5-1974

Meiotic and Mitotic Chromosomes of Fritillaria atropurpurea Nutt.

John Keith Archibald
Utah State University

MEIOTIC AND MITOTIC CHROMOSOMES OF *FRITILLARIA ATROPURPUREA* NUTT.

by

John Keith Archibald

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Botany

Approved:

_____
Major Professor

_____
Committee Member

_____
Committee Member

_____
Dean of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

1974

ACKNOWLEDGEMENTS

I wish to extend my appreciation to Dr. W. S. Boyle for his help, patience, and friendship ever since I have known him, and especially during the course of this study.

I would also like to thank Professor A. H. Holmgren for his help and suggestions, and also for supplying seed from Nevada which was used in this study. Thanks are also due to Dr. R. J. Shaw and Dr. Wade G. Dewey for their help and timely suggestions throughout this study.

To my wife, Linda, I also express thanks for her patience and encouragement during all aspects of work on this thesis.

John Keith Archibald

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Meiotic and mitotic chromosomes of

Fritillaria atropurpurea Nutt.

by

John Keith Archibald, Master of Science

Utah State University, 1974

Major Professor: Dr. W.S. Boyle
Department: Botany

The purpose of this paper is to provide information concerning the number, structure, and behavior of chromosomes in the plant species *Fritillaria atropurpurea*. The material used for this investigation included both floral buds and root-tips from actively growing naturally occurring plants collected from populations in both Utah and Nevada, and root-tips from seed germinated in the greenhouse. The results obtained include a karyotype analysis of mitotic chromosomes, a discussion of meiosis, a discussion of B-chromosomes, and a series of photographs depicting the stages of mitosis and meiosis in this species.

Fritillaria atropurpurea has a diploid chromosome number of 24, plus from zero to two B-chromosomes. Two

pair of chromosomes have median centromeres while the other ten pair have sub-terminal to terminal centromeres. Two pair have secondary constrictions and satellites. The length of the chromosomes in colchicine treated material ranges from 12.64 to 19.11 microns, with a width of 2.50 microns. During meiosis, pairing is about 95% complete, and an average of 2.50 chiasmata are formed per bivalent, with no localization of chiasmata observed. There is negligible terminalization between diplotene and anaphase I. Mitosis in this species is regular.

(86 pages)

INTRODUCTION

One of the continuing responsibilities of cytogeneticists is the investigation of the chromosome number and behavior of species where this information is lacking. The information currently available on the chromosomes of Fritillaria atropurpurea Nutt. is limited to pollen mother cell counts reported by Bottino (2), and Cave (4).

Furthermore, preliminary studies indicated that the chromosomes of this species are among the largest of any organism, plant or animal. The information acquired from this investigation should be of considerable interest to geneticists whose investigations may be greatly facilitated by new experimental materials with this advantage. A case in point is the probable importance of target size in understanding the effects of ionizing radiation on biological systems.

This investigation will supply information concerning chromosome number, structure, and behavior in this species by tracing the detailed chromosome behavior in meiotic and mitotic divisions, and by a detailed karyotype analysis.

REVIEW OF LITERATURE

The genus Fritillaria is widely distributed throughout Europe, Northwest Africa, temperate Asia, and Western North America (1, 30). Fritillaria atropurpurea Nutt. has been found only in North America, where, according to Beetle (1), it is found in all of the western states plus the Dakotas and Nebraska.

The number of species in the genus is reported by Turrill (30) as about 100, a number he feels will remain fairly constant; for while he admits some new species may yet be discovered, he feels some of the present species may be reduced to synonomy by taxonomists. Mehra and Kachroo (19) however, are taxonomically conservative and suggest that 50 species is a more reasonable estimate. The number of North American species described by Beetle (1) in her monograph is 17, plus two varieties. Two additional species, F. roderickii described by Knight (15) and F. gentneri described by Gilkey (14), have been reported since the publication of Beetle's work.

Darlington and Wylie (11) list chromosome counts for 44 different species of Fritillaria. Counts on five

additional species (2, 3, 20, 21, 23) were found in subsequent publications. The diploid number in most of the species is 24 (1, 4, 11, 13, 19). However, in F. ruthenica and F. nigra (11, 19) the diploid number is reported as 18. These two species are classified by Darlington and Wylie (11) as old world species. Noda (20, 21) working with species of Fritillaria in Japan has found two species, F. amabilis and F. japonica var. japonica, each having a diploid number of 22. The diploid number for F. falcata has been given as 24 (1, 11), but LaCour (17) reports examination of a plant with 25 chromosomes. Darlington and Wylie (11) report the diploid number for F. pudica as 26, while Beetle (1) and Turrill (30) give the number as 24 in some plants and 26 for others. Chromosome counts from pollen mother cells of F. atropurpurea have been reported by Bottino (2) and Cave (4). Both authors report 12 bivalents.

Polyploidy, although not common, is present in the genus. Triploid members of nine species have been reported (1, 4, 11, 29, 30), including triploid members of F. pudica having 39 chromosomes. One tetraploid plant of F. lanceolata has been collected by Stebbins in San Mateo County, Cali-

fornia, and reported by Beetle (1). This particular plant was four feet tall and occurred in a colony of diploid plants two and one quarter to three feet tall.

As it has already been pointed out, most species of Fritillaria that have been studied have a diploid chromosome number of 24. This number is considered to be the normal number in the genus by Beetle (1), Darlington (8, 11), Fogwill (13), and Mehra and Kachroo (19). Two types of chromosomes are distinguishable: two pair consist of long chromosomes with median centromeres, referred to as V chromosomes by Darlington (8) and Noda (20, 21), and as M chromosomes by Fogwill (13); the remaining 10 pair consist of short chromosomes with terminal to sub-terminal centromeres, referred to as I chromosomes by Darlington (8) and as S chromosomes by Fogwill (13). In this respect, Fritillaria chromosomes resemble those of Lilium (9, 13). Chromosomes from these two genera are among the largest known, having a length of up to 24 microns (7). Cave (4) reports the meiotic chromosomes of Fritillaria as being the largest in the Liliaceae family, and among the largest in the plant kingdom. She also reports a measurement of a mitotic metaphase chromosome in a pollen grain of a F. lanceolata-recurva hybrid of 34 microns.

Mehra and Kachroo (19) report secondary constrictions in the long arms of two pair of short chromosomes with subterminal centromeres in F. cirrhosa. Noda (20, 21) has observed secondary constrictions in five pair of chromosomes in F. amabilis, in two pair in F. japonica var. koidzumiana and in four pair in F. japonica var. japonica. Noda (21) further noted a variation in the number and position of secondary constrictions in chromosomes of F. japonica var. koidzumiana from different locations. The majority of plants showed karyotypes possessing two pair of satellited chromosomes, while in several plants from a location called Hokuriku, there was found a third pair of satellited chromosomes. Also, the satellites in one pair were at a slightly more distal position than in plants collected from other locations. In F. japonica var. japonica, aside from the presence of secondary nucleolar constrictions in four pair of chromosomes, an anucleolar constriction was found frequently in either homozygous or heterozygous condition in one pair of chromosomes.

In addition to the normal A-chromosomes, many species of Fritillaria contain one to 12 centric fragments or B-chromosomes. That fragments and B-chromosomes are the same thing is made clear by Ostergren (24). Many other

terms have been used to refer to these unusual chromosomes, but the terms "B-chromosomes", "accessary chromosomes", and "supernumerary chromosomes" are the most widely used. Ostergren (24) points out that "fragment chromosomes" is not an appropriate term because it leads to the misunderstanding that they have originated from the normal complement by simple fragmentation, a view which can be demonstrated to be incorrect. For this paper, the term "B-chromosomes" shall be applied to these extra and unusual chromosomes.

The western American species F. pudica, and F. recurva are reported by Beetle (1) to have B-chromosomes, and La Cour (17) reports two B-chromosomes in F. falcata. Snow (26) observed two, four, six and eight B-chromosomes in pollen mother cells of F. biflora, and Cave (4) reports observing B-chromosomes not only in F. pudica and F. recurva, but also in F. liliacea, F. agrestis, F. phaeanthera, and F. pinetorum. Neither Cave (4) nor Bottino (2) observed any B-chromosomes in F. atropurpurea. In addition, Darlington and Wylie (11) reports one to two B-chromosomes in the California species F. pluriflora. They also list three old world species, F. nigra, F. imperialis, and F. obliqua as having from zero to 12 of these chromosomes.

Noda (20, 21) reports zero to six B-chromosomes in F. amabilis, F. japonica var. koidzumiana, and F. japonica var. japonica, species present on the Japanese islands.

B-chromosomes are not only present in many plant species, they have also been reported in many animal species, limited however to flatworms and insects (18).

Reports (9, 10, 12, 24, 25) indicate that B-chromosomes are usually smaller than A-chromosomes, that they do not pair with the A's, but may pair with each other, and that they vary in number from population to population, from individual to individual, and sometimes even from cell to cell within an individual organism. Their presence cannot be detected in the external form of the plant, and they appear to be unnecessary to the organism or cell in which they are found. They do possess a centromere, but it may be weak and less functional than the centromere in A-chromosomes according to Darlington (10), and Ostergren (24). They also usually contain considerable heterochromatin.

Dawson (12) points out that a large number of B-chromosomes can be accumulated in an individual organism without affecting the phenotype. In maize, up to 34 per nucleus are sometimes found, but it is only when more than 15 are present that there is a noticeable loss of vigor and

fertility. Darlington (9) reports that large numbers of B's in Sorghum purpureo-sericeum lead to extra mitoses of the vegetative nucleus of the pollen and embryo-sacs. Swanson, Merz and Young (28) report that serious genetic disturbances result from B-chromosomes in Plantago coronapus, in that all individuals carrying them are male sterile.

