TECHNICAL SESSIO N

Chairman: C. E. Farnsworth

MEIOSIS IN LARIX LARICINA KOCH[1]

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INTRODUCTION

Small cones collected from trees of American larch (Larix laricina Koch) growing on the Boyce Thompson Institute farm at Yonkers New York yielded normal size seed However: 5,336 seed (298 cones) produced from cross-pollinations with L. laricina as a seed parent and 3,565 seed (44 cones) from cross-pollinations with L. laricina as pollen parent produced only three seedlings when planted in sphagnum in the greenhouse Forty cones from fully controlled self-pollinations gave four week seedlings while 580 seed from open-pollination gave no germination. To stimulate germination the open pollinated seed were stratified by mixing them with moist peat moss held in a refrigerator at 40° F for two months before planting in soil in the greenhouse. Upon dissection open-pollinated seed were found to be entirely empty or with only small rudimentary embryos which were dry and wrinkled.

A native stand of American larch was located on state hospital land at Wingdale, New York, by Earl D. Brockway, District Forester Natural regeneration was observed among these large trees which showed that cones with viable seeds were produced on these trees.

A study was initiated to compare the stages of meiosis found in material collected at the two locations As far as the writers are aware no detailed cytological investigation of meiosis has been made for this species of larch, Timberlake (10) studied the types and the formation of spindle fibers in L. decidua Mill and L. laricina but further reports were not published. Several authors have published their studies on L. decidua and L. dahurica. It was found by Allen (1), Belajeff (2), Devise (4) and Nemec (5) that pollen mother cells were in the early prophase stages of the first meiotic division during October and November. Prosina (6) working with chondriosomes in L dahurica, also found prophases in February.

MATERIAL AND METHODS

From the time when flower buds were discernible in September, branches were collected and brought into the laboratory at monthly intervals until February when more frequent collections were made until meiosis was completed. Acetic-orcein smear preparations were made of the pollen mother cells as soon as possible after the material was collected Strobili were also fixed in acetic-alcohol (1:3) for later studies The branches collected in the spring were placed in containers of water on the laboratory bench. Once the pollen mother cell had entered the diakinetic stage of meiosis all ensuing stages could be found in this material within the next few days. Following the method of Darlington and LaCour (3 p 126) permanent slides from the acetic-orcein smear preparations were made by floating off the cover glass in acetic-alcohol (1:3) and then passing the slides through two changes of absolute alcohol with one minute in each. A drop of Euparal was placed on the slide and a clean cover glass completed the mount, The original cover glass was inverted over a drop of Euparal on a clean slide thus giving two preparations from the original smear.

[1] Contribution from Boyce Thompson Institute for Plant Research Yonkers, N. Y.

RESULTS AND DISCUSSION

Meiosis, Yonkers Material

The prophase of the first meiotic division was of long duration. In September the sporogenous tissue was composed of angular cells. The nuclei of these cells were filled with chromatin granules which stained faintly with acetic-orcein. During October these cells became sperical and during November, December, and January the nuclei remained in an inactive state. By the first week in February the nuclei occupied at least two-thirds of the cell and the chromatin appeared more organized. Aggregates of the chromatic material were of various sizes and were connected by discontinuous threads or matrices (leptonema). It was evident that homologous chromosomes were beginning to pair lengthwise. Synapsis appeared at random along the entire length of the chromosomes (zygonema). The chromosomes soon shortened and appeared thicker (pachynema) as seen in Figure 1 A and B. By the end of the **week** the longitudinal separation of the homologous chromosomes showed that each bivalent consisted of four chromatids, and each chromosome had been completely duplicated (diplonema). The separation was not complete, however, for paired chromosomes were held together at one or more points along their length by chasmata (Fig. 1C). At the end of diplonema (last week in February) when the bivalents had contracted and at diakinesis when the contraction reached its maximum, chiasmata were clearly visible for the bivalents which were evenly distributed throughout the nucleus (Fig. 1 C). The 49 cells with a total of 588 chromosomes studied at this stage of meiosis showed 34 chromosomes with 0 chiasmata, 47 with 1, 314 with 2, 170 with 3, 22 with 4, and 1 chromosome with 5 chiasmata. A precocious terminalization of the chiasmata may have occurred to give the relatively high number of bivalents without chiasmata or it may be due to the loose association of bivalents. Sax (8) studied the chiasma frequency in L. decidua, L. leptolepis, and their hybrid L. eurolepis and concluded that the preponderance of the symmetrical arrangement of the chromatids in Larix suggests that there is little crossing-over even though the chiasma frequency is relatively high.

