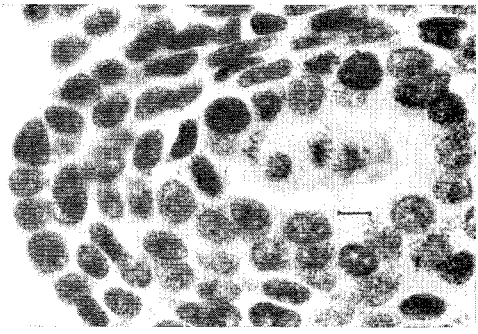

Fig. 2. The position and orientation of the embryo sac mother cell in the nucellus. The fixation was performed on April 19th, 1968. The line indicates a distance of 10 μ.

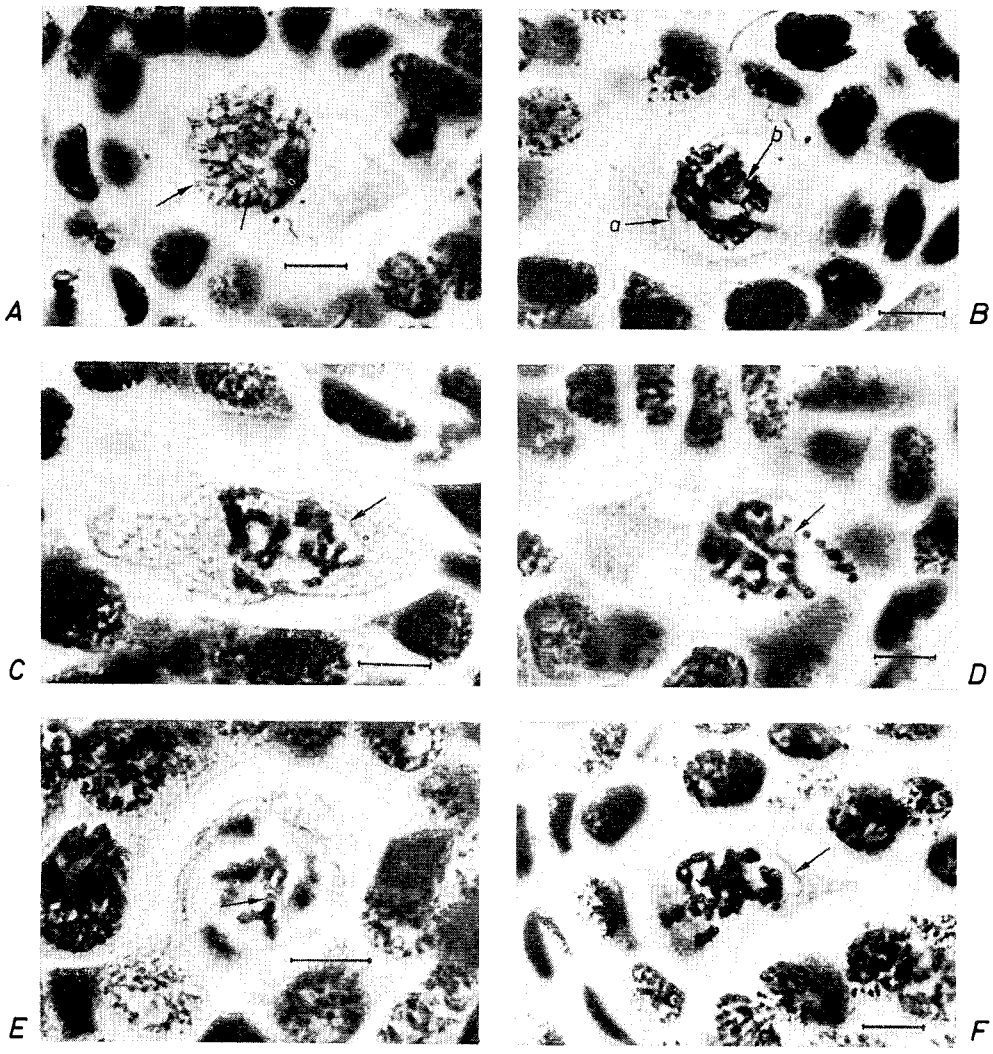

Fig. 3. Meiosis in the EMC of the Norway spruce clone with ordinary seed set. Prophase I. The lines indicate a distance of 10 μ.

A: Zygotene. The chromosomes are visible as slender threads; → marks positions in which the chromosomal pairing has started.
B: Pachytene. The chromosomes are paired; a → marks the nuclear membrane, b → marks a nucleolus.
C: Longitudinal section of an EMC during diplotene; → marks the nuclear membrane.
D: Longitudinal section of an EMC during diplotene; → marks a nucleolus.
E: Transverse section of an EMC during diplotene; → marks a bivalent.
F: The bivalents are strongly contracted during diakinesis; → marks the nuclear membrane.

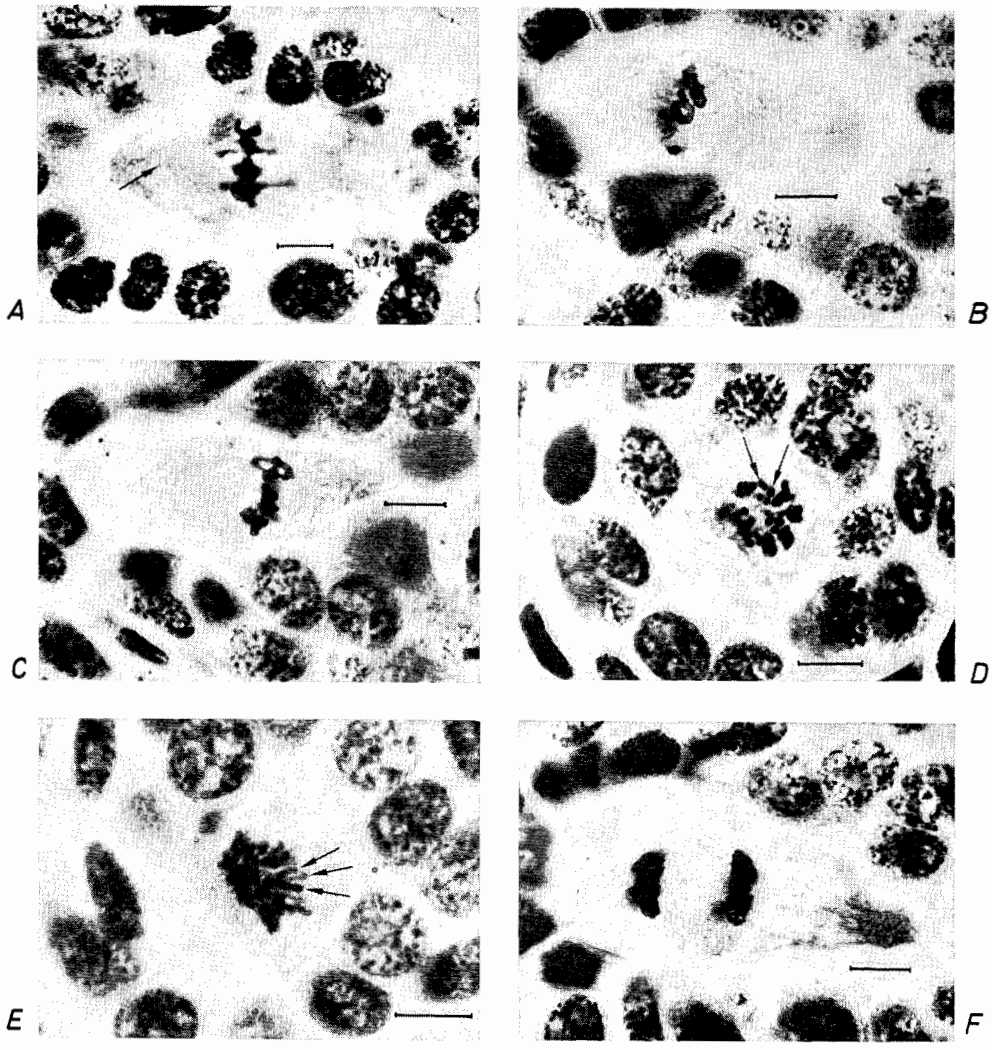

Fig. 4. Meiosis I in the EMC of the Norway spruce clone with ordinary seed set. The lines indicate a distance of 10 μ.

A: Metaphase I. The bivalents are located at the equatorial plane;→ marks the nuclear spindle.
B, C: Metaphase I. Two sections of the same EMC.
D, E: Anaphase I. Two transverse sections of the same EMC; → marks the chromatids.
F: Telophase I.