While Darlington (9) thinks B-chromosomes are derived from A-chromosomes, other workers (24, 28) report their origin as simply unknown. But while their origin is unclear, once they are present they seem to have genes for their own survival. Ostergren (24) even suggests that they may be parasites, utilizing their genes to insure survival. An example of this is preferential fertilization in corn, a phenomenon discussed by several authors (9, 12, 24). This means that of the two male gametes in each pollen tube, the gamete containing the B-chromosomes unites with the egg more often than expected statistically, some 60 times for the male gamete with the B's to only 40 times for the other gamete.

Darlington (9) thinks B-chromosomes must be of some use to the organism, or else he feels natural selection would have eliminated them. Ostergren (24) does not agree

with this. He feels they are of no use to the organism, but that they are "useful to themselves" and hence maintained in the population.

The behavior of B-chromosomes at meiosis is usually irregular. As pointed out earlier, they rarely synapse with A-chromosomes, but they do sometimes pair with themselves (25). Dawson (12) points out that in many species, the B's fail to divide synchronously with the A's. Because of this, their segregation into gametes is usually not regular and predictable. They are often lost at this point. Darlington (10) also states that B's are often lost during meiosis. He attributes this to weak centromeres present in these chromosomes. Ostergren (24) points out that B's are not always lost during meiosis. In Anthoxanthum, a grass genus he has studied extensively, the B's pair among themselves, and are only rarely lost during meiosis. He also points out that B's are rarely lost in the genera Sorghum and Godetia. He attributes this to the B's having centromeres only mildly inferior to those of the A's. Ostergren (24) has also obtained detailed information on the behavior at meiosis of a single B-chromosome in Anthoxanthum. At anaphase of the first division, the B univalent, like normal univalents, may either be included in one of the

polar groups, or may lag between them. They differ from normal univalents in that lagging B's never divide at first anaphase. They remain doubled, manifesting both chromatids. This lagging B univalent may later be included in one of the two normal nuclei, or it may form a small nucleus by itself. It does divide normally at second anaphase. Lacadena (16) reports two kinds of B-chromosomes in rye. The original B he calls a sub-telocentric, and it is about one-half the size of the normal chromosomes. A misdivision of the centromere of this normal B gives rise to another type of B he calls a B-isochromosome. Samejima (25) has observed three different kinds of B-chromosomes in Lilium medeoloides. He has designated them as E, e, and ee. Type E has a subterminal centromere with an arm length ratio of 1:5. Type e is smaller than type E, with a nearly terminal centromere. Its length is about twice the short arm of the type E. Type ee is characterized by a median centromere, with the length of each arm about equal to the length of an e type B-chromosome. When different sizes of B's are known for the same plant, Ostergren (24) reports that the smaller ones are more irregular in behavior during cell division than the larger ones.

Although B-chromosomes have been reported in many species of Fritillaria, detailed descriptions and behavior of them has been somewhat limited. Snow (26) has observed two, four, six, and eight B's in pollen mother cells of F. biflora. He noted some variation in the number of B's in pollen mother cells within the same bud, and further observed them to always be paired at metaphase I. The B's did not congress to the metaphase plate with the other bivalents, but instead were found to lie off in the cytoplasm above or below it. Lagging of the B's sometime occurred at anaphase I. Separation of a pair of B's sometimes occurred at anaphase I, with one or both chromosomes being included in a nucleus. Sometimes, however, a pair were included in a nucleus without separation taking place. Because of lagging and irregular separation, a nucleus at anaphase may receive from none to as many B's as the pollen mother cell contained. The B's he observed were all found to possess a subterminal centromere, but he points out that it is perhaps less efficient in divisions than the centromeres of the A-chromosomes. Snow is convinced that these chromosomes are not products of centromere mis-division. He feels that whatever their origin, they are the result of a single primary event of the past. He does not know

if the B's he observed in F. biflora are the same as those in other species of Fritillaria. He thinks B's may be present throughout the entire genus, in some species frequent, in others rarer. Their role in the population dynamics and evolution of the species, he feels, remains to be determined.

Noda (20) has found three different kinds of B-chromosomes in F. amabilis, designating them as B_1, B_2, and B_3. B_1 and B_2 are both small. They differ from each other in that in B_1 the centromere is subterminal, while in B_2 the centromere is terminal. B_3 is larger in size than either B_1 or B_2 and it possesses a median centromere. Observations were made of plants from two different populations in Japan, one from Mizunase, and the other from Hiko-San. Chromosomes B_1 and B_2 were found in both populations, but B_3 was confined to a single plant from the Mizunasi population. Noda observed a significant difference in the B-chromosome frequency between the two populations. In another study, Noda (21) found Fritillaria japonica var. koidzumiana to contain B_2 chromosomes, while F. japonica var. japonica was found to contain B_2 and B_4 chromosomes. The B_4 chromosomes occurred only in F. japonica var. japonica,

where they were present in about one-half of the plants examined. The B_2 chromosomes observed in both varieties of F. japonica appeared to be similar to the B_2 chromosomes observed in F. amabilis.

Due to the large size of the chromosomes in the genus Fritillaria, many species have been used in cytological and cytogenetic research work. While no significant irregularities of the mitotic cell division have been reported, there are some distinctive features of the meiotic division in Fritillaria species that deserve review. Darlington (6, 7, 8) has studied some 25 species of Fritillaria, some of which are Old World species, and others represent New World species. In all of the species he has studied, he has noted a lack of complete pairing of the homologous chromosomes at zygotene. Some bivalents would show complete pairing while others in the same nucleus would not. The degree of pairing ranged from about 50% complete in some to around 95% complete in others, the degree depending upon the species studied. He noted that pairing begins at the centromeres, and continues along to both ends of the bivalent, with the unpaired regions usually occurring at the distal ends. He also observed however, some intermittent areas where for some reason pairing occasionally would

not occur. Darlington has also pointed out a great variation in the location of chiasmata along the length of the bivalents in different species within the genus. In some species the chiasmata are localized to a greater or lesser degree around the centromere, while in others, they are positioned evenly along the entire length of the bivalent. He feels this localization of chiasmata is related to the degree of pairing between the chromosomes making up the bivalents. He (6) has grouped various species into three different groups, depending upon the degree of chiasmata localization, and the completeness of pairing between the homologous chromosomes at zygotene. In one group, there is no localization, and pairing is 95% complete. Some members placed in this group include F. imperialis, F. pallidiflora, F. eggeri, and F. pudica. In another group localization of chiasmata is intermediate, and pairing between homologous chromosomes is 75% complete. This group includes F. verticillata, F. obliqua, and F. oranensis. The last group is characterized by extreme localization, and pairing that is only around 50% complete. Examples from this group include F. acmopetala, F. pontica, and F. meleagris.

Darlington (5) reports the mean chiasmata frequency per bivalent in F. imperialis to be between 2.58 and 4.96.

He further notes that in the various species of Fritillaria that he has studied, the chiasmata frequency per bivalent was between 3 and 5. Fogwill (13), working with F. meleagris, reports from one to four chiasmata per bivalent. She further notes a higher chiasmata frequency in embryo sac mother cells than in pollen mother cells. She feels this could be due to the larger nuclei, or slower development of the embryo sac mother cells. In 1968, Noda (22) observed bivalents in pollen mother cells of F. amabilis, in which no chiasmata were distinguishable. Synapsis of homologous chromosomes occurred during early prophase I, and although no chiasmata were formed, the bivalents were maintained in parallel up to early anaphase I. He reports that while this has been observed in various insects, he thinks this was the first case reported in plant material. He noted that chiasmata were present in the embryo sac mother cells of the same plants, the mean frequency in these cells being 2.45 per bivalent at metaphase I.

Negligible terminalization has been reported by Darlington (5, 6, 7, 8) in all members of the genus he has worked with. While he admits that the position of certain chiasmata may change slightly, due to a small degree of repulsion, and hence some terminalization, he feels none of

the chiasmata are lost before anaphase I. If this is the case, the number counted at this stage will be the same as would be observed at diplotene. Also, there would be no great difference between diplotene and diakinesis, the most pronounced change probably being greater condensation of the bivalents at diakinesis. In the achiasmate bivalents Noda (22) observed in F. amabilis, the only repulsion between the two members of a bivalent was a small opening out localized in the region of the centromere. He points out that this amounts to suppression of the stages from diplotene through diakinesis.

Darlington (6) has also noted a short interphase between the two meiotic divisions in the species he has worked with.

MATERIALS AND METHODS

A large natural population of plants growing on a north facing hill at the mouth of Green Canyon near the Utah State University campus provided most of the material for this study. All of the buds used for the meiotic study came from this population, as did much of the seed and many of the root tips used for the mitotic study and the karyotype analysis. In addition, seed collected from populations in Humboldt and White Pine counties in Nevada as well as root tips collected from plants in Elko County Nevada were used for the mitotic study and the karyotype analysis.

Meiotic divisions were observed from pollen mother cells. Buds were collected from young plants during the early spring and placed in a fixing solution. If the buds were to be utilized within two to three weeks of their collection, a fixing solution composed of three parts absolute alcohol and one part acetic acid was used. Newcomers fixing solution was used for buds that would remain in fixative for a longer period of time. Slides for examination were made by the aceto-carmine anther squash technique. An anther is removed from a bud, and excess

fixative is drained off on an absorbent paper towel. The anther is then transferred to a small drop of aceto-carmine stain on a slide. The anther is then cut transversely and the two cut ends are placed together. The pollen mother cells are carefully pressed out with the flat edge of a scapel, and the empty sacs are discarded. The pollen mother cells are mixed around in the stain to facilitate staining, and a cover slip is applied.