In most of the cells, the 12 bivalents were oriented in the center of the cell at late prophase; however, an occasional bivalent lagged behind. During the second week of March metaphase ensued and was of short duration. The anaphase of the first meiotic division brought about the separation of homologous chromosomes almost immediately (Fig. 1 D). At this stage 51 cells were studied and in nine of these cells one chromosome was found lagging in the cytoplasm. In addition to lagging chromosomes (Fig. 1 E), an unequal distribution to the poles was observed. Instead of two equal groups of 12 chromosomes each, two to five groups were present in certain cells. Typical unequal chromosomal distribution in seven such aberrant cells was found as shown in Table 1. As many as six chromatin bridges were seen and occasionally chromosomal fragments or an entire chromosome were found lagging in the cytoplasm (Fig. 1 F).

Cytokinesis may or may not occur following first division. Saxton (9) reported a similar behavior for L. decidua var. pendula,. When two spores are forced the nucleus enters an interphase stage in which the chromosomal material becomes granular and well dispersed throughout the nucleus. Second division may occur shortly after the chromosomes reach the poles and again from one to seven chromosomes were observed lagging in the cytoplasm in 28 out of the 70 cells studied (Fig. 2 A). This alone would give 40 percent abnormal microspores. Unequal distribution of chromosomes at anaphase II (table 1) also contributed to further abortion of microspores.

