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The Meiotic Development in Male Aspen

Meiosutvecklingen hos hanträd av asp

by

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Introduction

A great interest in the possibility of using triploid aspens in silviculture was raised after the first detection of naturally occurring triploid aspen by NILSSON-EHLE 1935 (NILSSON-EHLE 1936). This was mainly due to the gigas form of the triploids. Meiosis in the first detected triploid aspen was studied by MÜNTZING (1936). Subsequently a few more observations of spontaneously occurring triploid aspens were reported (BLOMQVIST 1937, MELANDER 1938, JOHNSSON 1939 and 1940). In *Populus alba* and *P. canescens* triploids were described by PETO (1938).

In spite of the gigas character of the autotriploids they seem to be of limited practical interest as certain diploid and especially triploid hybrids *(tremula x tremuloides)* show still better growth than the autotriploids (*cf.* JOHNSSON 1956, 1958, and 1959). Thus JOHNSSON (1958) stated that a doubling of the wood production per unit area might be achieved by cultivating hybrid aspen. Besides this the hybrids are in some cases relatively resistent against some diseases (*cf.* VON WETTSTEIN 1937, JOHNSSON 1957, GUSTAFSSON and MERGEN 1964).

The possibilities of using triploids and hybrids in aspen breeding provoked some studies of meiosis. Due to the two types of breeding it is not surprising that the pairing of chromosomes during metaphase I of meiosis attracted the greatest interest in these studies. Therefore, metaphase I has carefully been described in the literature. Another line followed in the meiotic studies in *Populus* was the search for heterochromosomes.

BLACKBURN and HARRISON (1922) were the first who correctly reported the haploid chromosome number in aspen. This number (19) was later confirmed to be true also for several other species belonging to *Salicaceae* (BLACKBURN and HARRISON 1924). According to these authors the chromosomes of aspen could be divided into two groups according to their size. One group consisted of 9 small chromosomes of almost uniform size, whereas the second group consisted of 9 large chromosomes which varied in size. Besides these two groups they claimed the existence of heterochromosomes. Such chromosomes have also been claimed to occur in *Populus balsamifera, P. Simoni,* and *P. trichocarpa* by MEURMAN (1925) as well as for *Populus tremuloides*

by ERLANSON and HERMANN (1927). However, PETO (1938) and JOHNSSON (1940) seemed to be somewhat uncertain whether heterochromosomes existed or not.

The main purpose of the present investigation was to study meiosis in pollen mother cells (PMC) and to demonstrate the different stages by photomicrographs. Further we intended to study the duration of individual meiotic stages in catkins growing on twigs which were allowed to develop in a greenhouse. This is of especial importance for investigations where treatment of any kind has to be applied during a certain sensitive developmental stage. The present investigation might also be regarded as a part of a general investigation with the purpose of studying the meiosis of forest trees native to or introduced into Sweden.

Material and methods

Twigs of male aspen kindly provided by Dr M. SIMAK, The Royal College of Forestry, Stockholm, were placed in glass vials in the greenhouse on February 20th 1967. The day temperature varied between 19—25° C and the night temperature between 15—18° C. Fixations in acetic alcohol (1 : 3) were made at intervals until mature pollen grains appeared. The fixed material was stored under refrigeration until examination. The PMC were subsequently dissected and squashed in 4 % acetoorcein. This well-known staining technique is convenient both for routine and careful investigations of chromosomes in PMC of aspen.

In the catkins where the meiotic stages appeared the percentages of PMC in different developmental stages were determined in flowers growing in different positions of the catkin. In each slide 100 PMC were counted. Thus it was possible to follow the pattern of development within a catkin. Furthermore, with the aid of these percentages, it was also possible to estimate the duration of individual meiotic stages.

The pollen fertility was estimated by staining the pollen grains in a solution of methylene blue.

Meiosis in PMC

According to NICOLAEVA (1965) meiosis in the PMC starts at the end of winter or in early spring. At the time of the first fixation meiosis had not started as is evident from Table 1 where the distribution of stages is shown for the different times of fixation.