It was found that the pollen mother cells are undergoing meiosis when the anthers are three to four millimeters in length.

Representative plates of each of the stages of meiosis were photographed.

Root tips for mitotic study were obtained from actively growing naturally occurring plants, and from germinated seeds. The first attempt at obtaining root tips involved digging up young plants, transplanting them into pots, and moving them into the greenhouse under conditions thought to be conducive to rapid growth and cell division. After remaining in the greenhouse for a few days the plants would be carefully tapped out of their pots and root tips collected. This method had been successfully used earlier with grasses, but it failed to produce actively dividing root tips with

this plant. Moderate success was obtained by digging up a young plant, being careful to leave ample soil around the roots, and then carefully removing the soil either by hand or by soaking in water. Root tips were then collected as they were discovered, and while they did not show profuse mitotic activity, enough divisions could be found to make examinations of them worthwhile.

A much more successful method of obtaining root tips was found by germinating seed. At first many difficulties were encountered in getting the seed to germinate. Not only was a stratification period necessary, but the seeds required cold temperatures in which to germinate. Germination was made possible by mixing seed in moist peat moss in a plastic bag, and then placing the loosely closed bag in a refrigerator and leaving it there at 0 degrees centigrade for approximately 60 days. Under these conditions about 75% of the seed would germinate. However, if the seedlings were then transferred to the greenhouse they would fail to grow, and in three to four days they would die. The method of handling that gave the greatest success was to place the seedlings in very wet soil, and leave them for two to three days at 4 to 5 degrees centigrade. Root tips collected showed good mitotic activity.

For the karyotype analysis, root tips were placed in .1% colchicine solution for 12 hours and then transferred to fixing solution. The same fixing solutions discussed earlier in connection with buds were used. Aceto-carmine root tip smears were made by softening them in equal parts 95% alcohol and concentrated HCl for one to two minutes, washing in 50% alcohol and then squashing in a drop of aceto-carmine. The best metaphase plates were drawn with the aid of a camera lucida and careful measurements made of the chromosomes with the aid of ocular and stage micrometers under oil immersion lens. Good flat plates were also photographed.

A composite idiogram was prepared on the basis of 17 camera lucida plates, and photographs.

A series of photographs depicting the different stages of mitosis was also made from root tip squashes. For this work, root tips were placed directly in fixing solution, omitting the colchicine treatment. Slides were made using the feulgen technique, and the best plates were photographed.

RESULTS AND DISCUSSION

Karyotype Analysis

Fritillaria atropurpurea Nutt. has 12 pair or 24 somatic chromosomes ranging in length from 12.64 to 19.11 microns, and having a width of 2.50 microns. These figures are based upon slides made of root tip material that had been treated with a .1% colchicine solution for 12 hours. The size and number of chromosomes made it impossible to obtain plates from untreated material showing enough separation between chromosomes for accurate measurements to be made. Two pair of chromosomes have median to sub-median centromeres, while the remaining 10 pair all have sub-terminal centromeres. Of the 10 pair with sub-terminal centromeres, two pair have secondary constrictions and satellites. In addition to the 24 A-chromosomes, some plants have one to two B-chromosomes. The study of mitotic divisions used to prepare this karyotype revealed many plants having one B-chromosome, and measurements on it are included in the karyotype results. The subsequent study of meiosis in this

species revealed many additional plants with two B-chromosomes. In these plants, both B-chromosomes observed appeared to be very similar in size.

The length of the individual chromosomes, arm ratios and total chromosome length, of the 12 pair of A-chromosomes, and the one B-chromosome, are summarized in table 1. Numbers have not been assigned to the chromosome pairs because some of them have very similar measurements and arm ratios and it is not always possible to distinguish between them. The table is arranged by the over-all length of the chromosome pairs.

The two pair of chromosomes having the longest length possess median centromeres. These characteristics make them very easy to distinguish. The two pair of satellited chromosomes are also readily distinguishable. Of the remaining eight pair, the longest pair can be distinguished on the basis of length and centromere position which is very nearly terminal. The shortest of the eight pair can also be determined most of the time on the basis of their length and nearly terminal centromere position. The remaining six pair are all very similar in length and centromere position and it is not always possible to distinquish between them. Their total length shows a gradual decrease,

Table 1. Karyotype analyses of 17 camera-lucida plates of the chromosomes used in preparing composite idiogram of Fritillaria atropurpurea. Lengths are in microns, and based upon root tip material treated in .1% colchicine solution for 12 hours. Measurements on the B-chromosome are based upon 6 plates, each having one B-chromosome present.

Total Chromosome Length	Length Long Arm	Length Short Arm	Ratio Long/Short Arm
19.11	11.34	7.77	1.45
18.65	11.81	6.84	1.73
16.00	14.60	1.40	10.43
15.63	13.23	2.40	5.51
14.53	12.20	2.33	5.24
13.58	11.69	1.89	6.19
13.48	10.66	2.82	3.78
13.40	11.15	2.25	4.96
13.16	9.73	3.43	2.84
12.69	10.14	2.55	3.98
12.69	10.14	2.55	3.98
12.64	11.66	.98	11.90
B-Chromosome			
5.42	3.85	1.57	2.45

and they all have sub-terminal centromeres with similar locations. The similarity of these chromosomes presented a problem in determining measurements for the karyotype. Another difficulty encountered was the variation in measurements between different plates. For example, the longest pair of chromosomes has an average length of 16.00 microns, with individual measurements running from 13.66 to 18.30 microns. Similar variations were experienced for the other chromosome pairs. The following may account for this: 1) All plates measured may not have been at exactly the same stage. It is difficult to distinguish between late prophase and metaphase. Also, there may be a difference in chromosome length at different points in metaphase. 2) There may be some inconsistencies in chromosome measurement. The chromosomes are measured using an ocular micrometer and an oil immersion objective. Because chromosomes are rarely straight, difficulty arises in measuring curved or bent chromosomes. Thus, the more an arm of a chromosome is bent the harder it is to measure, and the greater the possibility of error. 3) The amount of pressure put on the cover slip in making the aceto-carmine smears probably varies from slide to slide. On a slide that has a great amount of pressure the chromosomes will be in a flatter

plane and hence will show less undulating of the chromosome arms. This would perhaps show a slightly longer length than one that has not had as much pressure applied. 4) There may be a difference in length due to a difference in differential coiling of chromosomes. Two chromosomes that have the same total chromonemata length, but different differential coiling, will not have the same total chromosome length. Stebbins (27) has suggested this as an inconsistency in total chromosome length. 5) A difference in the effect the colchicine may have on shortening the chromosomes. Although the colchicine concentration, and the duration of treatment were the same for all material used, it is not certain that all chromosomes were equally affected by it.

No irregularities were encountered in any of the stages of mitosis from the material observed. Colchicine treated metaphase plates showed well defined chromatids.

Meiotic Chromosomes

Prophase I of meiosis occurs with all of the stages represented that are diagnostic of this phase. Based upon the low frequency of prophase I plates encountered in the material studied, it would seem logical that this whole

phase must occur relatively rapidly, with no appreciable delays. The most difficult stage to obtain plates of was diakinesis. Of the great number of slides prepared of meiotic material, less than a half dozen showed diakinesis.

Pairing of homologous chromosomes seems to be nearly complete in this species. Darlington (6, 7, 8) has noted a lack of complete pairing in all the Fritillaria species he has studied, the degree of pairing ranging from about 50% complete in some species to about 95% complete in others. Fritillaria atropurpurea would fit in the latter group. Many plates were observed in which pairing was 100% complete for all 12 bivalents. Occasionally plates would be encountered showing one and sometimes two bivalents with no pairing on one side of the centromere.

Darlington has also noted a great variation in chiasmata location in the species with which he has worked, with many species showing various degrees of localization. Fritillaria atropurpurea does not show localization of chiasmata, and in this respect is like F. imperialis, F. pallidiflora, F. eggeri, and F. pudica, a group in which pairing is about 95% complete, and no localization of chiasmata is present.

The number of chiasmata per bivalent ranged from one to four, with the average chiasmata number per bivalent in a complete metaphase plate ranging from 2.17 to 2.75. These figures are based upon chiasmata counts taken from 10 metaphase plates where all 12 bivalents were separated so that accurate counts could be made. The average chiasmata frequency per bivalent for all 10 plates worked out to be 2.50. These data are consistent with the chiasmata frequency reported for other species of Fritillaria by both Darlington (5) and Fogwill (13).

The number of good clear plates showing diplotene and diakinesis was somewhat limited, but of the ones obtained, no bivalents having more than four chiasmata were observed. Clearly then, virtually no terminalization occurs between diplotene and the end of metaphase I. Further evidence of this is found by observing bivalents at diakinesis and metaphase I and noting the lack of repulsion between the two chromosomes making up the bivalent. Rarely are loops of any kind found between successive chiasmata along the length of the bivalent. In fact, the two chromosomes are so close together during metaphase I that it is often difficult to determine for certain where the chiasmata are located. This condition persists right up to the beginning

of anaphase I. Again, this behavior in Fritillaria atropurpurea is consistent with Darlington's reports (5, 6, 7, 8) wherein he has noted negligible terminalization in all species he has worked with. He admits that the position of chiasmata may change slightly due to a small degree of repulsion between the two chromosomes making up the bivalent, but he feels that no chiasmata are lost before anaphase I.