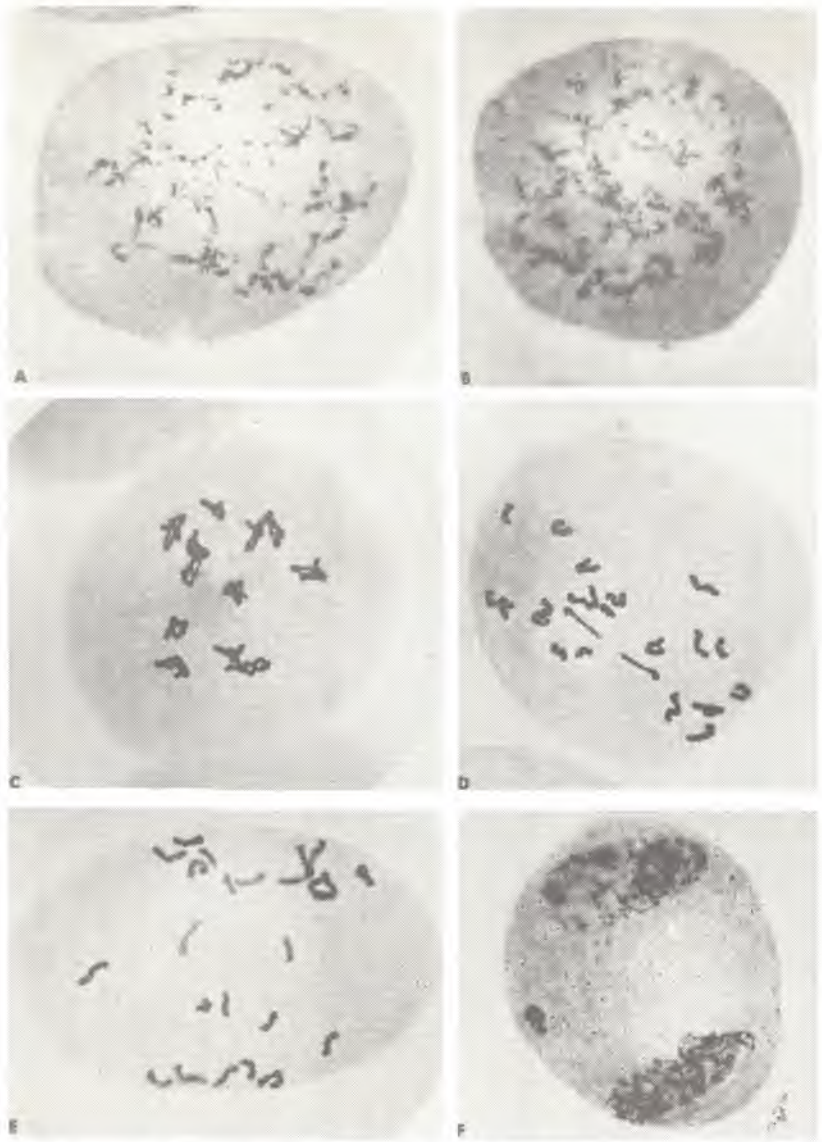

Figure 1.--Meiosis in L. laricina (Yonkers). A. Early diakinesis (Feb. 16). B. Diakinesis (Feb.26). C. Late diakinesis (Feb. 23). D. Anaphase I (Feb. 21). E. Lagging chromosomes at anaphase I (March 7). F. Interphase with extraneous chromatin material in cytoplasm (March 29). (X 657).