As early as 6—7 hours after the twigs had been placed in the greenhouse the pachytene stage was reached which indicates the rapid development taking place in the PMC. During this stage the chromosomes are faintly stained and highly elongated. There is no possibility of identifying any of the chromosomes during this stage. Usually only one nucleolus is present during pachytene and at least one chromosome pair is associated with the nucleolus. This chromosome pair contains heavily stained parts close to the nucleolar organizing region as is revealed in Fig. 1 A—B.

The contraction of the chromosomes proceeds and during the diplotene stage (Fig. 1 C) the individual bivalents could be distinguished. During this stage it ought to be possible to study the localization and occurrence of chiasmata.

At diakinesis (Fig. 1 D) the chromosomes are even more contracted than during the previous stage and the 19 bivalents are easily recognized. During this stage it is also easy to see that the size of the different chromosomes varies considerably. During this as well as during the subsequent metaphase I (Fig. 1 E—F) it is not possible to recognize the two individual chromosomes of a bivalent, which confirms earlier observations (*cf. e.g.* JOHNSSON 1940). In a few cells at diakinesis and metaphase I, 20—21 chromosome units seemed to appear. It is not possible to determine whether they all were bivalents or if some were univalents due to the uniform appearance of the small chromosomes. MEURMAN (1925) who made similar observations believed this to be due to the compound nature of the chromosomes in *Populus*. As high a number of univalents as 24, found in some of the aspen clones studied by JOHNSSON (1940), was obviously not present in our material.

In the literature metaphase I is the most frequently described stage of meiosis in *Populus* (*cf.* above). Our observations confirm the earlier findings. Thus frequently one large X-shaped bivalent is visible during

Table 1. Percentage of cells in different stages and the number of cells examined from catkins fixed at different times following the placing of aspen twigs in greenhouse.

Hours in greenhouse	Catkin no.	% cells in each stage														Numbers of cells examined
		prepachytene	pachytene	diplotene	diakinesis	metaphase I	anaphase I	telophase I	interphase	prophase II	metaphase II	anaphase II	telophase II	tetrad	microspores	
0	2	100														300
6.5	3 A	62	38													300
	3 B	67	33													300
11.5	4 A		100													500
	4 B		100													1,000
24	5 A		100													1,000
	5 B		82.3	11.3	1.1	5.3										1,000
26	6 A		100													700
	6 B		41.7	49.5		7.3	0.6	0.6	0.3							1,000
28	7 A		61.8	21.8	5.8	8.5	0.7									1,000
	7 B		49.3	14.6	4.2	14.1	0.9	3.6	5.0	0.7	2.7	2.3	2.6			1,800
32	8 A		13.8	2.8	0.2	21.0	1.7	5.2	9.6	3.1	4.4	3.4	4.1	13.0	17.7	1,200
	8 B		7.7	26.4	4.1	16.1	0.7	1.3	10.7	1.9	2.9	1.6	0.7	23.5	2.4	1,500
34.5	9 A		10.8	8.7	5.3	26.0	2.2	4.5	9.3	2.7	5.2	2.8	2.4	12.4	7.7	1,800
	9 B		5.0	5.6	3.5	5.5	0.9	1.6	4.5	1.2	7.2	4.3	3.6	31.6	25.5	1,700
37.5	10								0.4		0.4	0.3	0.5	15.5	82.9	1,900
50.5	11														100	500
53.5	12														100	500
71.5	13														100	500
81.5	14														100	500
145	15													mature pollen		2,000