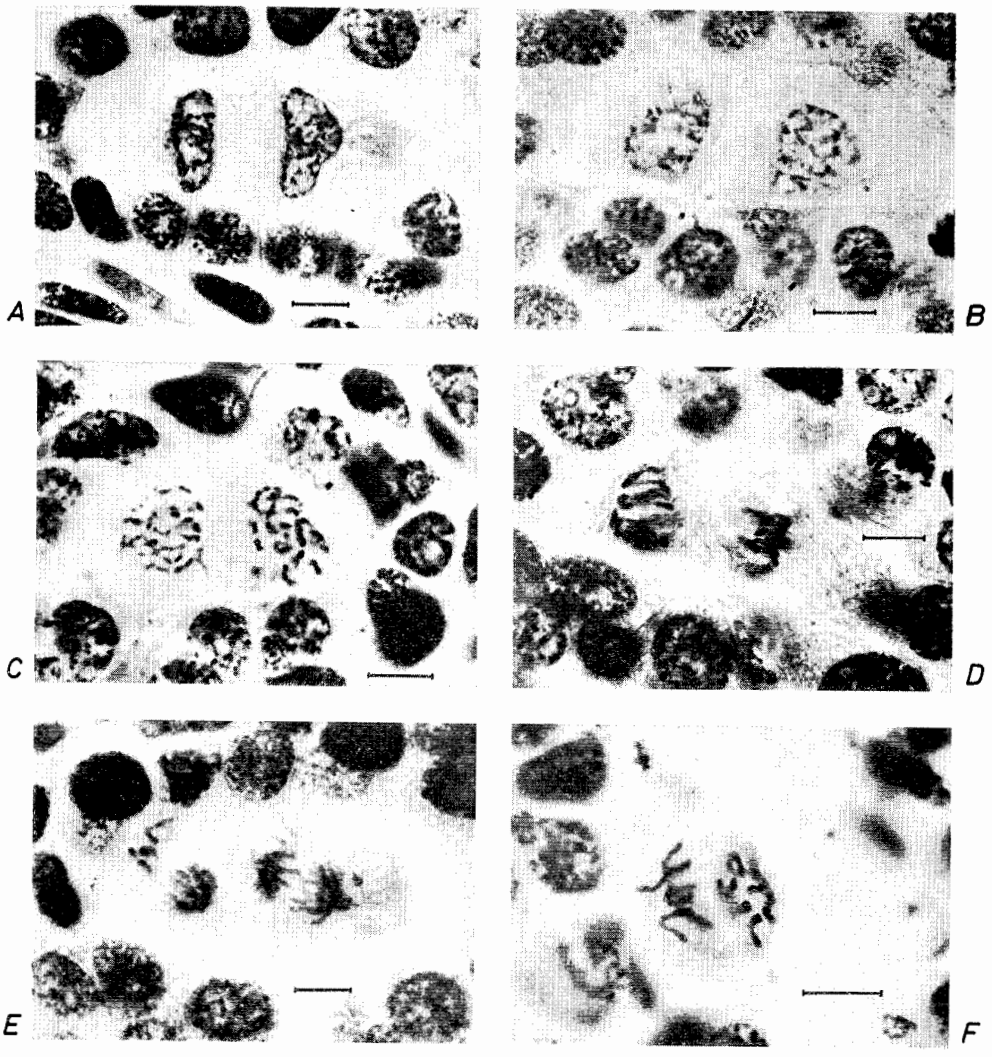

Fig. 5. Meiosis in the EMC of the Norway spruce clone with ordinary seed set. The lines indicate a distance of 10 μ.

A: Interkinesis.
B: Interkinesis with wall formation (dyad).
C: Prophase II.
D: Transition from prophase II to metaphase II.
E: Anaphase II.
F: Anaphase II in one of the daughter cells—the long and slender chromosomes are clearly visible.

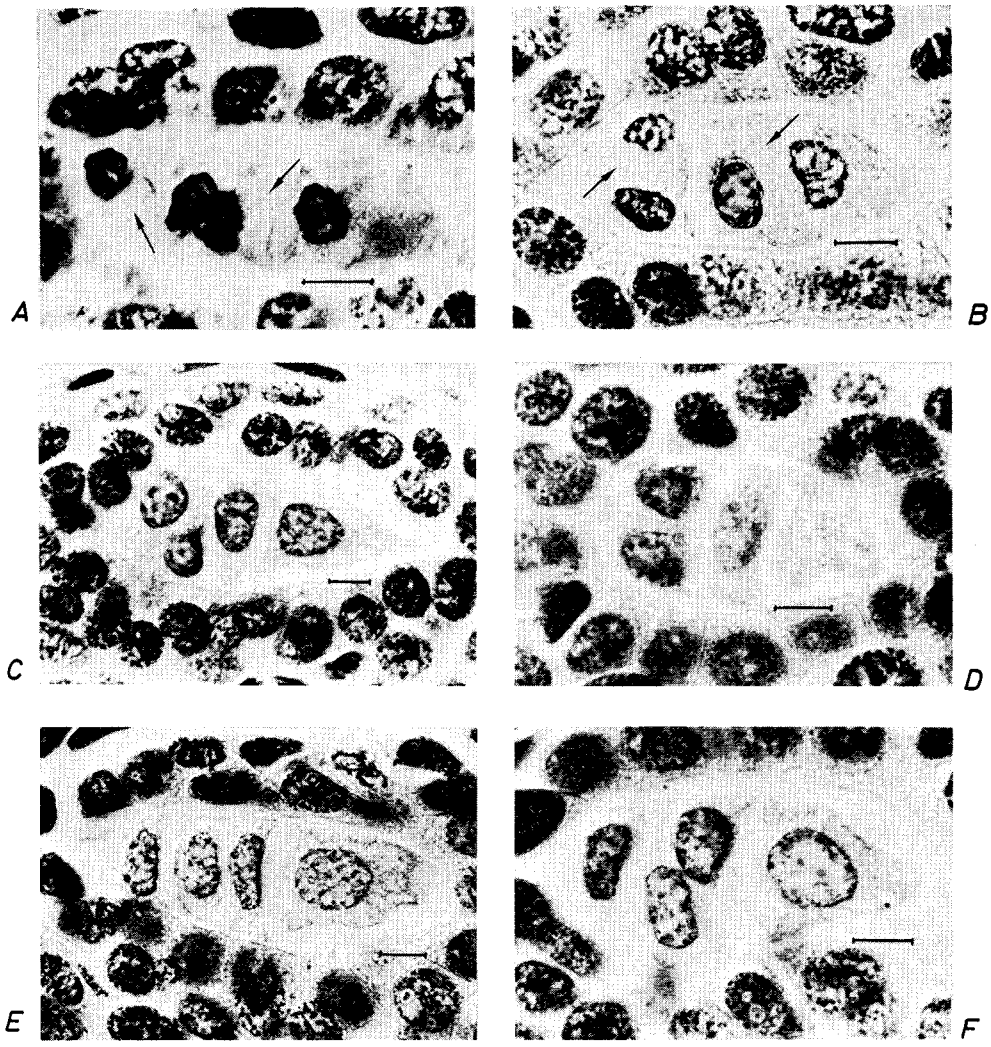

Fig. 6. Meiosis in the EMC of the Norway spruce clone with ordinary seed set. All microphotographs are oriented in such a way that the apex of the nucellus is situated to the left. The lines indicate a distance of 10 μ.

A: Telophase II; → shows where the wall formation has started.
B: Telophase II. The nuclear spindle of the apical daughter cell is oriented perpendicularly to the longitudinal axis of the nucellus; → shows starting wall formation.
C—F: Different orientations of the megaspores during the tetrad stage.