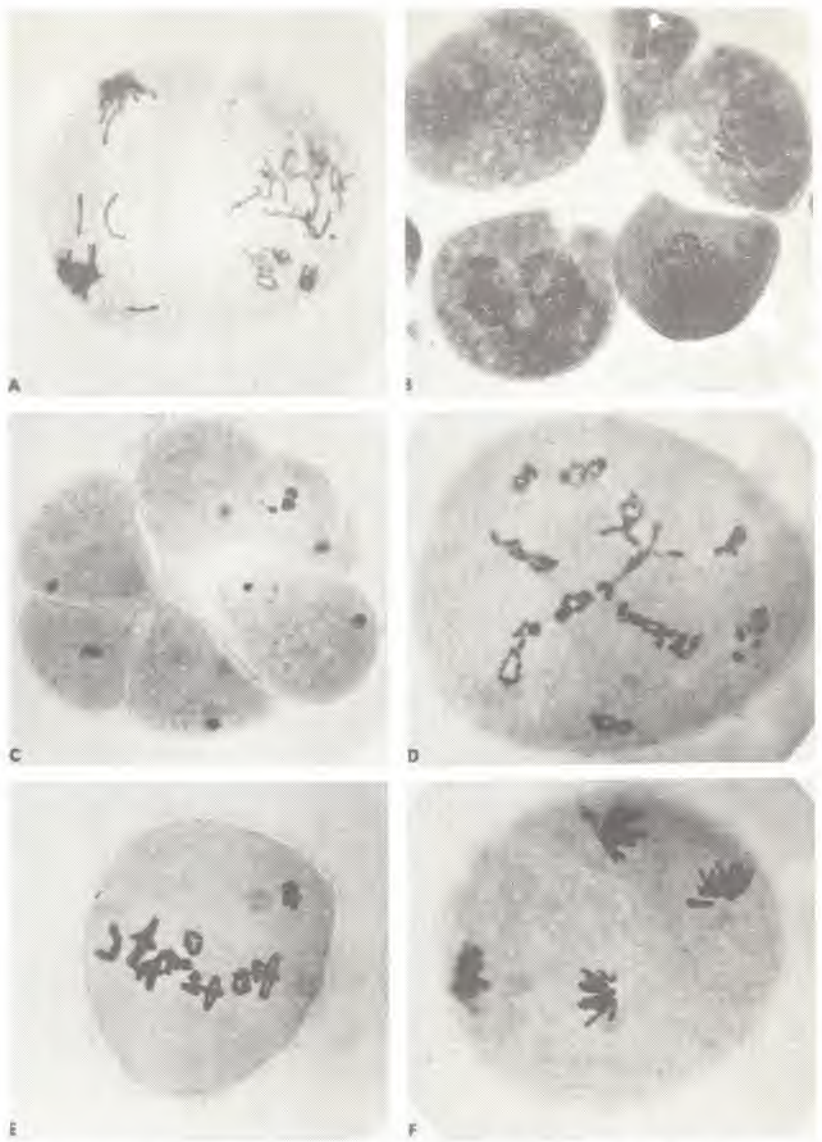

Figure 2.--Meiosis in L. laricina. A to C (Yonkers). D to F (Wingdale). A. Anaphase II (March 25). B and C. Microspores within a pollen mother cell wall (March 28). D. Diakinesis showing chiasma (March 5). E. Terminalization of chiasma (March 3). F. Anaphase II without lagging chromosomes. (X 657).

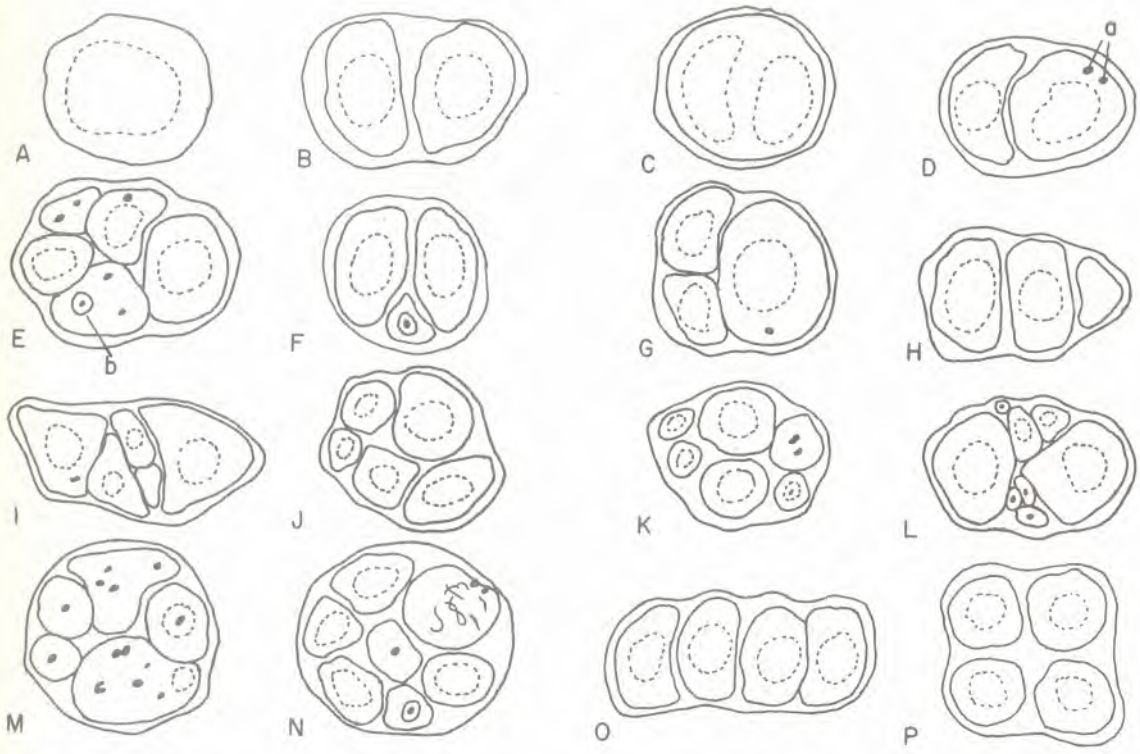

Figure 3.--Camera lucida drawings of aberrant microspores formed from single pollen mother cells. D-a. Microcysts. E-b. Microcyte. (X 211).

Table 1.--Unequal distribution of chromosomes at anaphase I and II in Yonkers material.