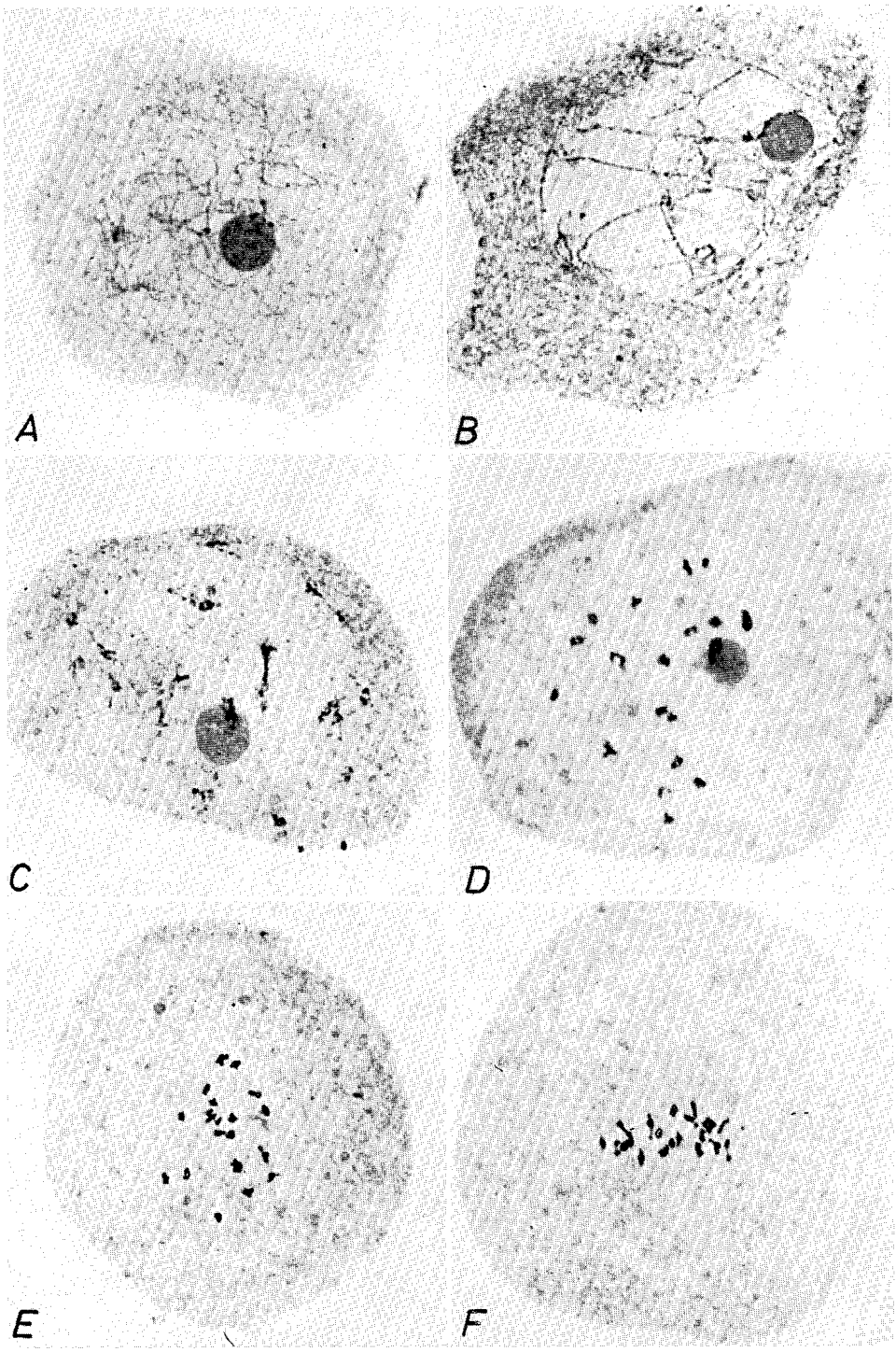

Figure 1. Pachytene to first metaphase in pollen mother cells of aspen. A. Mid-pachytene B. Late pachytene. In A—B the darkly stained regions of the nucleolar organizing chromosome pair are seen. C. Diplotene. D. Diakinesis. In A—D the darkly stained nucleolus as well as the nucleolar organizing chromosome pair are seen. E. Polar view of metaphase I F. Side view of metaphase I. In D—F the majority of the bivalents show terminally localised chiasmata