As can be seen from figure 29, there is an interphase present between the two meiotic divisions.

At the end of the first meiotic division a rather thick wall is formed, separating the two members of each dyad. This wall starts to form during late telophase I, and is fully formed by prophase II. It can be readily observed during any stage of the second division. Chromosome movement in both members of a dyad are nearly always perfectly synchronized. Only very rarely was a plate observed in which the chromosomes in one member of the dyad were at a later stage than the chromosomes in the other member. When this condition was observed, usually one member was in anaphase II while the other member was still in metaphase II. During late telophase II, the second cell wall, perpendicular to the first, begins to form. This

too becomes a very thick wall. In the tetrad stage, all four microspores are visible, along with extremely thick cell walls that separate them from one another.

Based on the frequency of second division plates observed, it would appear that the entire second meiotic division takes place rapidly, with no delays. Of all the meiotic phases, metaphase I was most frequently encountered, giving the impression that there may be some short delay at this phase.

B-Chromosomes

Although a complete and thorough study of the frequency and behavior of B-chromosomes in Fritillaria atropurpurea is beyond the scope of this study, much information regarding them was obtained, and will be reported here.

B-chromosomes were found in plants from two of the four areas from which material was collected. Most of the plates showing B-chromosomes came from material collected in Green Canyon, near Logan, Utah. Many slides of root tip material showed the presence of one B, as did many meiotic slides of pollen mother cells in material taken from this area. A number of root tips taken from germinated seed

collected from plants in Humboldt County in Nevada also had one B-chromosome present. Only a limited number of seeds from plants in White Pine County Nevada, along with a limited number of root tips collected from material growing in Elko County Nevada were available for study. This material failed to show any cells having B-chromosomes present. This does not necessarily mean B's are not present in these two populations. Had more material from these areas been available, B-chromosomes may have been observed. It is also possible that had more material been observed from these and other populations, some plants may have been discovered having more than two B-chromosomes present.

The B-chromosomes observed in mitotic material corresponds to the type designated by Noda (20) as a B_1 chromosome. It has a sub-terminal centromere, and a length of 5.42 microns as opposed to 12.64 microns which is the length of the shortest of the 24 A-chromosomes. In colchicine treated metaphase plates, the morphological details of the B-chromosome are similar to those of the A-chromosomes. The centromere can be easily seen, and the individual chromatids can be readily distinguished. The only observable distinction between the B and the A-chromosomes is size. The B appears as a miniature A-chromosome.

It was not possible to follow the behavior of this B-chromosome through the entire mitotic sequence. In untreated material it was never possible to distinguish the B-chromosome from the other chromosomes in the complement, due to the number and large size of the A-chromosomes. The B was observed only in colchicine treated metaphase plates.

In meiotic plates of pollen mother cells, B-chromosomes were observed at metaphase I, and anaphase I. Again, it was not possible to follow the behavior of these chromosomes during the entire division, and they were never observed during any phase of the second meiotic division. This was probably due in part to the relatively small number of second division plates observed compared to a much larger number of first division plates studied. The B-chromosomes observed were present individually, they were never observed to be paired. They were always located very close to but off to one side of the A-chromosomes. This would suggest that their centromeres may not be as active as the centromeres of the A-chromosomes in the same cells. Lateral views of metaphase I in pollen mother cells having two B's present would sometimes show both B's on the same side of the A complement, while other plates would show one B on

one side and the second B on the other side of the complement. Similar observations were made of anaphase I plates. During anaphase I, B's were never observed lagging, but rather they seemed to precede the A-chromosomes to the poles. From these observations it is doubtful that B's are ever lost during anaphase I and telophase I. Where two B's were present on the same side of the A complement at metaphase I and anaphase I, it seems logical that they would both be included in the same member of the dyad at telophase I, while the other member of the dyad would receive none. Similarly, if one B was present on each side of the complement at metaphase I and anaphase I, it should then follow that each dyad would contain one B at telophase I.

How these chromosomes segregate during the second meiotic division, and if they are ever lost during anaphase II and telophase II could not be determined from this study.

SUMMARY AND CONCLUSIONS

Plants having large chromosomes are very valuable to the work of geneticists and cytogeneticists. In this respect the genus Fritillaria has been most useful, for its members possess some of the largest chromosomes known. While cytological information is available on a large number of species in this genus, only very limited information has been available on the chromosomes of Fritillaria atropurpurea. The purpose of this study has been to provide this information.

The material used for this study came from natural populations collected from four different areas in Utah and Nevada.

Meiotic chromosomes were observed from pollen mother cells taken from anthers between three and four millimeters in length. Buds collected were placed in either Newcomers fixing solution if they would remain in fixitive for three or more weeks, or in a fixing solution composed of three parts absolute alcohol and one part acetic acid if they would be utilized within three weeks of collection. Slides were made using the aceto-carmine anther squash technique.

Root tips for mitotic study were obtained from actively growing naturally occurring plants, and from germinated seeds. For the karyotype analysis, root tips were treated with a .1% colchicine solution for 12 hours before being transferred to fixing solution. The same two fixing solutions used for buds were also used for the root tip material. Slides were made using the aceto-carmine root tip squash method.

Photographs depicting the different stages of mitosis were made from root tips that had not been treated with colchicine. The slides were made using the feulgen method.

Fritillaria atropurpurea Nutt. has a diploid chromosome number of 24. Two of the 12 pair of chromosomes have median centromeres, while the other 10 pair have sub-terminal to nearly terminal centromeres. In addition, two of the 12 pair have secondary constrictions, and satellites. The length of the chromosomes ranges from 12.64 to 19.11 microns, with a width of 2.50 microns. These measurements are based upon slide material that had been treated with a .1% colchicine solution for 12 hours.

Present in many individual plants along with the A-chromosomes was one to two B-chromosomes. These chromosomes have sub-terminal centromeres, and are from one third

to one fourth the length of the A-chromosomes. These chromosomes are morphologically similar to the A-chromosomes and differ only in size. In mitotic material, the B-chromosomes were observed only in colchicine treated metaphase plates, and never was more than one observed in any one cell. The length of this chromosome, based upon six camera-lucida drawings, is 5.42 microns, which is less than half the length of the shortest pair of A-chromosomes. In meiotic slides of pollen mother cells, B-chromosomes were observed at metaphase I and anaphase I. Many cells had only one B present, but frequently two B's were observed in the same cell. When two were present in the same cell, they were never observed to be paired. They were always located very close to but off to one side of the A-chromosomes, suggesting that their centromeres may not be as active as the centromeres in the A-chromosomes. Additional information is needed concerning the frequency, number, behavior, and purpose of these chromosomes in the species.

During meiosis, pairing of homologous chromosomes was found to be about 95% complete. Only occasionally were cells encountered showing one and sometimes two bivalents with no pairing on one side of the centromere.

One to four chiasmata were observed in each bivalent, with the average number of chiasmata per bivalent being 2.50. No localization of chiasmata was observed in the species.

As is true with other species in the genus, there is negligible terminalization between diplotene and anaphase I. There may be a small degree of repulsion between the two chromosomes making up a bivalent, and the position of the chiasmata may change slightly, but it appears almost certain that no chiasmata are lost before anaphase I.

Very thick cell walls are formed at the end of both telophase I and II. During the second meiotic division, the thick wall separating each dyad is constantly visible, and in the tetrad stage each microspore is separated from the other three by the presence of these thick cell walls.

There is a short interphase present between the two meiotic divisions.

Fritillaria atropurpurea should prove to be a useful plant for genetic investigations, especially for workers in the Western states where this species can be found in abundance.

LITERATURE CITED

1. Beetle, Dorothy E. (1944) A monograph of the North American species of Fritillaria. Madrono 7: 133-159.

2. Bottino, P.J. (1965) Documented chromosome numbers in plants. Madrono 18: 122-126.

3. Cave, M.S. (Ed.) (1961) Index to plant chromosome numbers. University of North Carolina Press, Chapel Hill, North Carolina. 2:110.

4. Cave, M.S. (1970) Chromosomes of the California Liliaceae. University of California Press, Berkeley, California. 58p.

5. Darlington, C.D. (1930) Chromosome studies in Fritillaria III, chiasmata formation and chromosome pairing in Fritillaria imperialis. Cytologia 2: 37-55.

6. Darlington, C.D. (1935) The internal mechanics of the chromosomes. The Proceedings of the Royal Society of London. Series B. 118: 33-96.

7. Darlington, C.D. (1936) The external mechanics of the chromosomes. The Proceedings of the Royal Society of London. Series B. 121: 264-319.

8. Darlington, C.D. (1937) Recent Advances in Cytology. Second edition. The Blakiston Company, Philadelphia and Toronto. 671p.

9. Darlington, C.D. (1956) Chromosome Botany. George Allen and Unwin Ltd., London. 186p.

10. Darlington, C.D. (1965) Cytology. Little, Brown and Company, London. 768p.

11. Darlington, C.D., and A.P. Wylie (1955) Chromosome Atlas of Flowering Plants. Second edition, George Allen and Unwin Ltd., London. 519p.

12. Dawson, G.W.P. (1962) An Introduction to the Cytogenetics of Polyploids. Blackwell Scientific Publications, Oxford. 96p.

13. Fogwill, Mary (1958) Differences in crossing over and chromosome size in the sex cells of Lilium and Fritillaria. Chromosoma 9(6): 493-504.