Meiotic phase	No. groups in cell	No. chromosomes/group	Total number of chromosomes
Anaphase I	2	7+17	24
	3	12+11+1	24
	3	8+15+1	24
	3	11+11+2	24
	3	3+16+5	24
	4	2+5+5+12	24
	5	6+1+3+3+11	24
Anaphase II	9	1+1+3+4+4+5+6+8+16	48
	9	1+1+2+2+5+6+7+10+14	48
	10	2+2+3+4+5+5+5+7+7+8	48
	12	1+2+2+2+2+3+4+5+6+7+7+7	48

The numbers of microspores within a pollen mother cell wall varied from one to as many as ten (Fig. 2, B and C). A single large spore with one large chromatin mass was seen occasionally (Fig. 3 A). More frequently two large spores of equal size within the pollen mother cell wall, indicating the failure of second division were observed (Fig. 3 B). Each of these spores had a single large nucleus. Two nuclei were also found within a single spore which resulted from the failure of cytokinesis following first division and the lack of further division of the chromatin material for second division (Fig, 3 C). Entire chromosome or chromatin fragments were seen in the cytoplasm. These homogeneous bits of chromatin material were usually rounded up in small masses, frequently referred to as microcysts (Fig. 3D). Other extraneous chromatin material was accompanied by a small amount of cytoplasm surrounded by its own cell wall and identified as microcytes (Fig. 3 E). Some of the microcytes found after first division was completed were reproduced during the heterotypic division of the other chromatin material in the cells.

A study of the microspores within the pollen mother cell wall showed a variation in the number and size of the spores produced as shown in Figure 3 A to N. As many as ten microspores of various sizes and two extra chromatin units were observed within a single pollen mother cell wall.

Division II may occur in the same plane as Division I although division in a plane at a right angle was more frequent (Fig. 3 O and P).

Mature pollen varied greatly in size. About 35 percent of the 375 grains observed were aborted. About one-half of these grains were empty. Another 25 percent were unusually large. Some of these grains were 122μ in diameter, Occasionally two large spores were seen within a pollen mother cell wall which indicates the failure of the second division of the pollen mother cell. Only 40 percent of the grains were of average size (71.4 to 81.6μ) and should function in hybridization pollinations unless the abnormality in chromosome numbers was not reflected in the size of the pollen grains.

The first division of the nucleus of the microspore took place shortly after their release from the pollen mother cell wall. A further division gave rise to the second prothallial cell, both of which became disorganized and degenerated. The third division formed the generative cell and vegetative nucleus (Fig. 4 B to F). These divisions were found in the average size microspores which formed normal size pollen grains. All divisions were regular without any further lagging of chromosomes. All three divisions were of short duration occurring with 24 hours.

Preliminary studies of meiosis in two other species, L. decidua Mill. and L. leptolepis Gord., growing in Yonkers showed that stages in meiosis were regular as shown in Fig. 5 A to F.

Only American larch growing in Yonkers showed irregular meiotic behavior which was probably due to unfavorable environmental conditions for this species of larch or to its genetic origin.

Meiosis, Wingdale Material

All stages of meiosis were approximately one week later at Wingdale, New York, which is 50 miles north of Yonkers. The early prophase stages of meiosis were identical with those described for the Yonkers material. At diakinesis there appeared to be a closer association of bivalents in this material (Fig. 2 D, E). In the 37 cells studied (444 chromosomes) the numbers of chiasmata were 49 chromosomes with 1 chiasma, 230 with 2 chiasmata, 138 with 3 and 27 with 4. These numbers of chiasmata are very similar to those reported by Sax (7) for L. decidua.