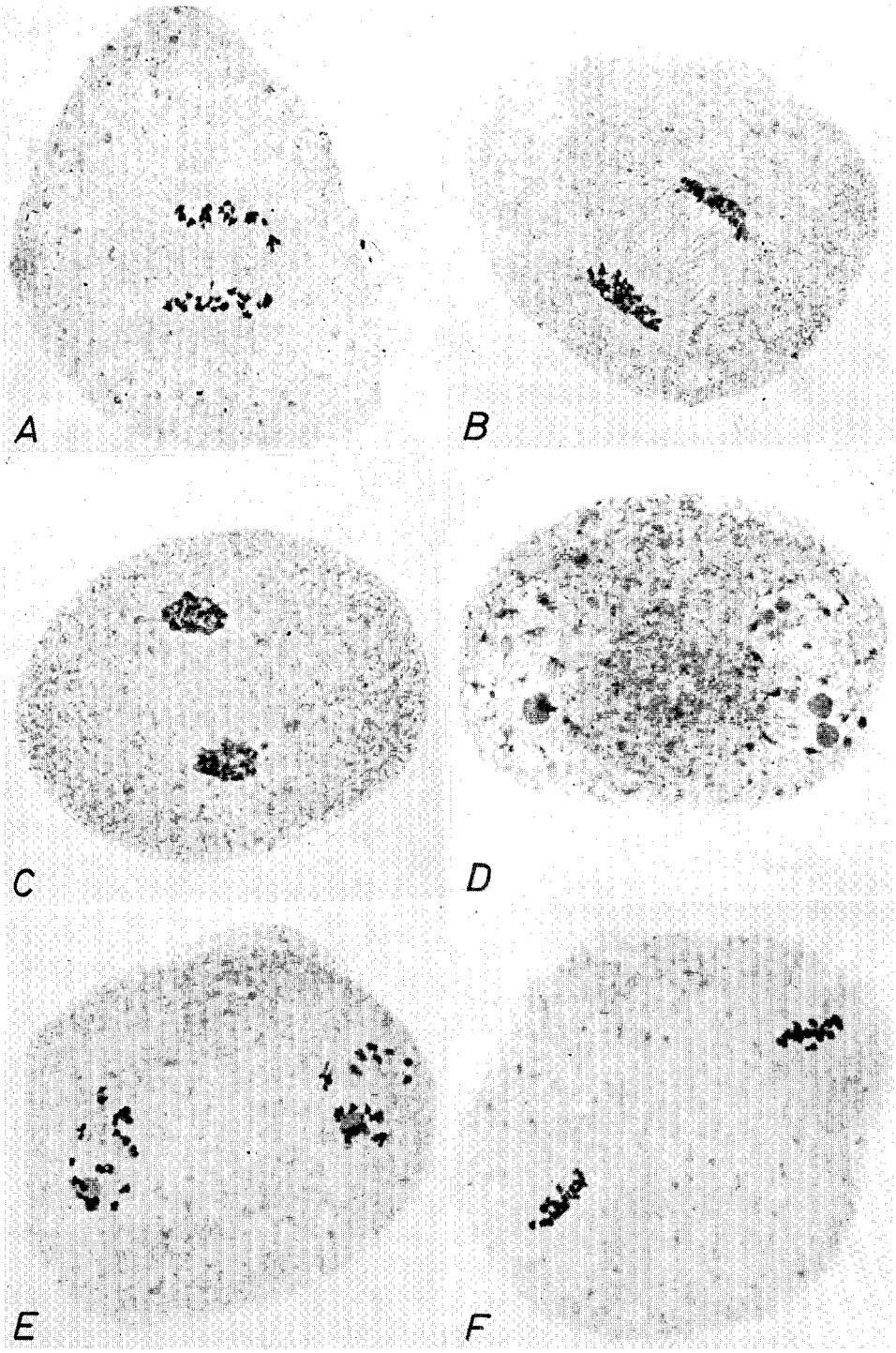

Figure 2. First anaphase to second metaphase in pollen mother cells of aspen. A. Anaphase. I B. Late anaphase I. C. Telophase I. D. Interphase showing several nucleoli. E. Prophase II containing one nucleolus in each daughter nuclei. F. Metaphase II