14. Gilkey, Helen M. (1951) A new Fritillaria from Oregon. Madrono 11(3): 137-141.

15. Knight, W. (1967) A new Fritillaria from Medocino county California. Four Seasons 2:2, 14-16.

16. Lacadena, J.R. (1966) Meiotic pairing between two different types of Rye B-chromosomes, a comparative study. Anales De La Estacion Experimental De Aula Dei. 8(1-2): 168-176.

17. La Cour, L.F. (1951) Heterochromatin and the organization of nucleoli in plants. Heredity 5: 37-50.

18. Makino, Sajiro (1951) Chromosome Numbers in Animals. Second edition. The Iowa State College Press, Ames, Iowa. 290p.

19. Mehra, P.N., and P. Kachroo (1951) Chromosome morphology of some species of Lilium and Fritillaria. phytomorphology (Delhi) 1(½): 64-66.

20. Noda, Shozo (1964) Cytology in the genus Fritillaria I, Variations in karyotypes and B-chromosomes in F. amabilis. Bulletin of Osaka Gakuin University 2: 125-132.

21. Noda, Shozo (1968) Cytology in the genus Fritillaria II, Karyotypes and B-chromosomes in F. japonica var. japonica and var. koidzumiana. Bulletin of Osaka Gakuin University 10: 127-141.

22. Noda, Shozo (1968) Achiasmate bivalent formation by parallel pairing in PMC's of Fritillaria amabilis. Bot. Mag. Tokyo 81: 344-345.

23. Ornduff, R. (Ed.) (1967) Index to plant chromosome numbers. International Bureau for Plant Taxonomy and Nomenclature of the International Association for Plant Taxonomy. Utrecht, Netherlands. 59: 39.

24. Ostergren, G. (1947) Heterochromatic B-chromosomes in Anthoxanthum. Hereditas 33: 261-296.

25. Samejima, Junichico (1958) Meiotic behavior of accessary chromosomes and their distribution in natural populations of Lilium medeoloides. Cytologia 23: 159-171.

26. Snow, Richard (1959) Chromosome numbers of California plants, with notes on some cases of cytological interest. Madrono. 15: 81-89.

27. Stebbins, G.L. (1957) Discussion following Dr. Swaminathan's paper. India Journal of Genetic Plant Breeding. 17: 412.

28. Swanson, Carl P., Timothy Merz, and William J. Young (1967) Cytogenetics. Prentice-Hall Inc. Englewood Cliffs, New Jersey. 194p.

29. Takizawa, Senzi (1954) Meiosis in triploid Fritillaria camschatcensis (L). Ker-Gawler. Japanese Journal of Genetics. 30: 34-43.

30. Turrill, W.B. (1950) Character combinations and distribution in the genus Fritillaria and allied genera. Evolution 4: 1-6.

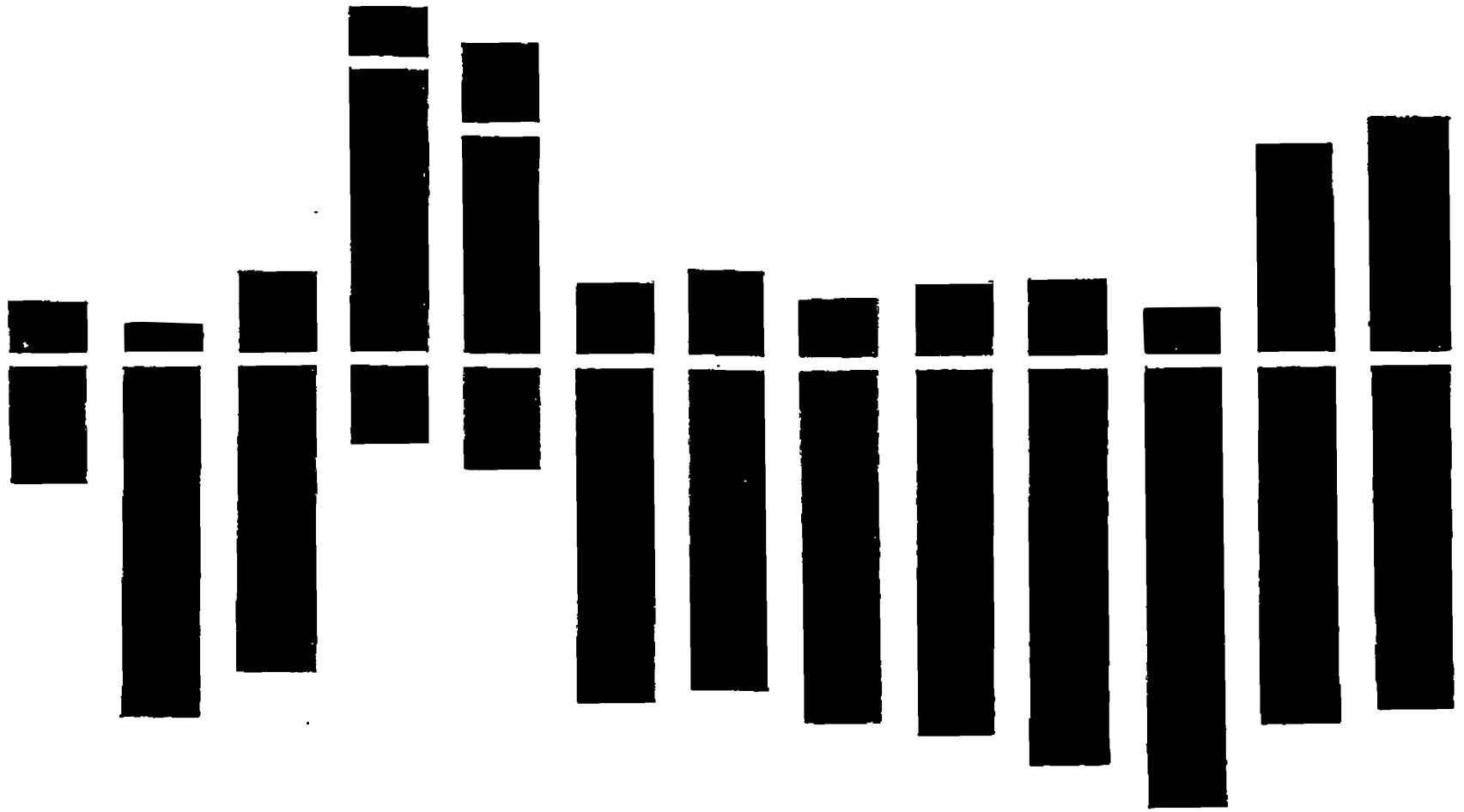

Figure 1. Idiogram of the chromosomes of Fritillaria atropurpurea including one B-chromosome. Based upon analysis of 17 camera-lucida drawings. 4400X

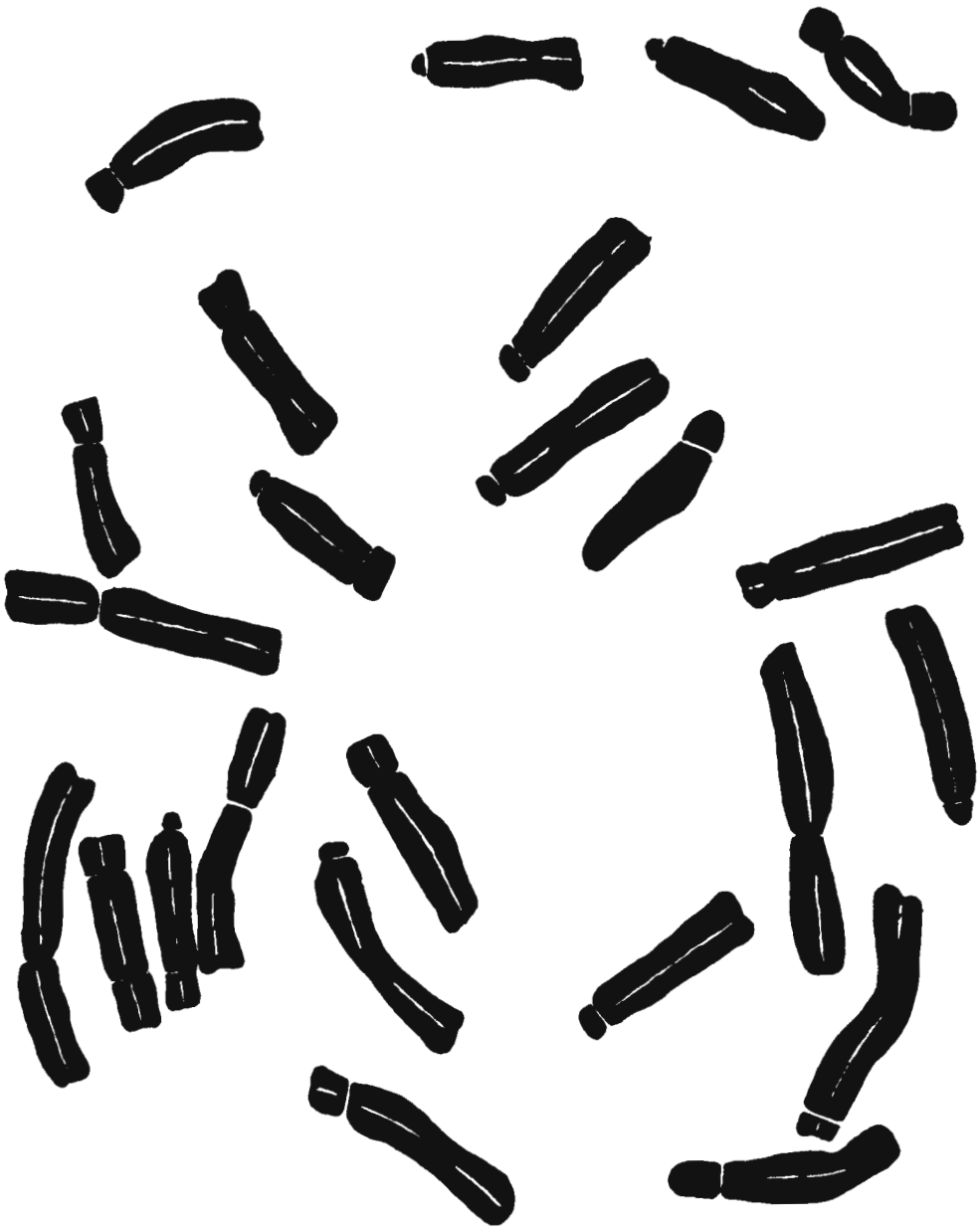

Figure 2. Camera-lucida drawing of metaphase chromosomes from colchicine treated root tip cell. 2400X