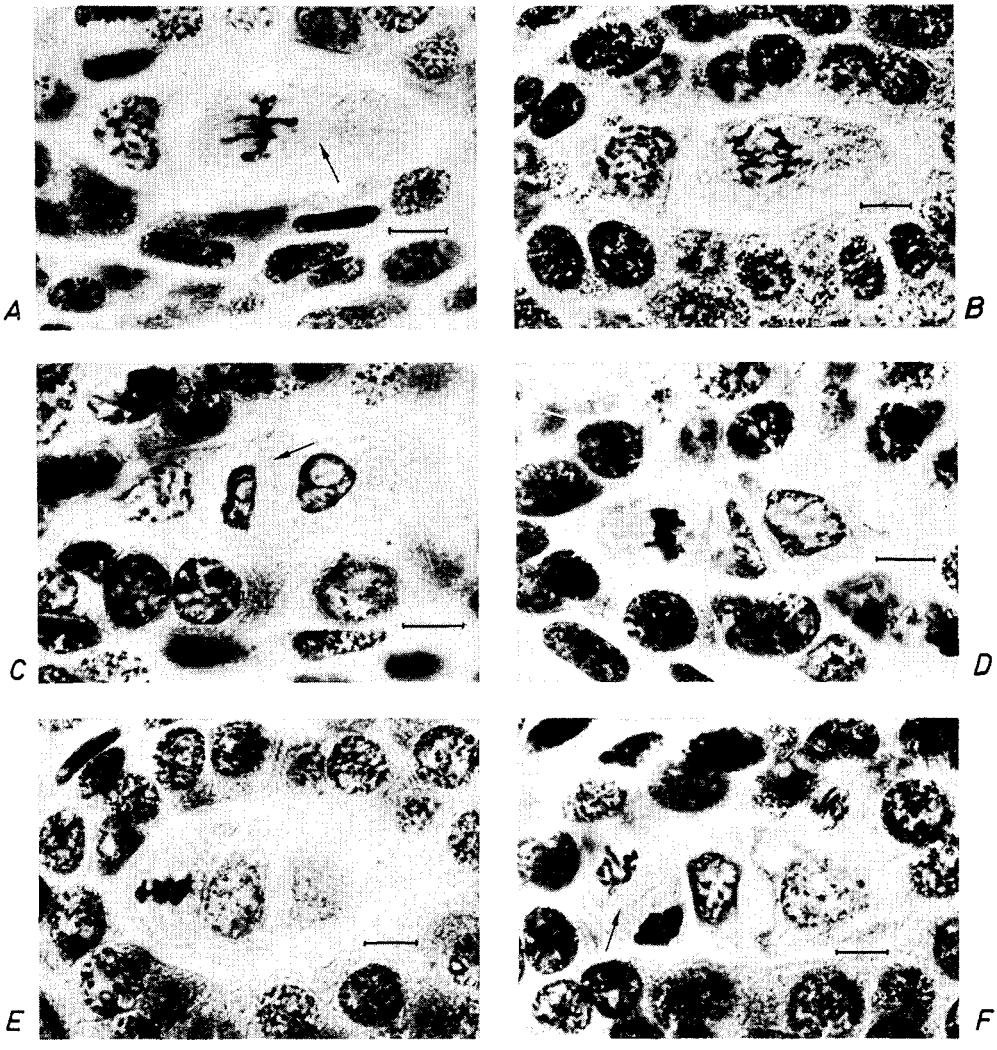

Fig. 7. The meiosis in the EMC of the Norway spruce clone with ordinary seed set. Non-synchronous second divisions. The difference in size between the two daughter cells is conspicuous—the basal one is much larger than the apical one. All microphotographs are oriented in such a way that the apex of the nucellus is situated to the left. The lines indicate a distance of 10 μ.

A: Prophase II/metaphase II; → marks the nuclear spindle in the basal daughter cell.
B: Prophase II/anaphase II.
C: Prophase II/telophase II; → marks the starting wall formation in the basal daughter cell.
D: Metaphase II/2 megaspores.
E: Metaphase II/2 megaspores—a rare case in which the nuclear spindle of the apical daughter cell is oriented perpendicularly to the longitudinal axis of the nucellus.
F: Telophase II/2 megaspores; → marks the wall formation in the apical daughter cell.

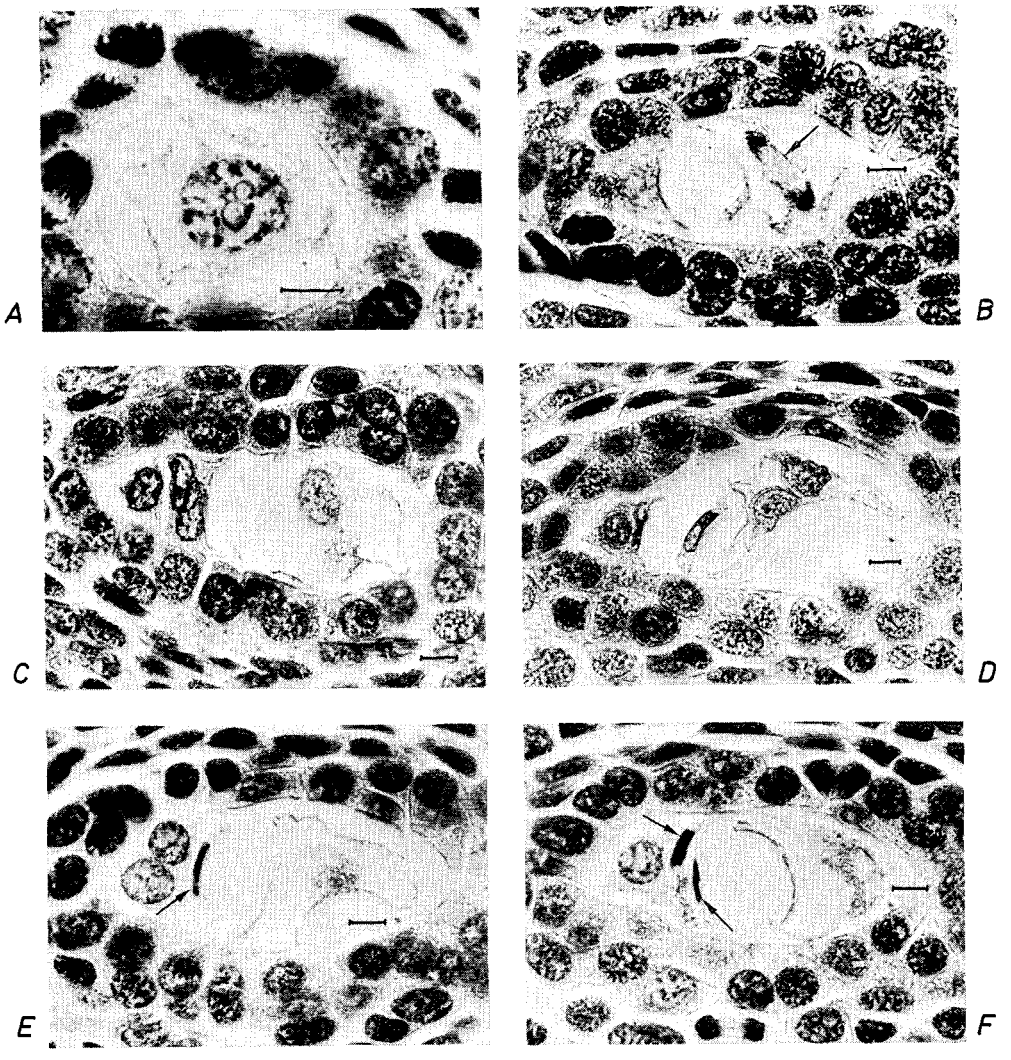

Fig. 8. Postmeiotic development in the Norway spruce clone with the ordinary seed set. All microphotographs with the exception of A are oriented in such a way that the apex of the nucellus is situated to the left. The lines indicate a distance of 10 μ.

A: The basal megaspore has grown considerably and the first mitotic division subsequent to meiosis has started. The sectioning was done more or less perpendicularly to the longitudinal axis of the nucellus.

B: Anaphase of the first mitotic division; → marks the nuclear spindle which is obliquely oriented against the longitudinal axis of the nucellus.

C, D: Binucleate embryo sacs, the three sister megaspores are starting their degeneration.

E, F: Enlarged embryo sacs with the three sister megaspores.

E— → marks the innermost megaspore of the three degenerating ones. This cell has become flattened out probably owing to pressure from the growing embryo sac.

F— → marks the two inner of the three nonfunctional megaspores which have been compressed by the growing embryo sac.

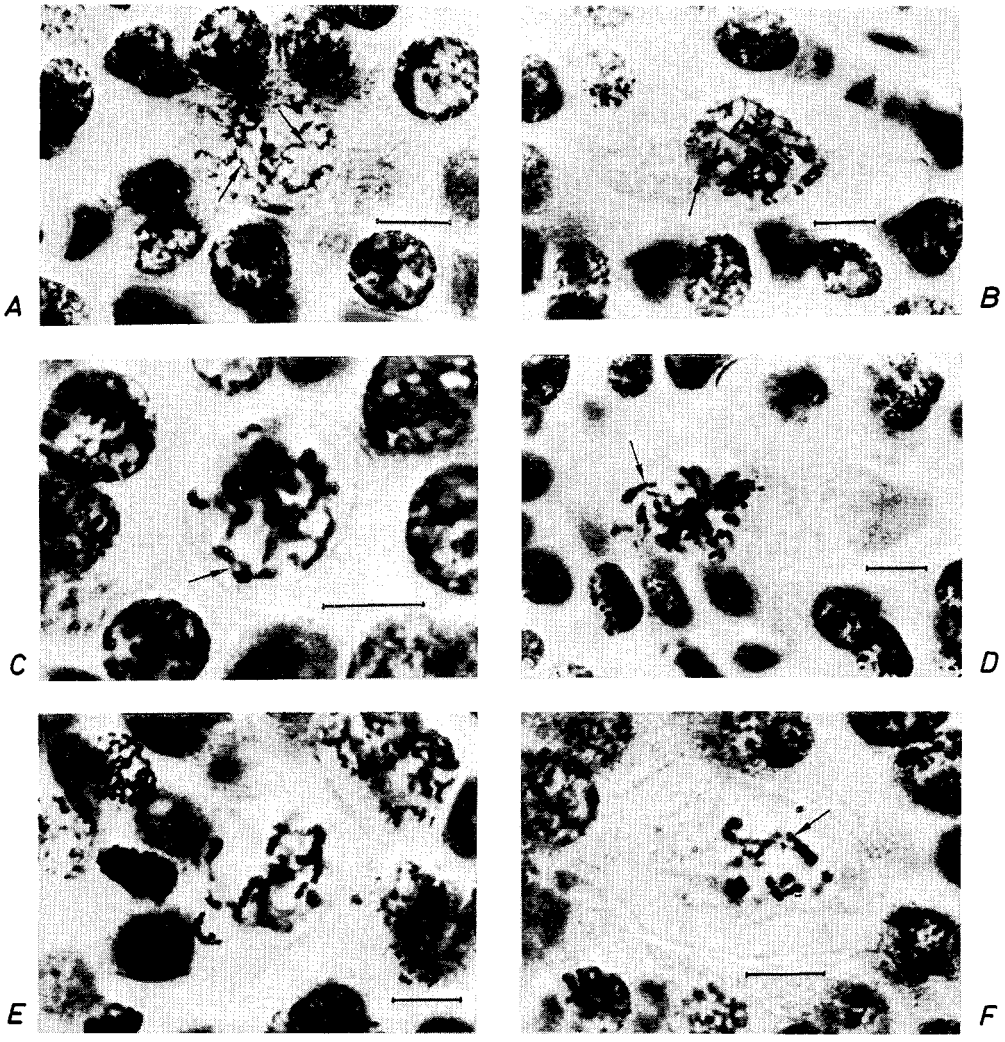

Fig. 9. Meiosis in the EMC of the desynaptic clone of Norway spruce. Prophase I. The lines indicate a distance of 10 μ.