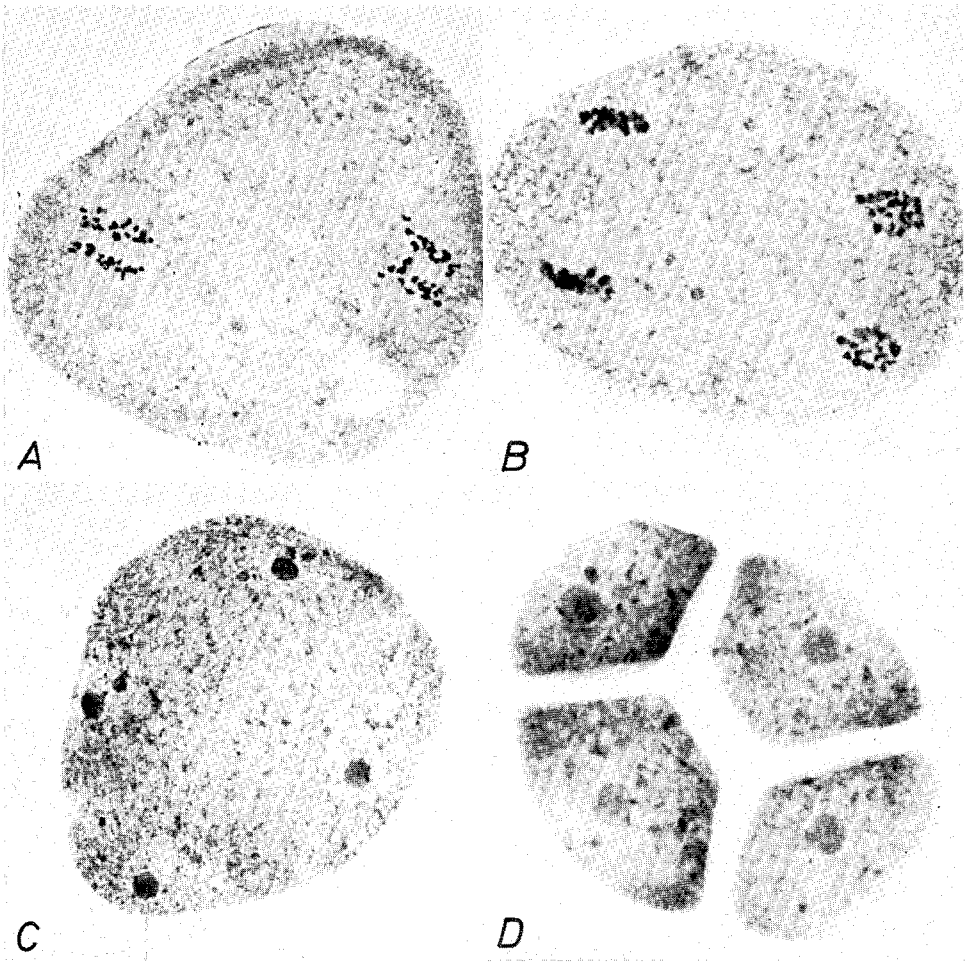

Figure 3. Second anaphase to the tetrad stage in pollen mother cells of aspen. A. Early anaphase II. B. Late anaphase II. C. Telophase II. D. Tetrad

this stage (*cf.* Fig. 1 F). Due to the contracted appearance of the bivalents it was impossible to determine if heterochromosomes were present or not. Two bivalents which contained satellites were revealed.

The separation of the chromosomes proceeded in a normal way in the overwhelming majority of cells during anaphase I (Fig. 2 A—B). Lagging chromosomes were never observed in the more than 100 anaphase I cells studied. (Due to the short duration of anaphase I this stage is relatively rare.) However, a few cases of chromosome bridges occurred during this and the following stage.

Telophase I (Fig. 2 C) was not followed by a wall formation, which always seemed to take place after the completion of the second meiotic division.

During the interphase several small nucleoli were present (*cf.* Fig. 2 D). The chromosomes stained only faintly or not at all during this stage.

During prophase II (fig. 2 E) only one nucleolus in each daughter nucleus could be seen. The many small nucleoli had probably fused during the interval of interphase to prophase II. The chromosomes appeared as small X-shaped bodies during this stage in similarity with the appearance of maize chromosomes of this stage (*cf.* RHOADES 1950).