Figure 3. Camera-lucida drawing of metaphase chromosomes, including one B-chromosome, from a colchicine treated root tip cell. 1600X

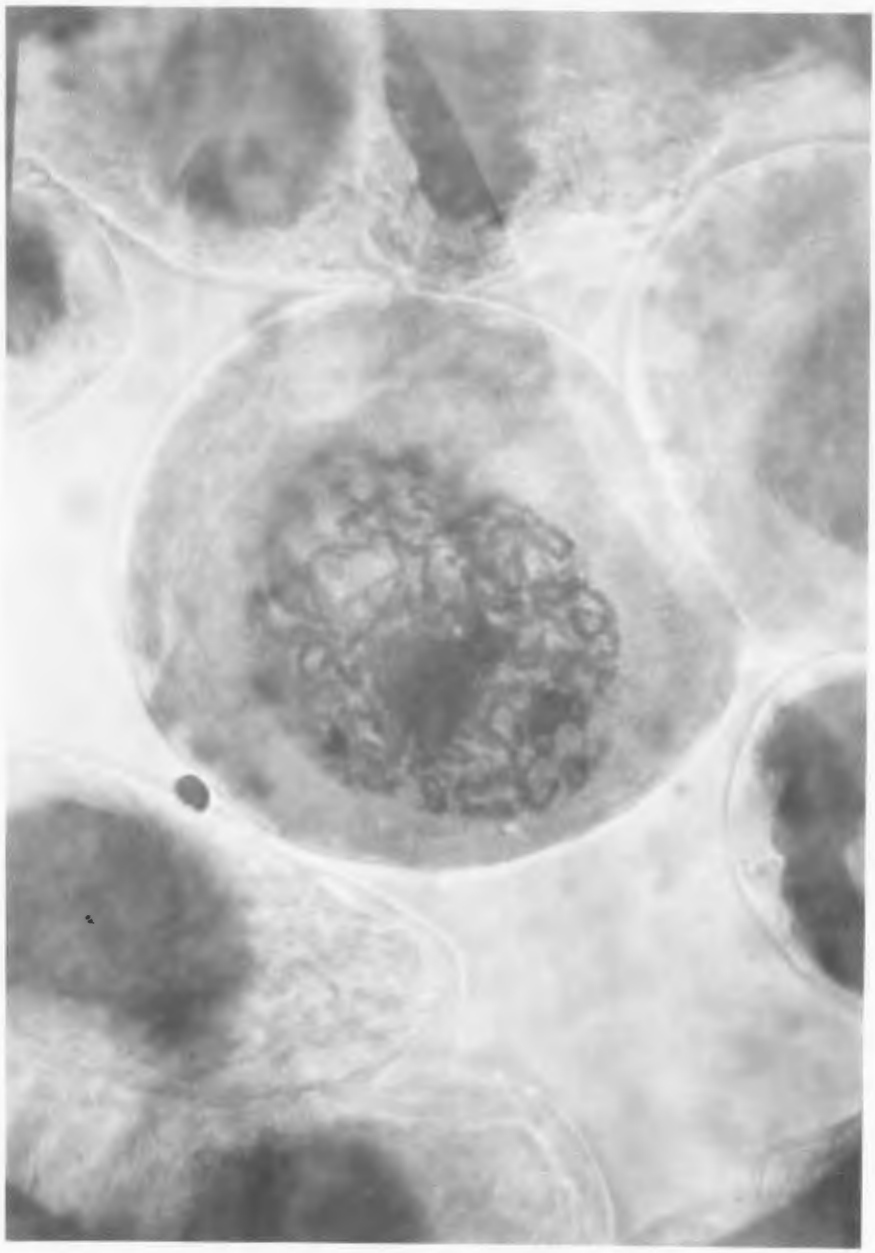

Figure 4. Prophase in an untreated root tip cell. 1500X

Figure 5. Metaphase in an untreated root tip cell. 1500X

Figure 6. Metaphase in a colchicine treated root tip cell. 1600X

Figure 7. Metaphase in a colchicine treated root tip cell. 1600X

Figure 8. Metaphase in a colchicine treated root tip cell. 1600X