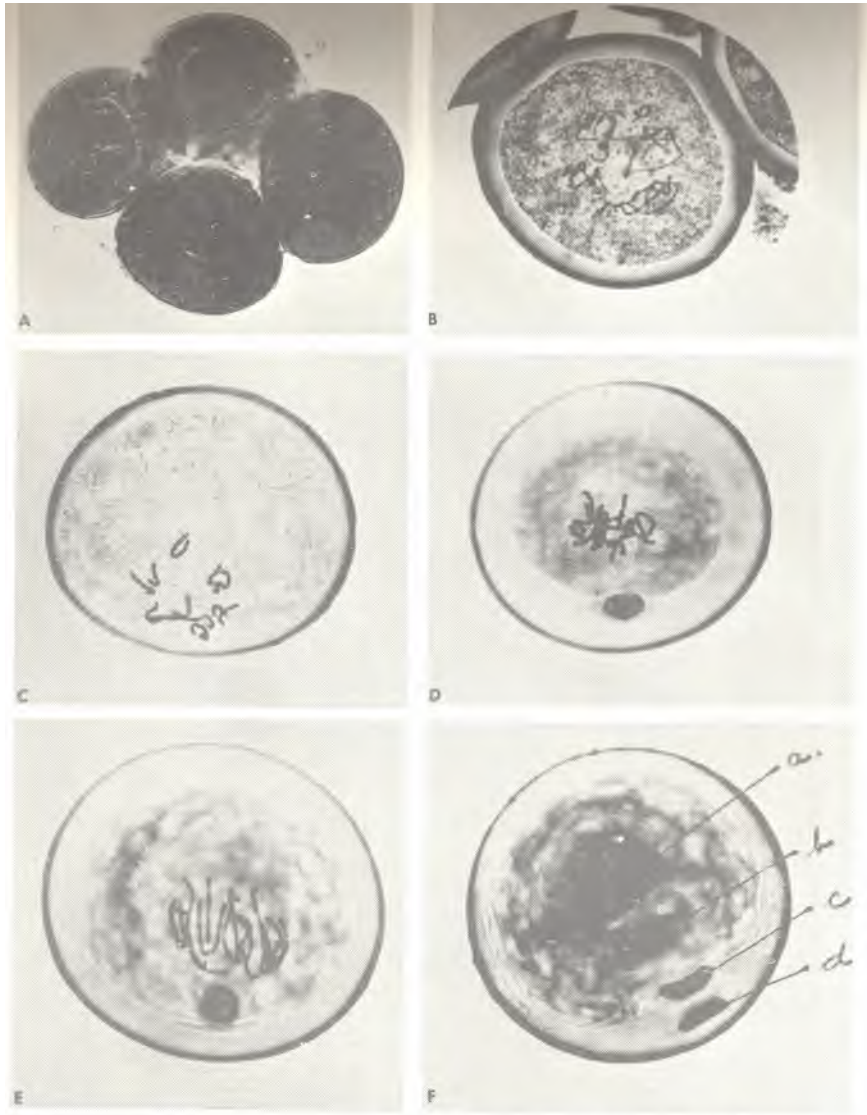

Figure 4.--Meiosis in L. laricina (Wingdale). A. Tetrad of four microspores (April 3). B. Prophase, first division of microspore nucleus. C. Early metaphase of first division. D. Metaphase of second division. E. Prophase of third division. F. Microspore. a, Vegetative nucleus; b, generative cell; c and d, prothallial cells. (X 657).