A: Zygotene. The chromosomes are partially paired; → indicates positions with paired chromosomes.
B: Pachytene. The chromosomes are completely paired; →marks a nucleolus.
C—F: Diplotene; → marks clearly visible bivalents.

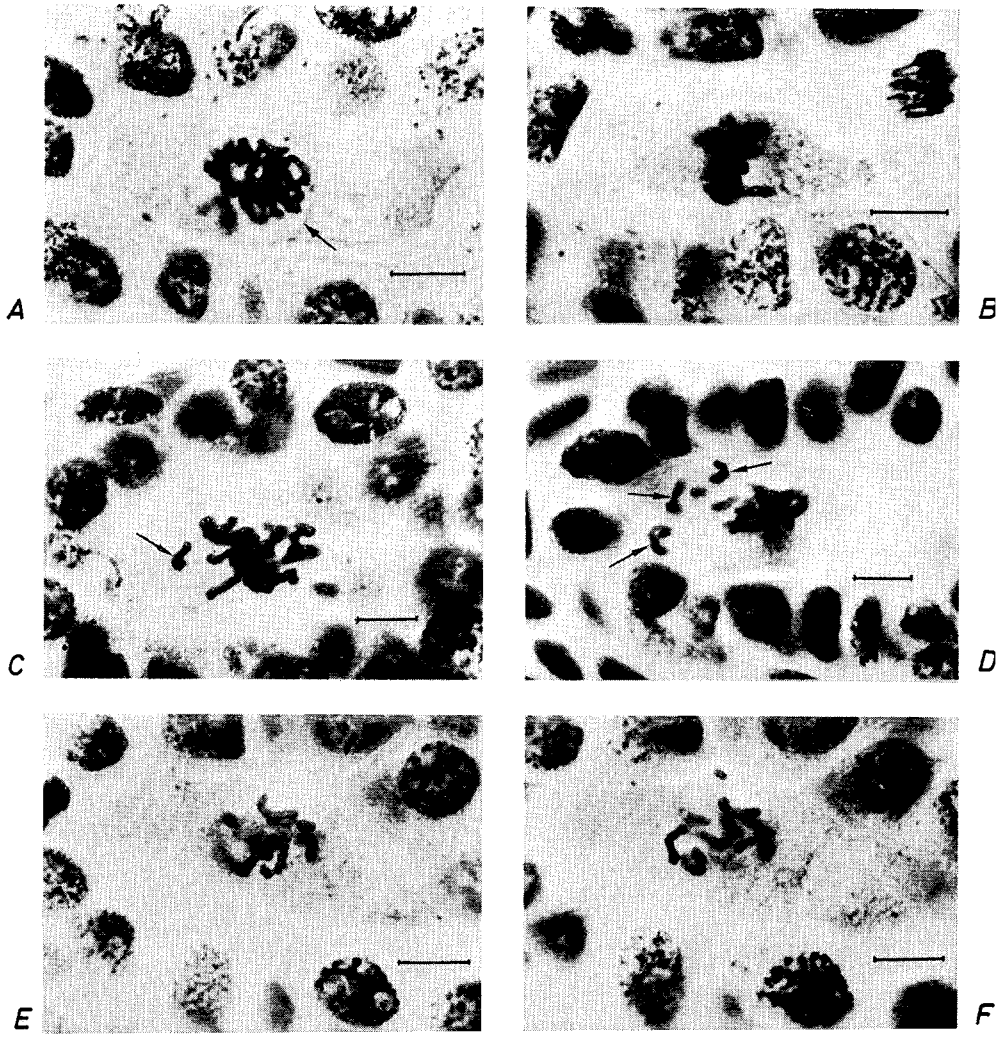

Fig. 10. Meiosis I in the EMC of the desynaptic clone of Norway spruce. The lines indicate a distance of 10 μ.

A: Late diplotene. The homologous chromosomes have started their separation, both univalents and bivalents are present; → marks the nuclear membrane.
B: Metaphase I of ordinary appearance.
C, D: Metaphase I—anaphase I; → marks univalents distributed in the cytoplasm.
E, F: Metaphase I—anaphase I containing univalents; two sections of the same EMC.

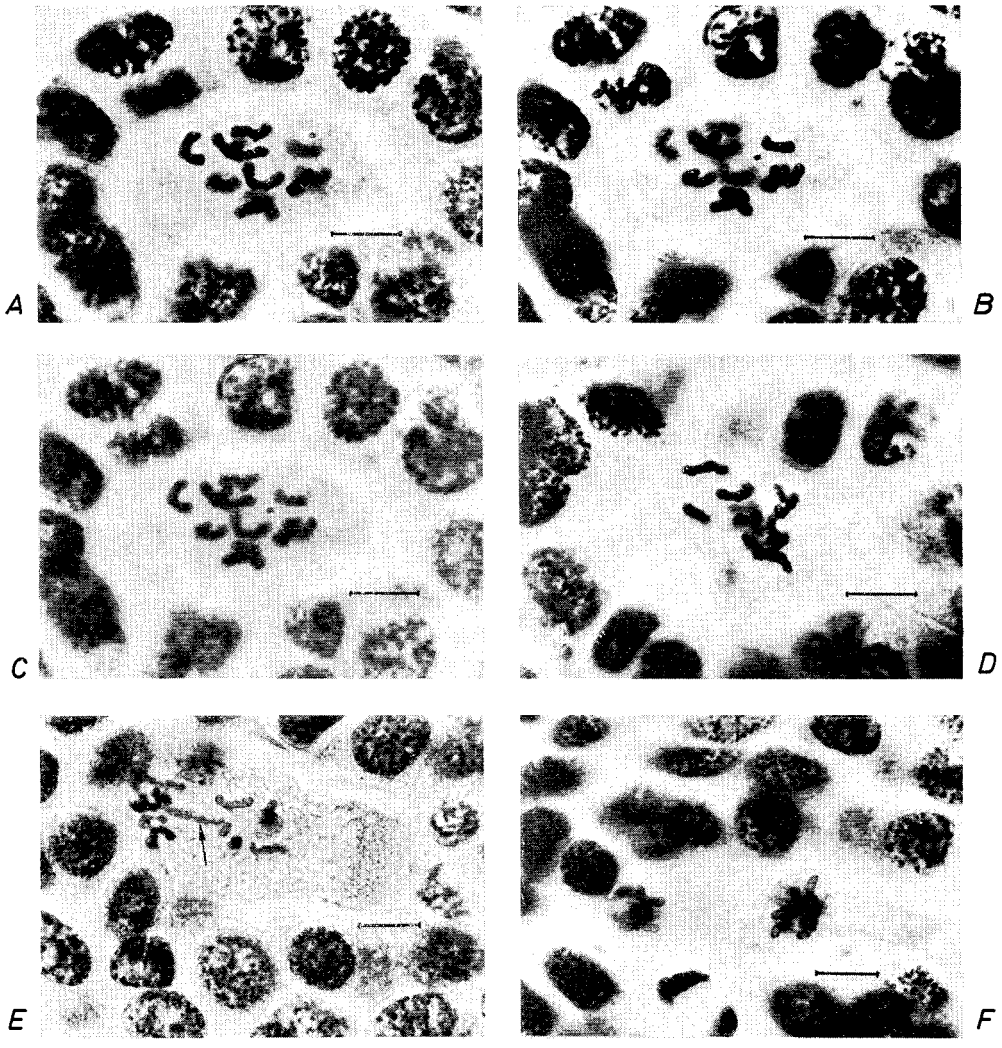

Fig. 11. Meiosis I in the EMC of the desynaptic clone of Norway spruce. The lines indicate a distance of 10 μ.

A—D: Metaphase I—anaphase I containing univalents.
A, B—microphotographs of one section at two different focal levels.
C—the same section as in A, B but this microphotograph was obtained by focusing at different levels during the exposure.
D—another section of the EMC illustrated in A—C. This EMC contained at least 22 univalents.

E: Early anaphase I; → marks a rod bivalent.

F: Telophase I of ordinary appearance.