The extremely small size of the chromosomes during metaphase II (Fig. 2 F) did not permit any evaluation concerning the existence of heterochromosomes.

Only a few abnormalities or peculiarities such as bridges were detected during the two following stages, anaphase II (Fig. 3 A—B) and telophase II (Fig. 3 C).

After the completion of the second meiotic division the wall formation started. As the two spindles of the second division mostly were perpendicular to each other the typical appearance of the tetrad stage was as demonstrated in Fig. 3 D.

Only one pollen mitosis occurred before pollen maturity as described by NICOLAEVA (1965). The mature pollen grains were of relatively uniform size. Only rarely were giant grains observed. The pollen fertility was high amounting to 92 %.

Developmental rate of meiosis

In Table 1 the percentage of cells in various stages of development at different time intervals is shown. A few hours in the greenhouse were enough to provoke the onset of meiosis as seen from this table. In some of the catkins all meiotic stages were present simultaneously. Also within individual flowers several meiotic stages might simultaneously be present although the variation was less pronounced than within a catkin. To demonstrate the pattern of development within a catkin the data from one of the analyzed catkins are demonstrated in Table 2 and Fig. 4. The positions of the analyzed flowers within the catkins were numbered from the base to the apex. From Table 2 and Fig. 4 it is evident that the development starts in the base and proceeds upwards. Some fluctuations occurred as is seen from the curves for diplotene and metaphase I.

In Fig. 5 the total duration of meiosis as well as the duration of pachytene is illustrated. From this figure it is evident that pachytene

Table 2. Percentage of cells of various stages in different flower position in catkin 8 B. The fixing was performed after the twig had been 32 hours in greenhouse.

Flower position	% of cells in different stages												
	pachy-tene	diplo-tene	dia-kinesis	meta-phase I	ana-phase I	telo-phase I	inter-phase	pro-phase II	meta-phase II	ana-phase II	telo-phase II	tetrad	micro-spores
1										1		75	24
2									1	2		97	
3										3		97	
4				1			1		1	1	1	83	12
5		1		21	1	1	31	11	13	14	7		
6				20	5	3	50	10	11			1	
7				13	4	11	53	6	11		2		
8		53	1	13		3	18	1	7	4			
9		10	12	71		1	5		1				
10		60	10	29	1								
11		28	14	57		1							
12		73	15	11			1						
13		100											
14	15	70	10	5									
15	100												

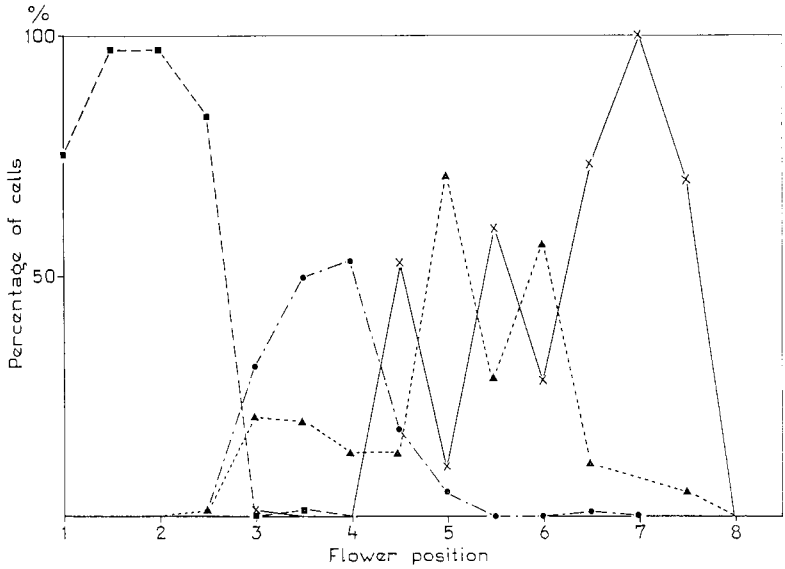

Figure 4. The distribution of pollen mother cells in aspen in different flower positions in catkin no. 8 B. The flowers were numbered from the base to the apex
X = diplotene ▲ = metaphase I ● = interphase ■ = tetrad