Figure 9. Anaphase in an untreated root tip cell. 1500X

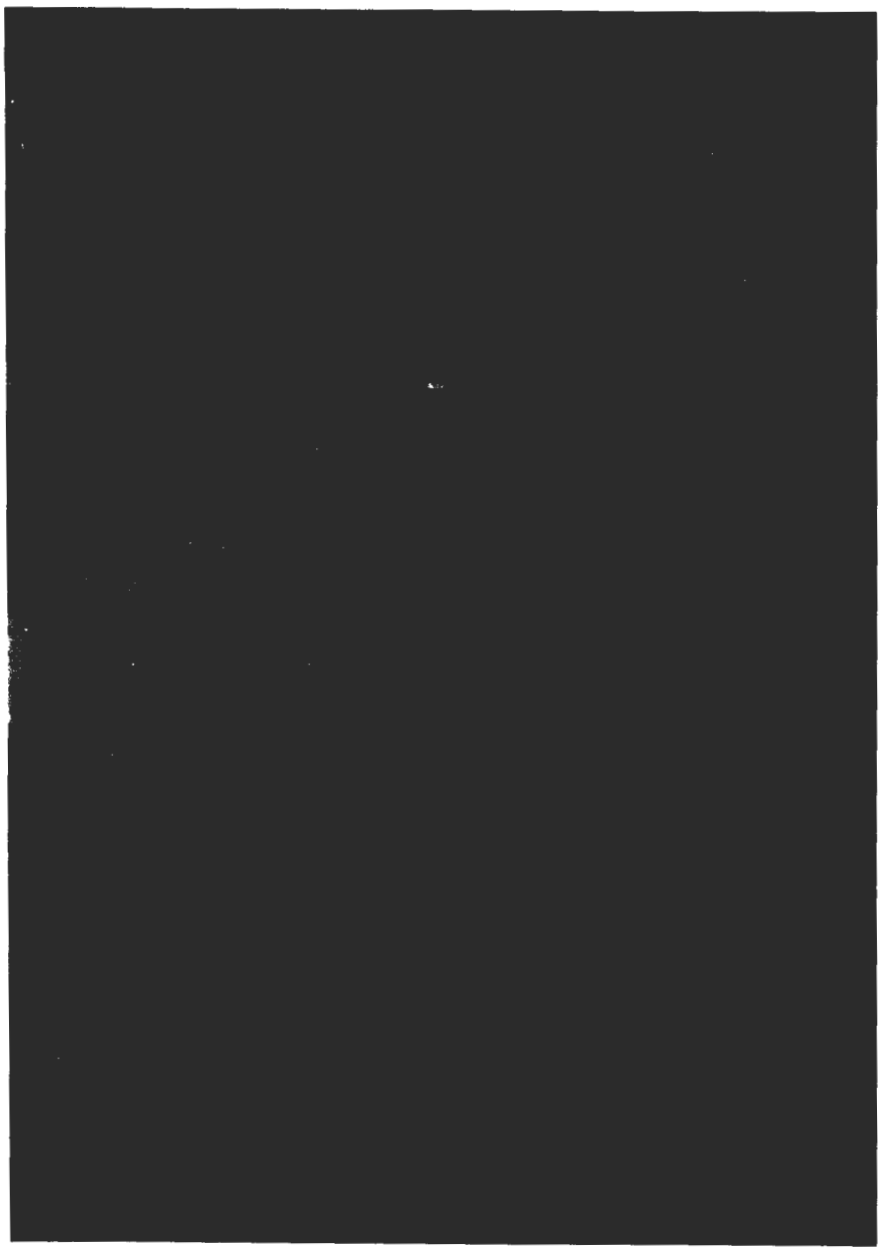

Figure 10. Anaphase in an untreated root tip cell. 1500X

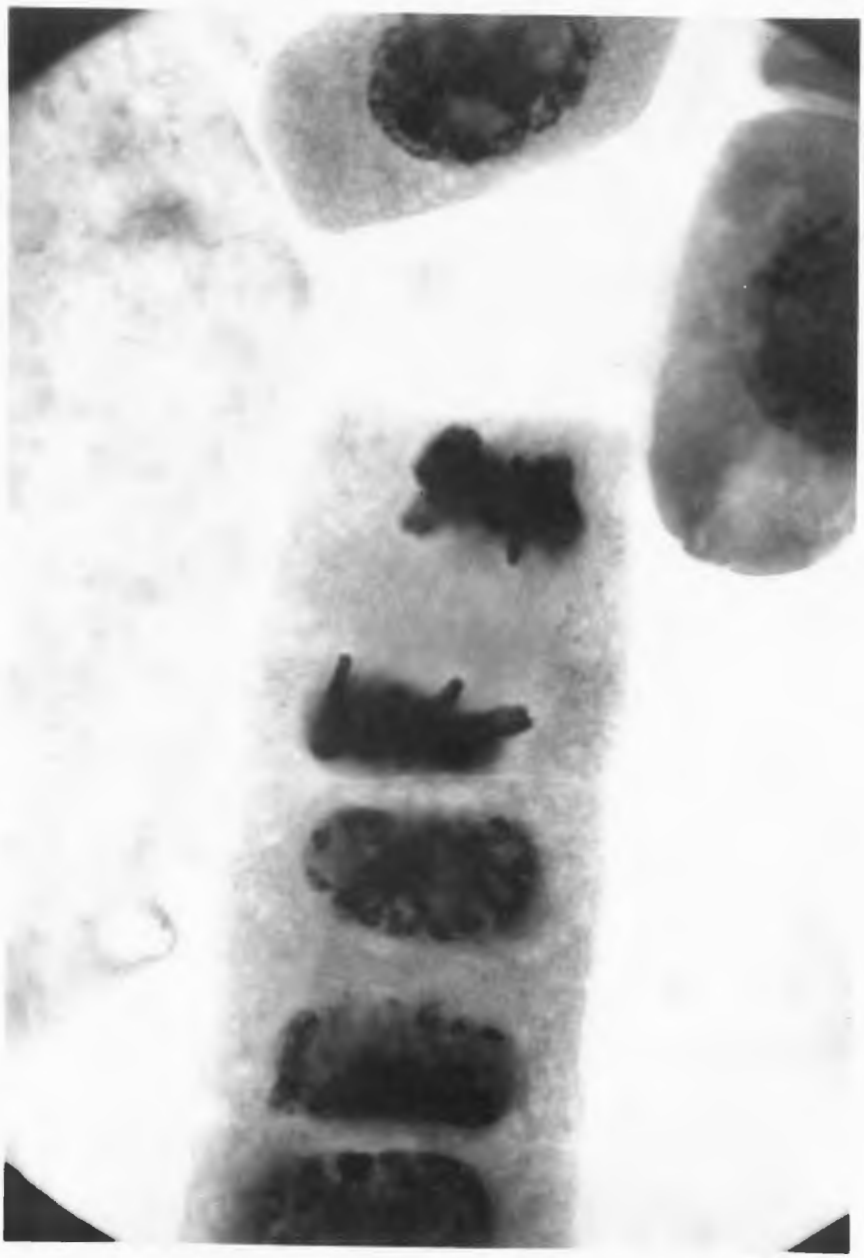

Figure 11. Telophase in an untreated root tip cell. 1500X

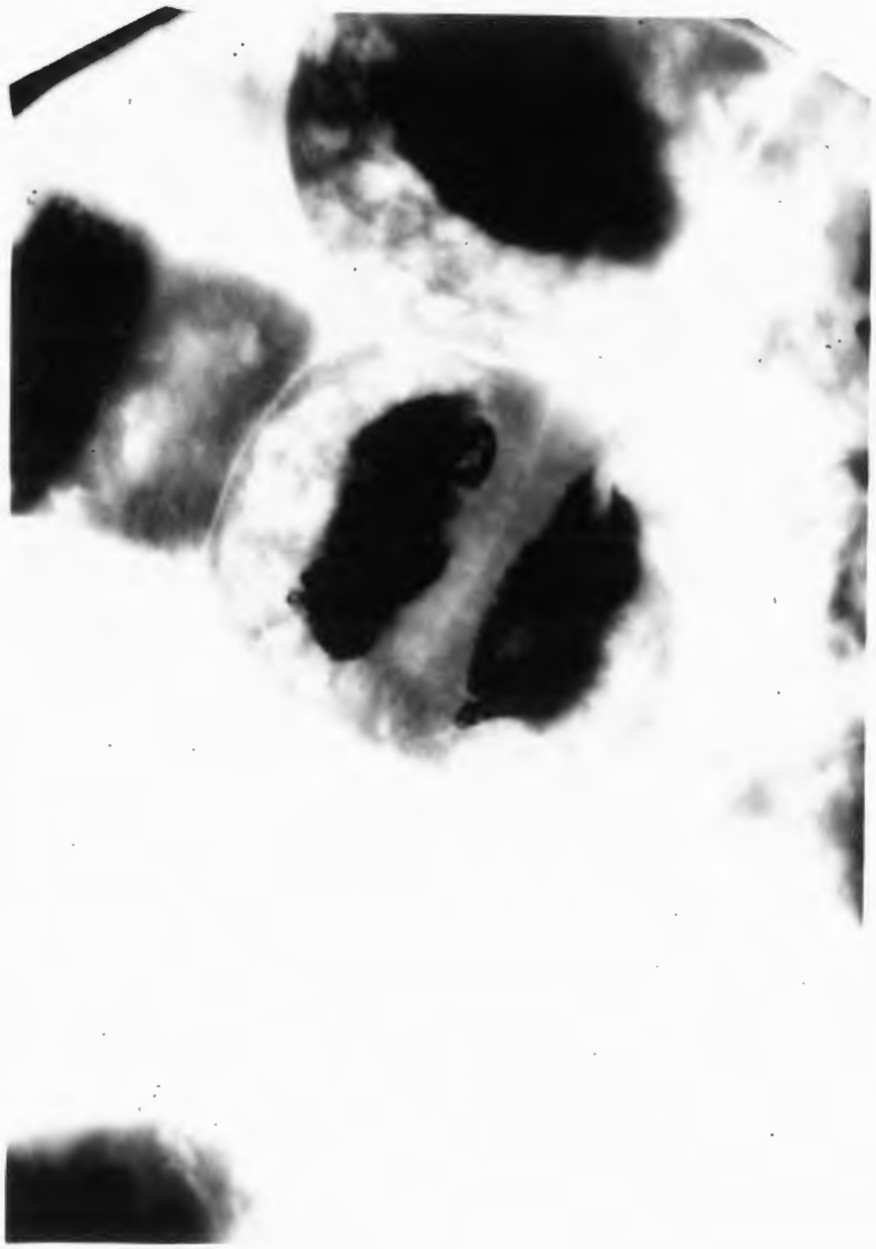

Figure 12. Telophase in an untreated root tip cell. 1500X

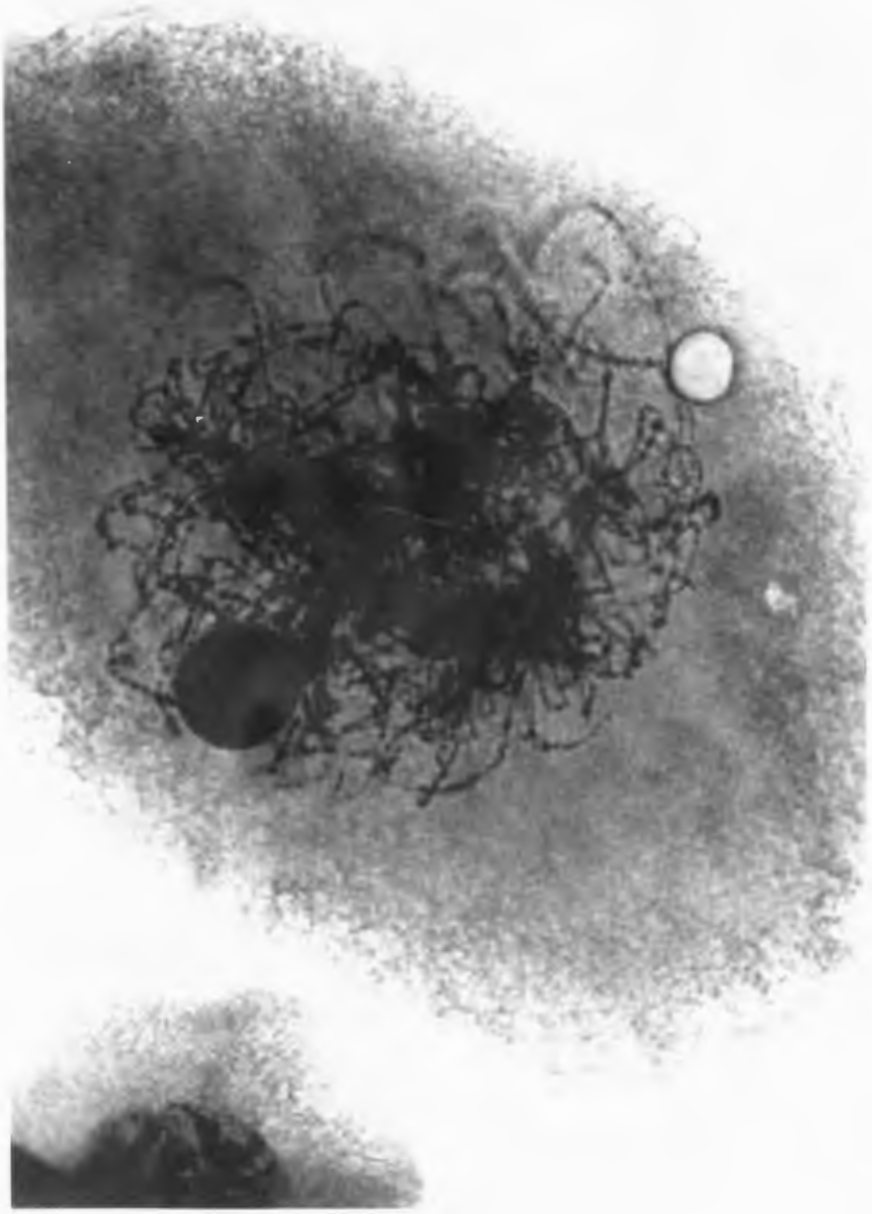

Figure 13. Leptotene in a PMC. 1500X