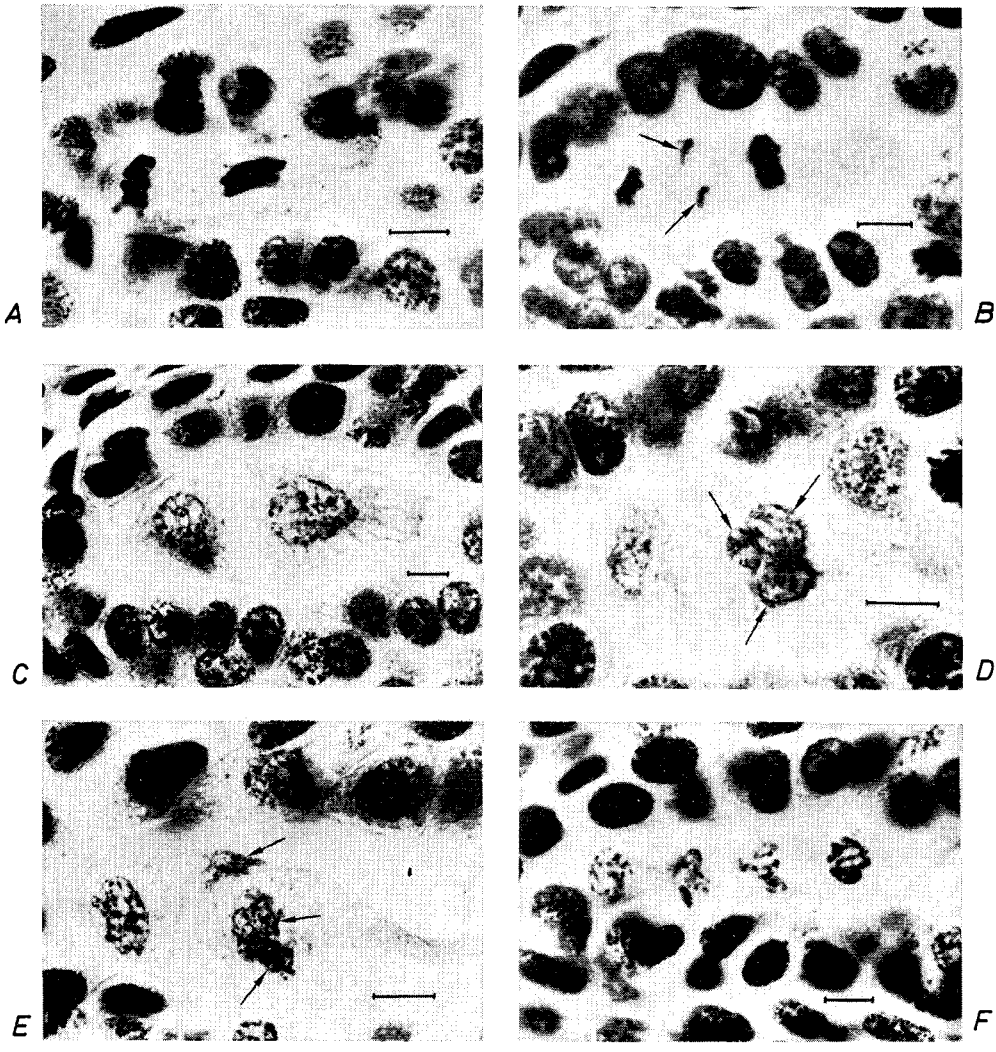

Fig. 12. Meiosis in the EMC of the desynaptic clone of Norway spruce. All microphotographs are oriented in such a way that the apex of the nucellus is situated to the left. The lines indicate a distance of 10 μ.

A: Telophase I of almost normal appearance.
B: Telophase I; → marks two lagging chromosomes.
C: Interkinesis of ordinary appearance.
D, E: Polynuclear interkinesis; → marks the three nuclei which have arisen instead of one single basal nucleus.
F: Telophase II of ordinary appearance.

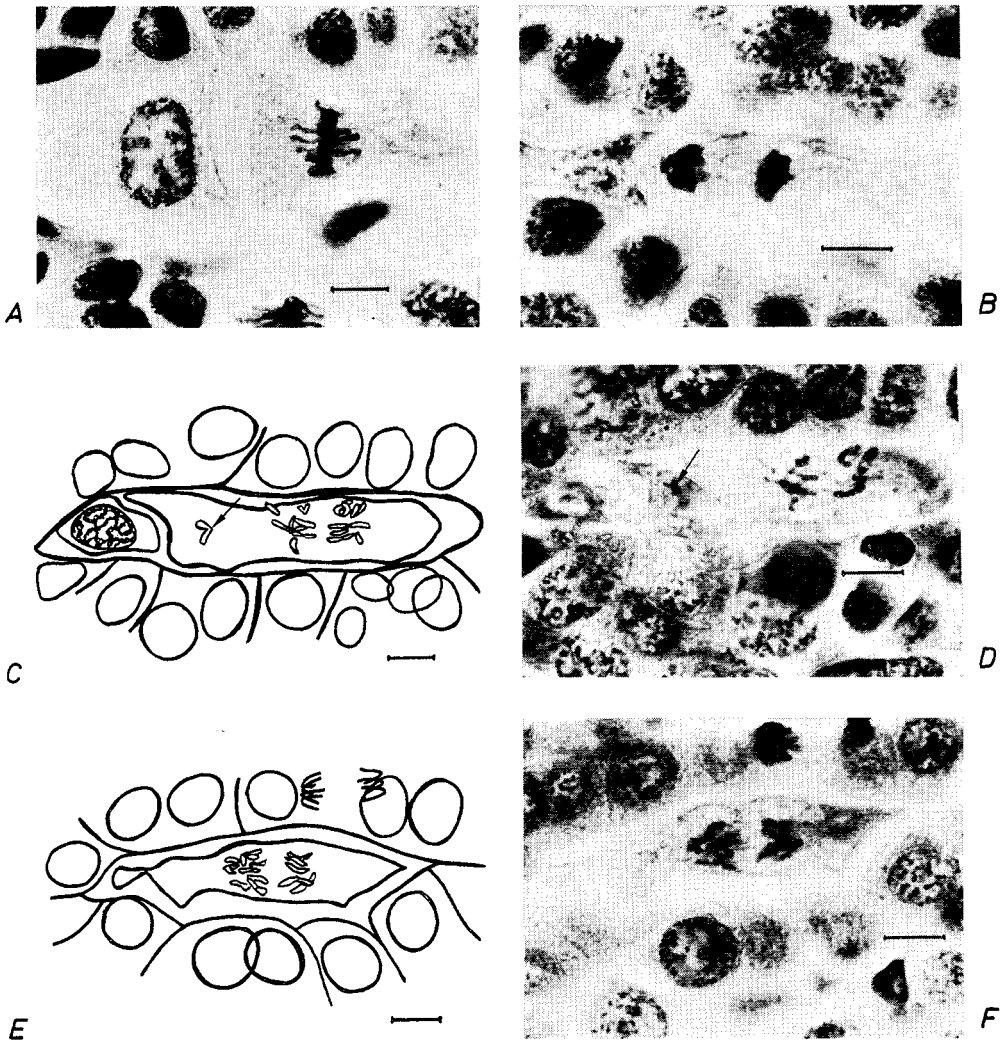

Fig. 13. Meiosis in the EMC of the desynaptic clone of Norway spruce. Nonsynchronous second division. All microphotographs are oriented in such a way that the apex of the nucellus is situated to the left. The lines indicate a distance of 10 μ.

A: Prophase II/metaphase II of almost ordinary appearance.
B: Prophase II/telophase II.
C, D: Drawing and corresponding microphotograph of prophase II/anaphase II; → marks a lagging chromosome.
E, F: Drawing and corresponding microphotograph of anaphase II in the basal daughter cell. E, F constitutes another section of the EMC shown in C, D.