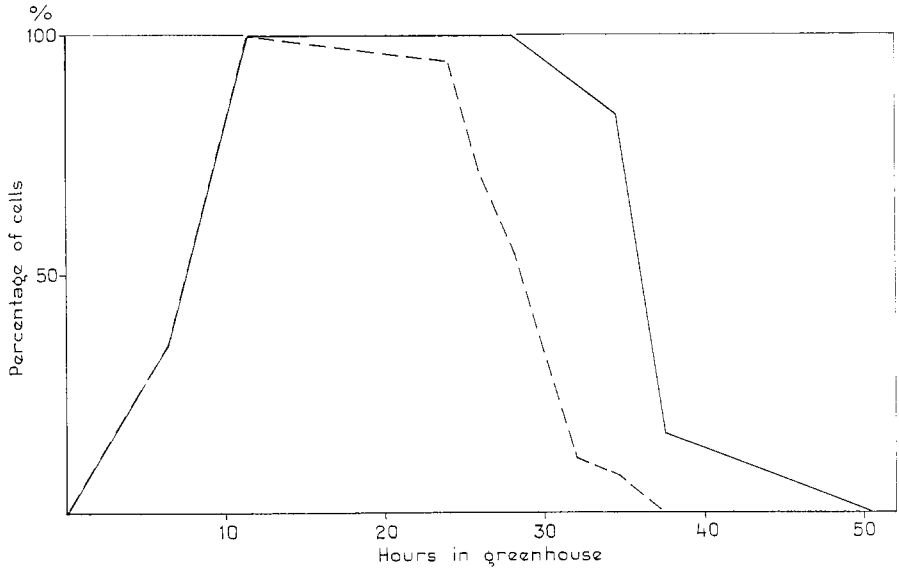

Figure 5. The duration of pachytene (dotted line) and the stages pachytene—tetrads (unbroken line) in twigs of aspen growing in glass vials in greenhouse.

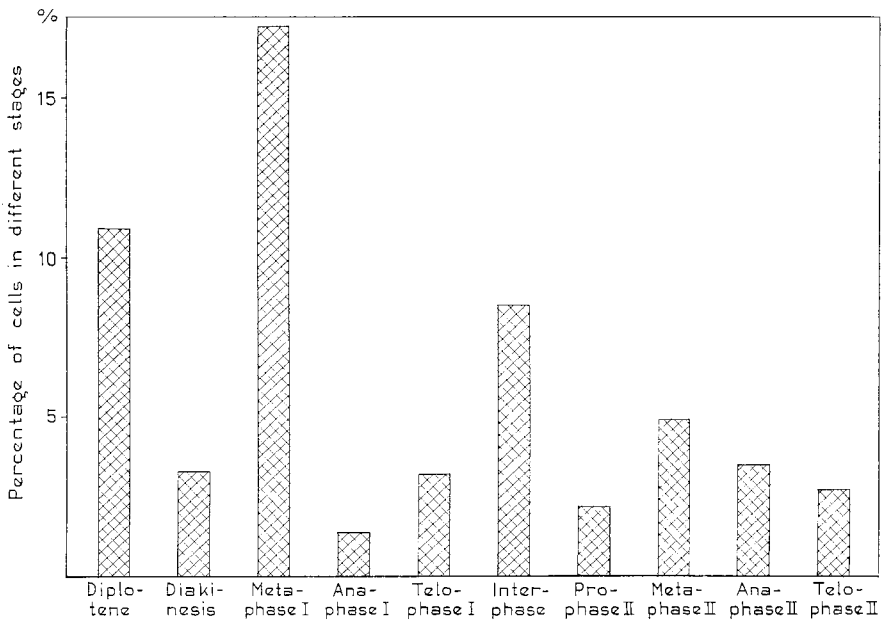

Figure 6. Average percentage of cells in different meiotic stages from 4 catkins where all meiotic stages from pachytene to microspores were represented. The relative durations of the individual stages are reflected by the percentages (*cf.* text)