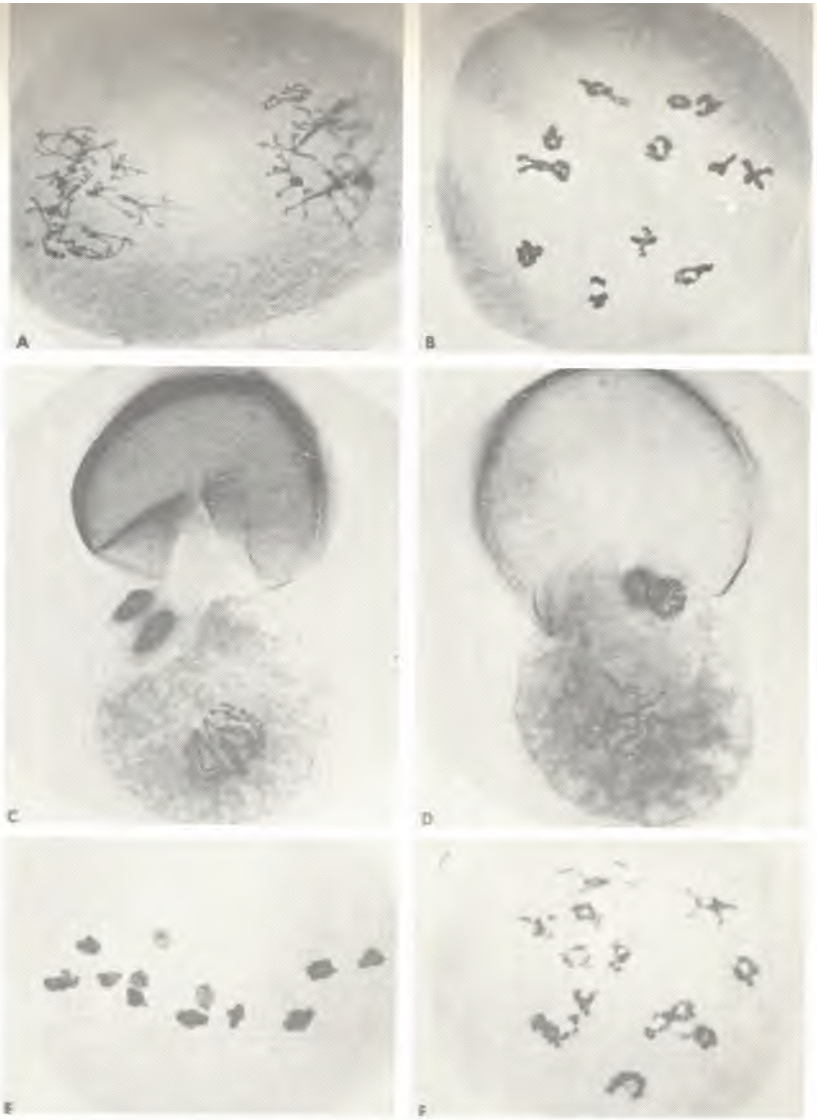

Figure 5.--A to D. L. decidua. A. Separation of chromosomes for second division. B. Chiasmata at diakinesis. C and D. Division of microspore nuclei. E and F. L. Kaempferi. E. Late diakinesis just prior to metaphase plate. F. Chiasmata at late prophase. (X 657).

Both the heterotypic and homeotypic divisions were regular which resulted in four groups of 12 chromosomes each (Fig. 2 F). Neither lagging chromosomes nor fragmentation of chromosomes was observed in the material from Wingdale. Four microspores of equal size were formed within a pollen mother cell wall (Fig. 4 A). The subsequent divisions of the microspore nuclei ensued as already described and produced pollen which, when collected from freshly dehisced anthers and mounted in a drop of 1 percent acetic-orcein, was very uniform in size. Less than 3 percent abortion of grains was observed. The spheroidal grains were 71 4 to 81.6μ in diameter.

Too much emphasis cannot be placed upon the importance of the critical evaluation of pollen prior to its use in a breeding program,

SUMMARY

American larch, Larix laricina Koch, growing at Yonkers, New York, and Wingdale, New York, provided material for the study of meiosis in this species. The study was initiated in order to determine the cause of the failure of the Yonkers trees to yield viable seed to open and fully controlled self- and cross-pollinations, while fully viable seeds were produced on the Wingdale trees to open pollinations. The various stages during meiosis of the two types are reported, Irregularities at both the heterotypic and homeotypic divisions in the Yonkers material resulted in about 60 percent abortion and large pollen grains. It is believed that this may account for the failure of viable seed production. Pollen abortion may be due to the physiological condition of the trees which were growing on dry land rather in moist areas which are more favorable for growing this species, or to its genetic origin. Pollen from Wingdale trees was uniform in size with only three percent abortion.

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