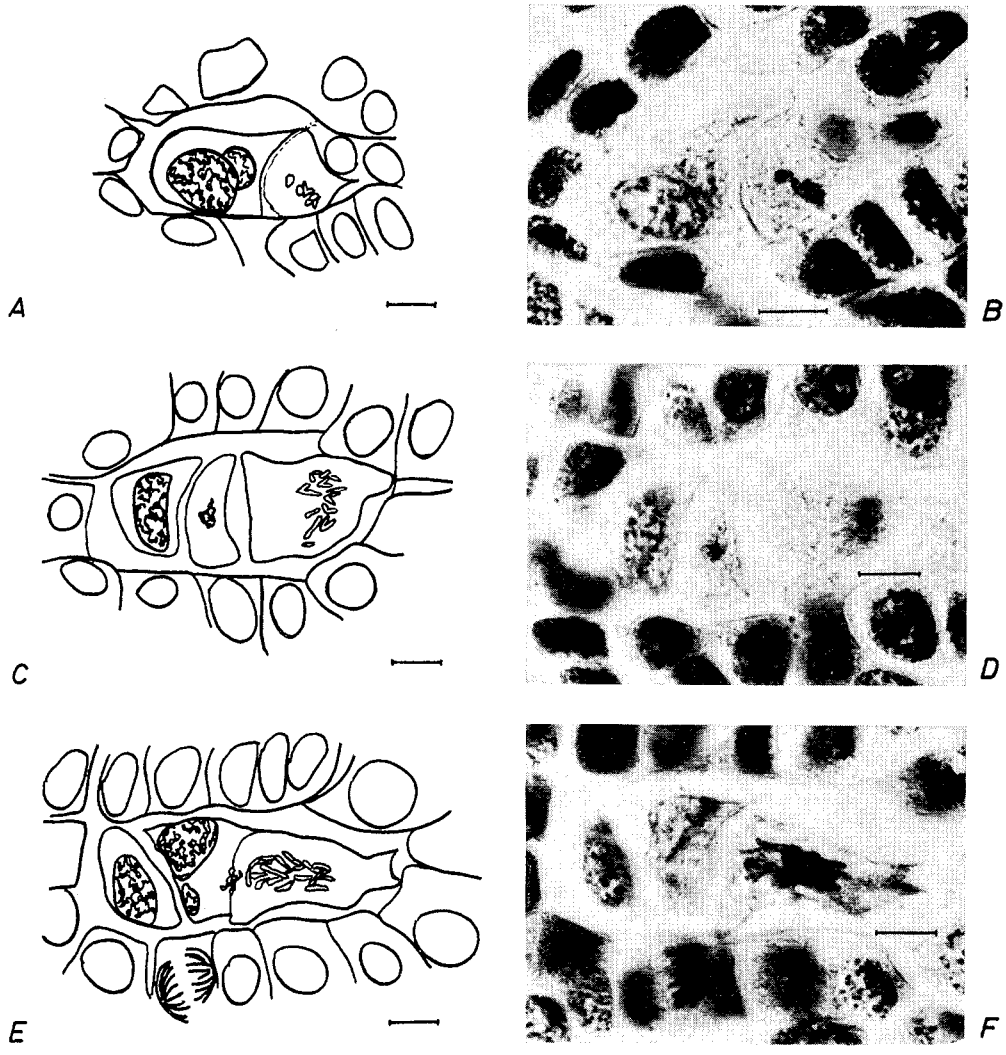

Fig. 14. Meiosis in the EMC of the desynaptic clone of Norway spruce. The further development of polynuclear interkinesis. Nonsynchronous second division. All microphotographs are oriented in such a way that the apex of the nucellus is situated to the left. The lines indicate a distance of 10 μ.

A, B: Drawing and corresponding microphotograph of a binucleate prophase II/metaphase II.

C, D: Drawing and corresponding microphotograph of prophase II (with two nuclei of different size)/anaphase II.

E, F: Drawing and corresponding microphotograph of prophase II (with three nuclei)/anaphase II.

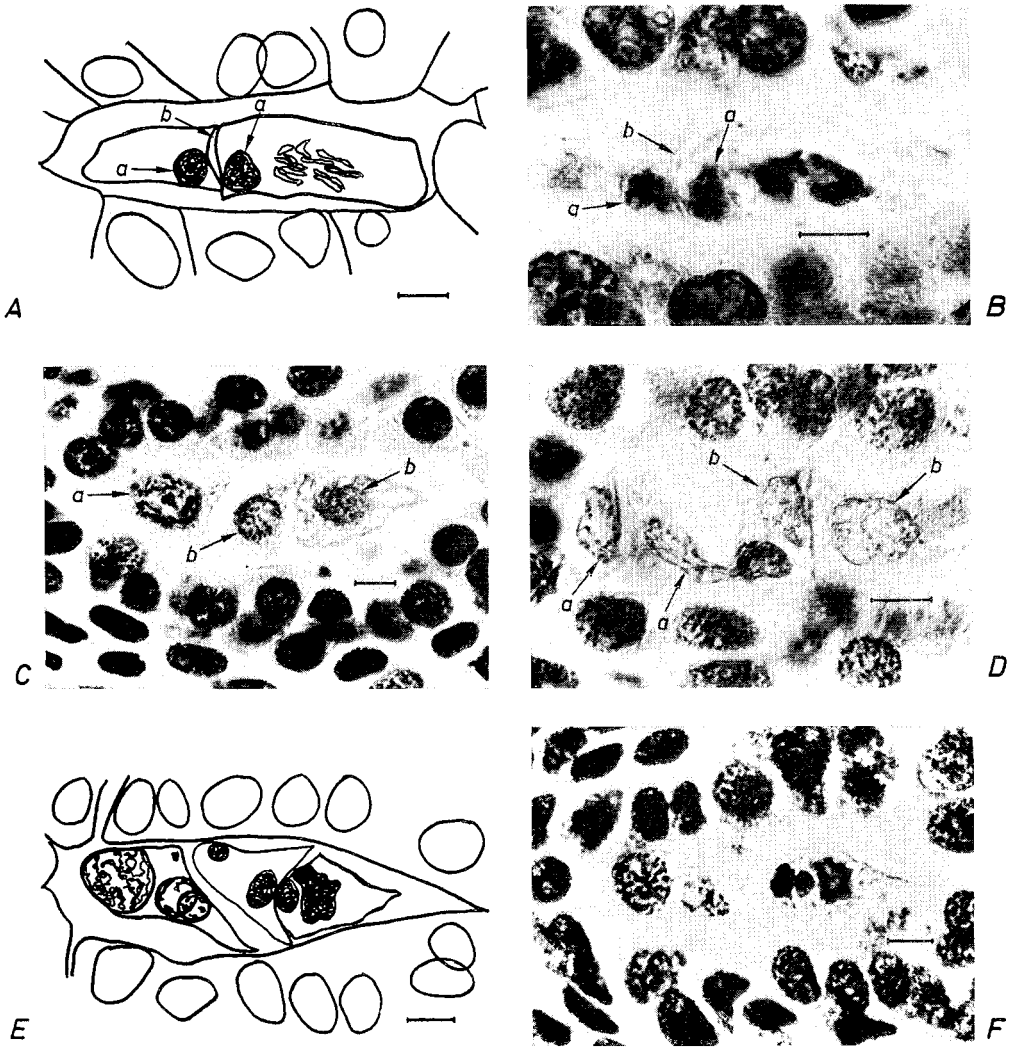

Fig. 15. Meiosis in the EMC of the desynaptic clone of Norway spruce. All microphotographs are oriented in such a way that the apex of the nucellus is situated to the left. The lines indicate a distance of 10 μ.

A, B: Drawing and corresponding microphotograph of prophase II/anaphase II; a → marks degenerating prophase II nuclei; b → marks the cell wall between them.

C, D: Prophase II/2 megaspores; a → marks the prophase II nuclei; b → marks the megaspores.
C—prophase II of normal appearance.
D—one of the two nuclei of prophase II seems to be sticky.

E, F: Drawing and corresponding microphotograph of prophase II (three nuclei + one micronucleus)/4 degenerating nuclei.

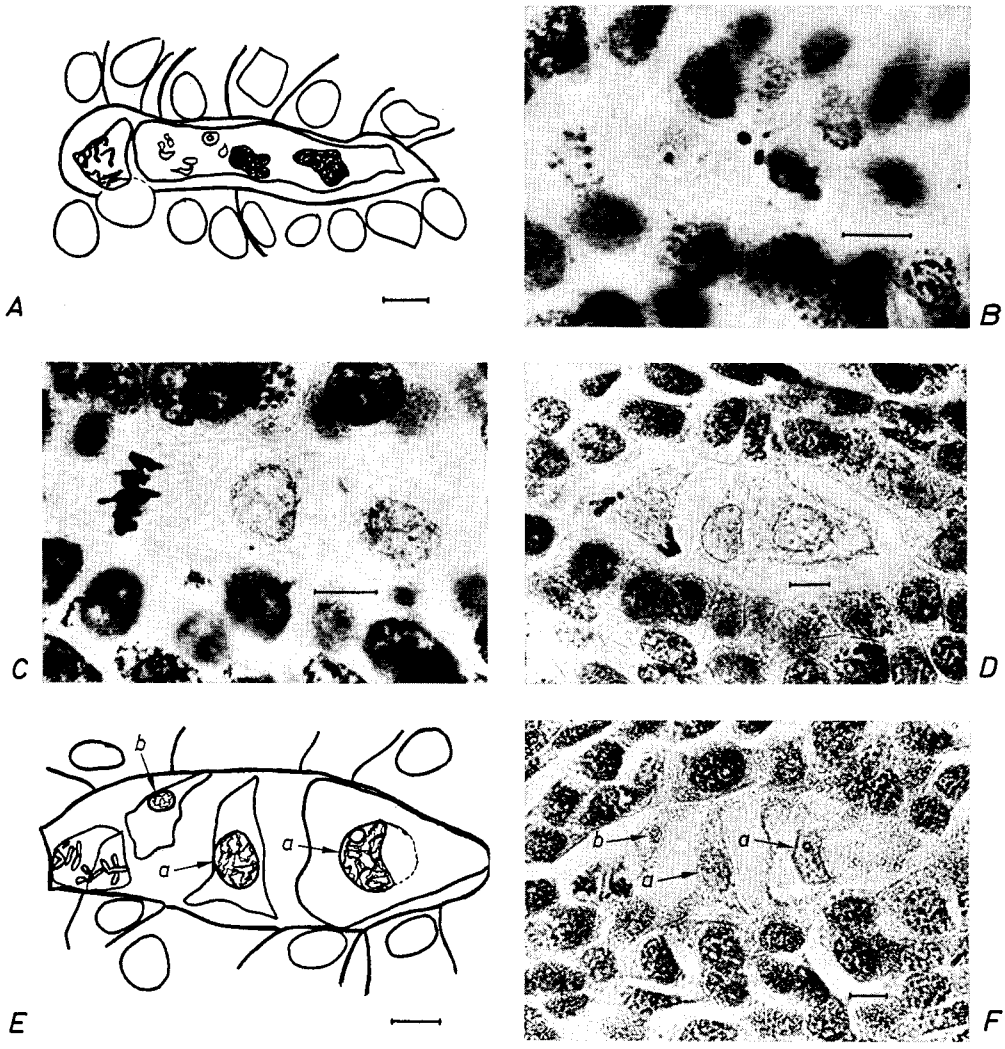

Fig. 16. Meiosis in the EMC of the desynaptic clone of Norway spruce. All microphotographs are oriented in such a way that the apex of the nucellus is situated to the left. The lines indicate a distance of 10 μ.