is the longest lasting of all stages. The average durations will be equal to the integrals of the respective curves for the percentages. The simplest estimation of this integral is to determine the weight of the area surrounded by the curve and the abscissa. The durations of pachytene and total meiosis (pachytene-tetrad) were thus estimated to be around 22 and 30 hours, respectively. As at most two catkins from each fixation were examined a calculation of the durations in hours of the other individual meiotic stages is omitted. The relative durations is reflected by the percentages in those catkins where all stages from pachytene to tetrads are present, as was the case for the 4 catkins fixed following 32 and 34.5 hours in the greenhouse. The average percentage of cells in different stages in these 4 catkins is demonstrated in Fig. 6. The long duration of metaphase I is evident. Besides metaphase I, diplotene and interphase constitute relatively long lasting stages compared to the other stages. Taken together the second anaphase and telophase seem to proceed somewhat slower than the first anaphase and telophase.

Discussion

Meiosis in PMC in aspen shows no peculiarities but follows the general pattern of meiosis described for many plants. Due to the small size of the chromosomes detailed analysis of chromosomal irregularities can only be carried out to a limited extent during meiosis. However, studies of the occurrence of bridges during anaphase or the formation of multivalents during metaphase I should be possible.

If special treatments are to be carried out during particular meiotic stages, the aspen is less advantageous as it has clearly been demonstrated in the present investigation that the stage of meiosis varies extremely within a catkin as well as within individual flowers (*cf.* Tables 1, 2 and Fig. 4). Therefore, in radiation experiments, an irradiation of individual meiotic stages will be difficult to carry out. Due to the great variation in radiosensitivity between meiotic stages (*cf.* ERIKSSON and TAVRIN 1965 and lit. cit.) great care must be taken in the evaluation of results following an irradiation of a population consisting of cells of several meiotic stages. The same is probably also true for any kind of treatment during meiosis.

Although the meiotic stages were passed within such a short time as 30 hours (on an average) it is interesting to note that chromosomal irregularities only rarely appeared. Due to this observation the high pollen fertility obtained was expected. The rare occurrence of giant pollen grains can be explained by the absence of stickiness during meiosis, which might have given rise to restitution nuclei. It can be concluded that the conditions prevailing in the greenhouse during meiosis were favourable for an appropriate development of the pollen grains.

Som information on the duration of meiosis in other plant species is available (*cf.* TISCHLER 1951). Mostly meiosis is passed within 6—7 days but exceptions occur. Among the exceptions *Larix* seems to be most extreme as meiosis of the PMC usually is extended over more than half a year (*cf.* EKBERG and ERIKSSON 1967 and ERIKSSON *et al.* 1967). In some cases observations of the duration of individual meiotic stages have been reported. As a rule the relative durations agree with our observations. Thus the first prophase is of long duration compared to the total duration of the stages metaphase I—

telophase II (*cf.* TISCHLER 1951). In similarity with the observations in aspen metaphase I seems to be of relatively long duration (*cf.* JARETZKY 1930, ROSENTHAL 1936, EKBERG *et al.* 1967).

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Summary

Meiosis in PMC of aspen in twigs allowed to develop in greenhouse, was studied and demonstrated by photomicrographs. During the conditions prevailing in the greenhouse the development from pachytene to microspores took place within around 30 hours. Pachytene was shown to be the longest lasting stage followed by metaphase I, diplotene and interphase. The development starts in the base of the catkin and proceeds upwards. Several meiotic stages might simultaneously be found in individual flowers.

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Sammanfattning

Meiosutvecklingen hos hanträd av asp

Meiosutvecklingen hos pollenmoderceller av asp studerades hos kvistar vilka drevs i växthus. De enskilda stadierna har beskrivits och illustrerats med mikrofotografier. Vid de förhållanden som var rådande i växthuset förlöpte utvecklingen från pachytenstadiet till mikroporstadiet inom loppet av 30 timmar. Pachytenstadiet visade sig vara det mest långvariga följt av metafas I, diploten och interfasen. Utvecklingen startar i basen av hänget och fortlöper mot spetsen. Flera meiosstadier påträffas vanligtvis samtidigt i de enskilda blommorna.