A, B: Drawing and corresponding microphotograph of prophase II/2 degenerating nuclei and several lagging chromosomes.
C: Metaphase II/2 megaspores.
D: Anaphase II/2 megaspores.
E, F: Drawing and corresponding microphotograph of an abnormal anaphase II (containing micronucleus)/2 megaspores; a → marks the megaspores; b → marks the micronucleus.

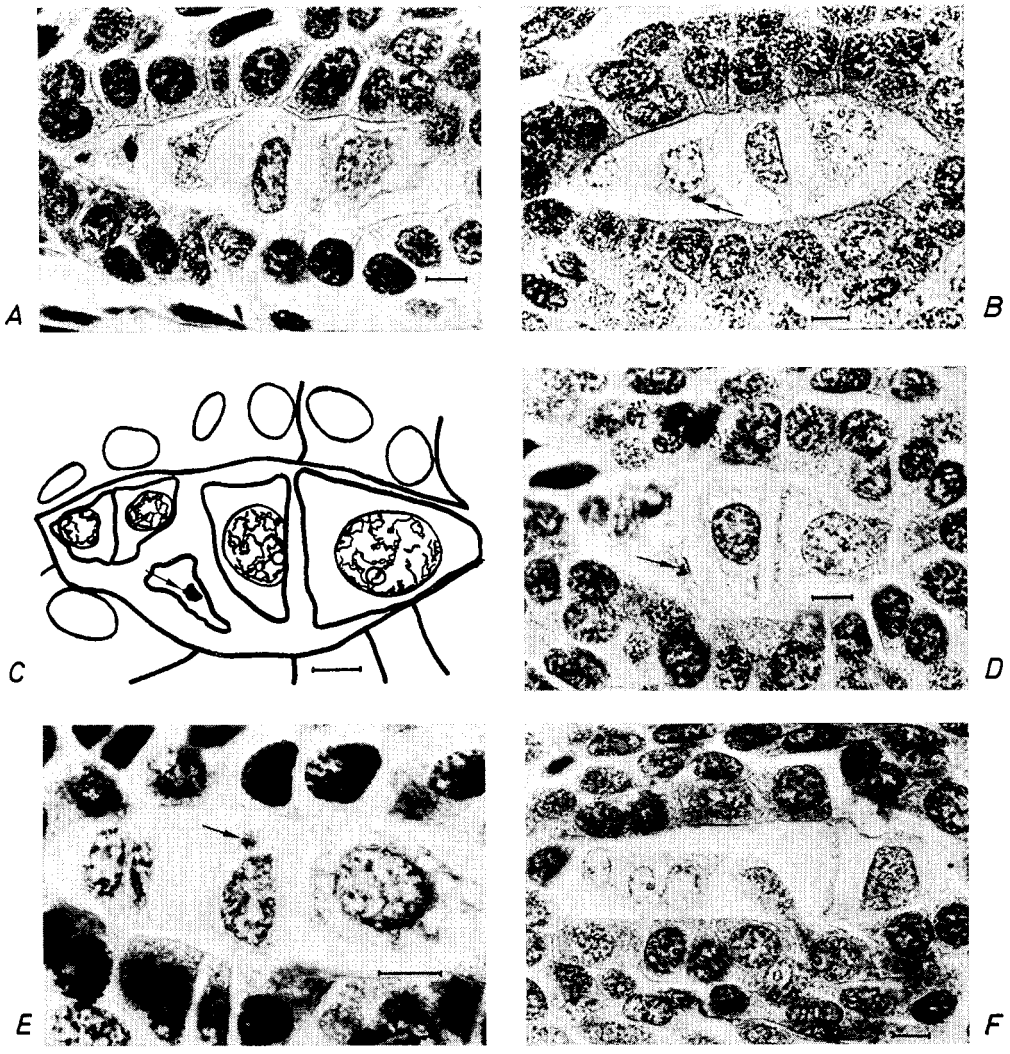

Fig. 17. Tetrad stage in the desynaptic clone of Norway spruce. All microphotographs are oriented in such a way that the apex of the nucellus is situated to the left. The lines indicate a distance of 10 μ.

A: Tetrad stage—three micronuclei and two megaspores.
B—E: Slightly damaged tetrads; C constitutes a drawing of the microphotograph of D;→ marks micronuclei.
F: A pentade.

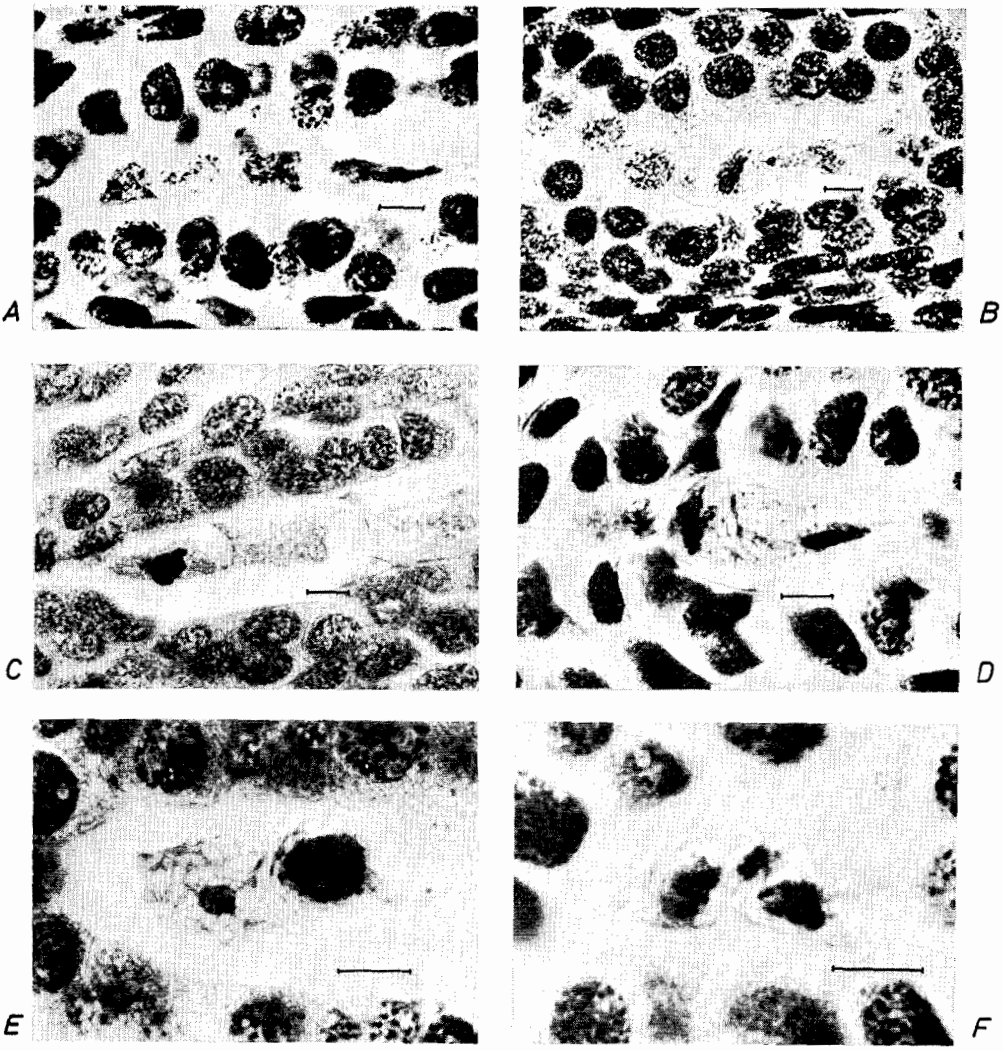

Fig. 18. Meiosis in the EMC of the desynaptic clone of Norway spruce. The EMC are completely collapsed owing to irregularities during the course of meiosis. The lines indicate a distance of 10 μ.

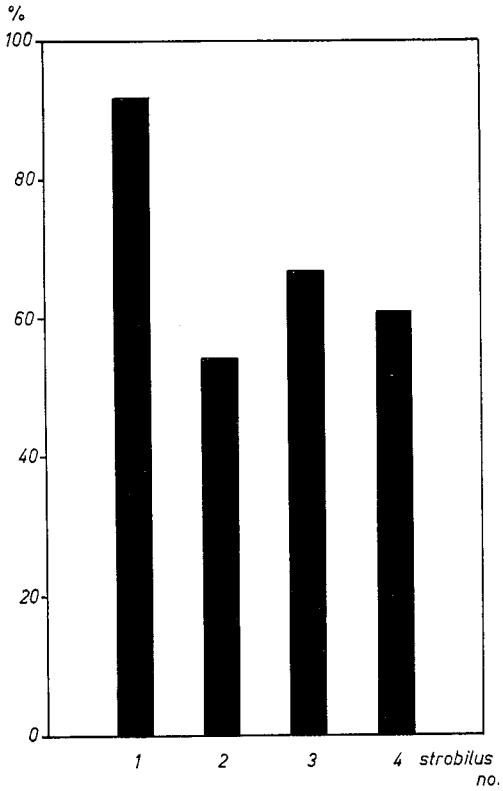

Fig. 19. The percentage of nonsynchronous second divisions in EMC from four strobili growing on the Norway spruce with an ordinary seed set. The material was fixed on April 19th, 1968 (n = 77).

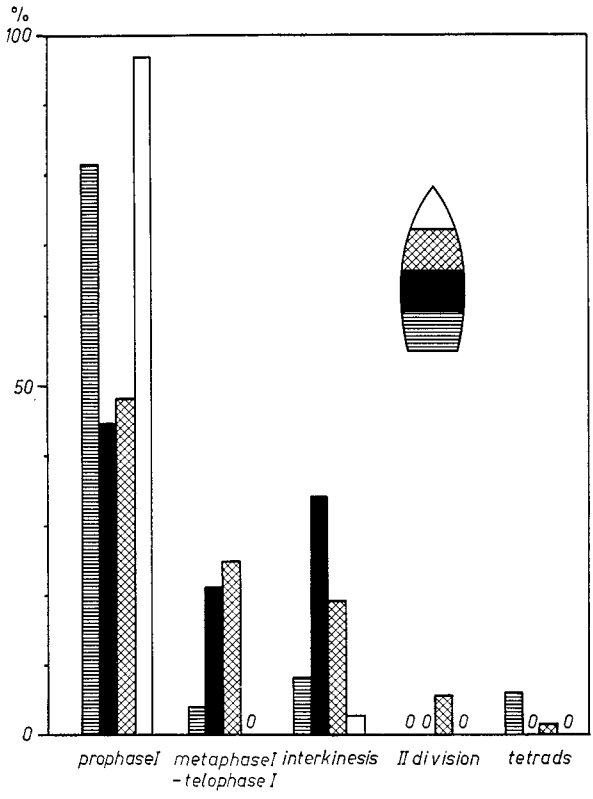

Fig. 20. The distribution of meiotic stages within a strobilus of the spruce with an ordinary seed set. The material was fixed on May 8th, 1969 (n = 182).

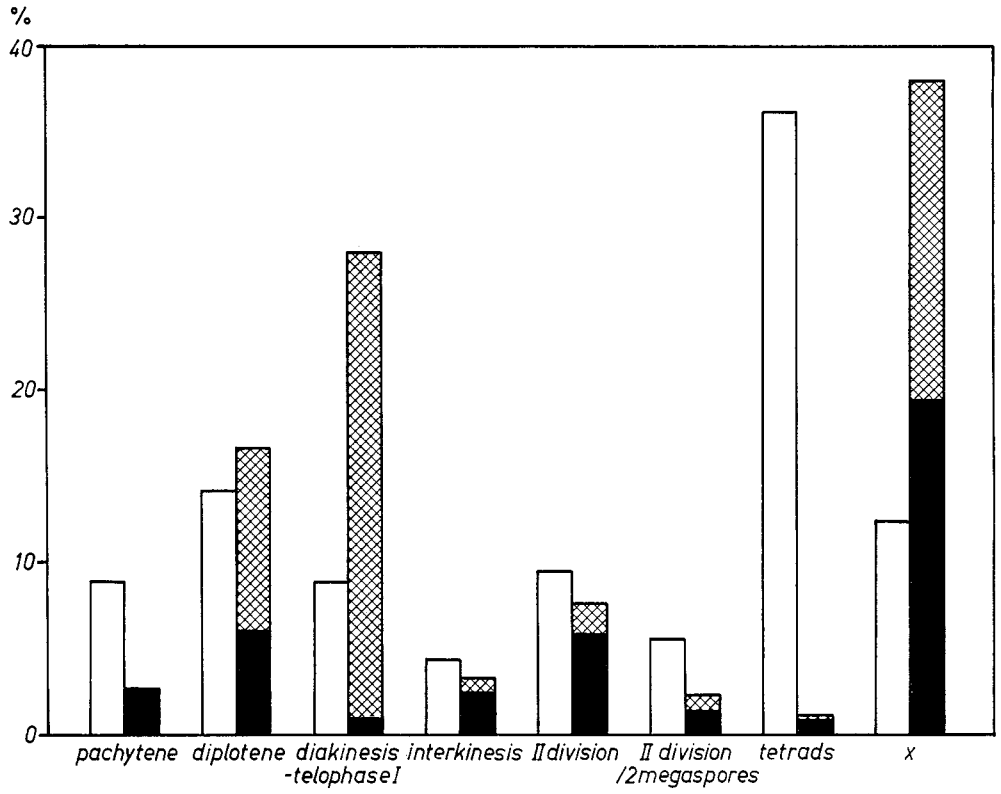

Fig. 21. The percentage of EMC in different meiotic stages of the Norway spruce with the ordinary seed set (white columns; n = 506) and of the desynaptic Norway spruce (black columns; n = 482). The fixation was performed on April 19th, 1968. In some of the EMC the meiotic stage could not accurately be determined. These EMC are shown in the columns above *x*. The hatched part of the columns indicates the percentage of irregularities during the meiotic division. II division/2 megaspores means that there was a nonsynchronous division, the apical daughter cell remained in the second division whereas the basal daughter cell had already formed two megaspores.

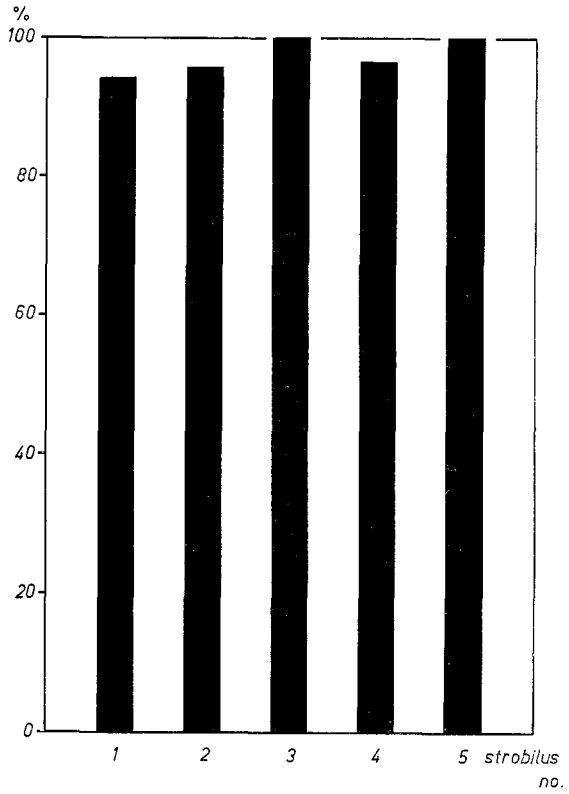

Fig. 22. The percentage of EMC containing one or more univalents during metaphase I—anaphase I of five female strobili from the desynaptic graft. The material was fixed on April 19th, 1968 (n = 133).

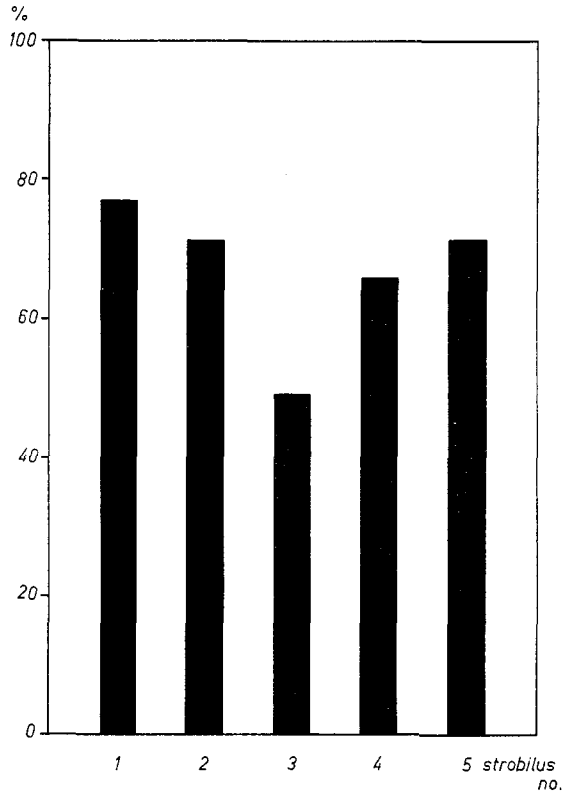

Fig. 23. The percentage of EMC with irregular meiosis of five female strobili from the desynaptic graft. The material was fixed on April 19th, 1968 (n = 299).