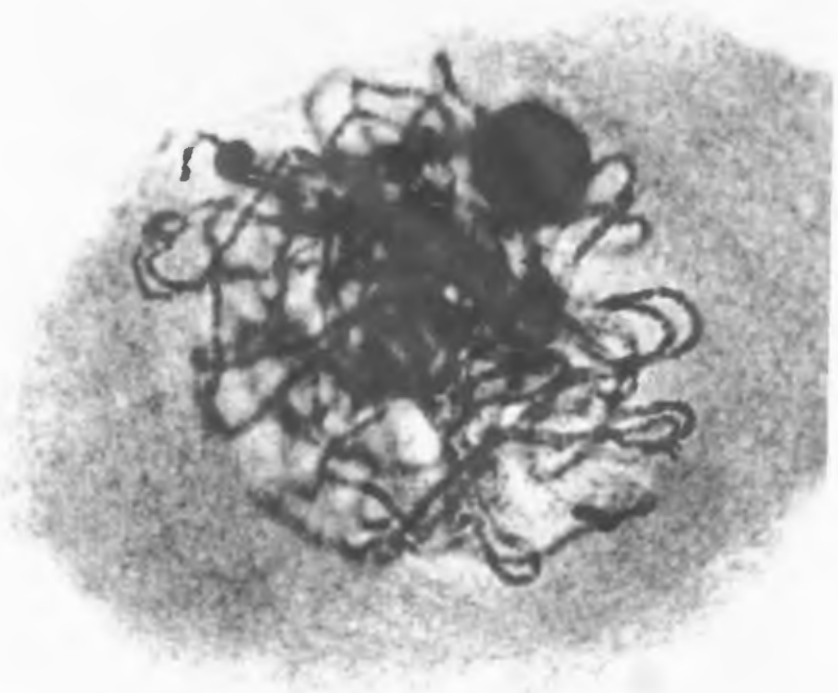

Figure 14. Zygotene in a PMC. 1500X

Figure 15. Diplotene in a PMC. 1500X

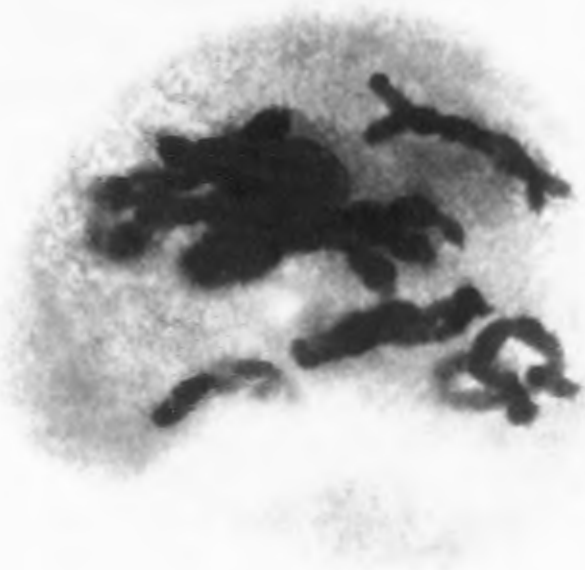

Figure 16. Diakinesis in a PMC. 1500X

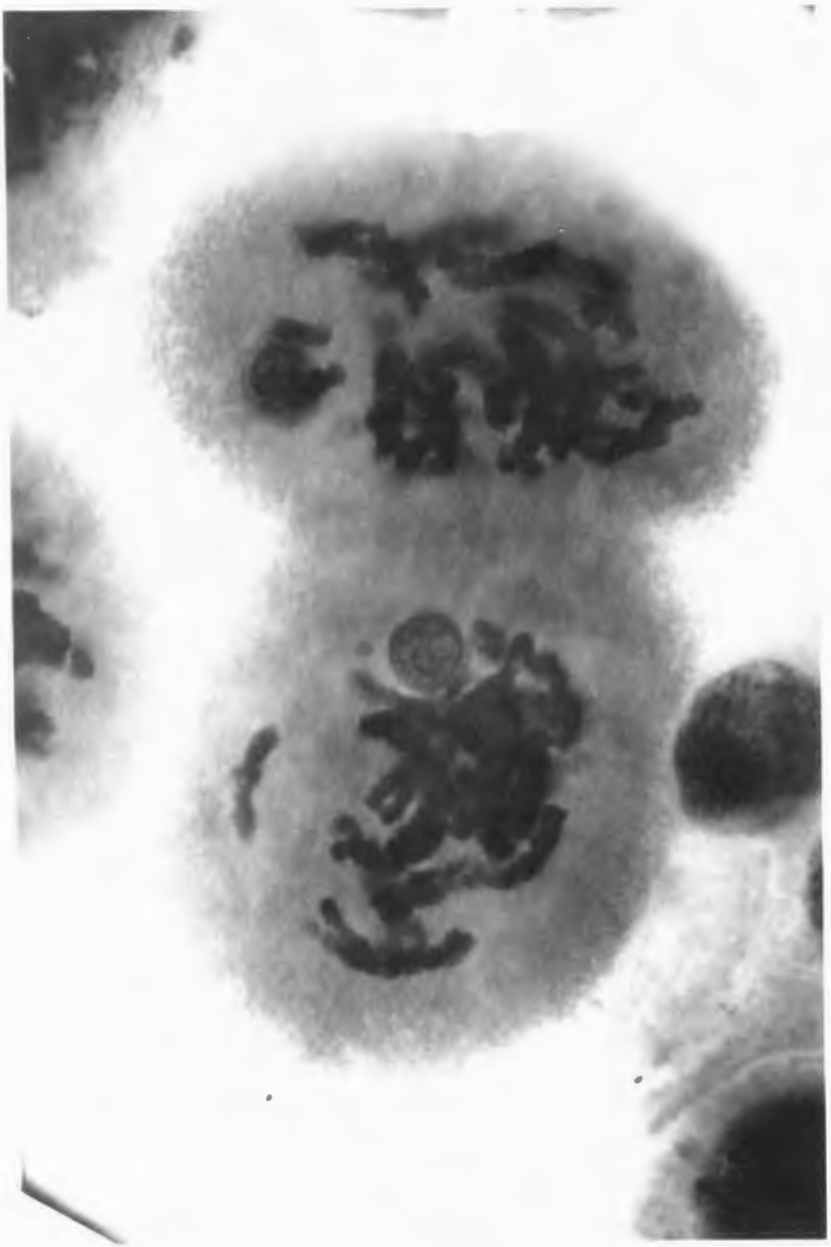

Figure 17. Diakinesis in a PMC. 1500X

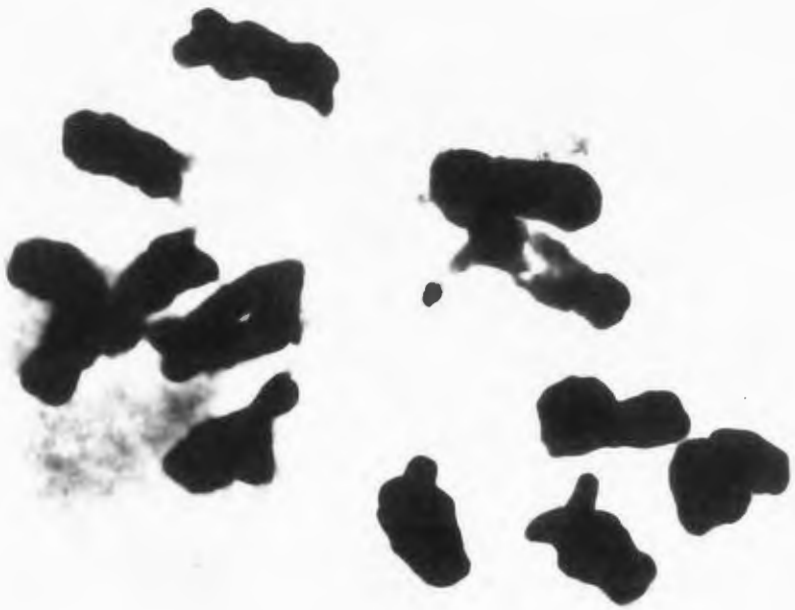

Figure 18. Metaphase I in a PMC. 1500X

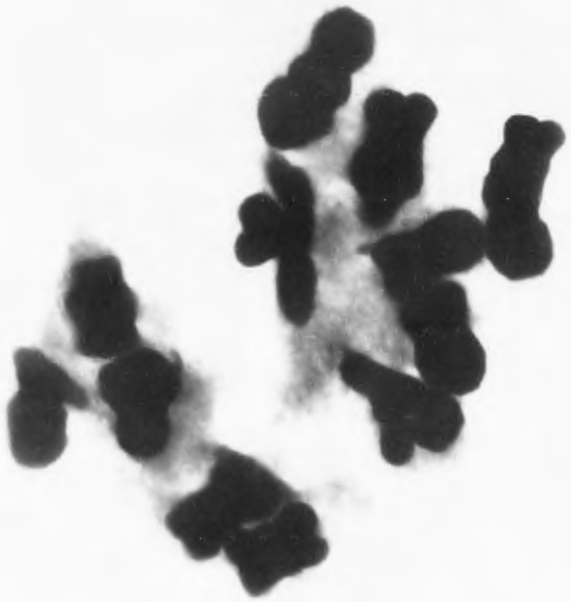

Figure 19. Metaphase I in a PMC. 1500X

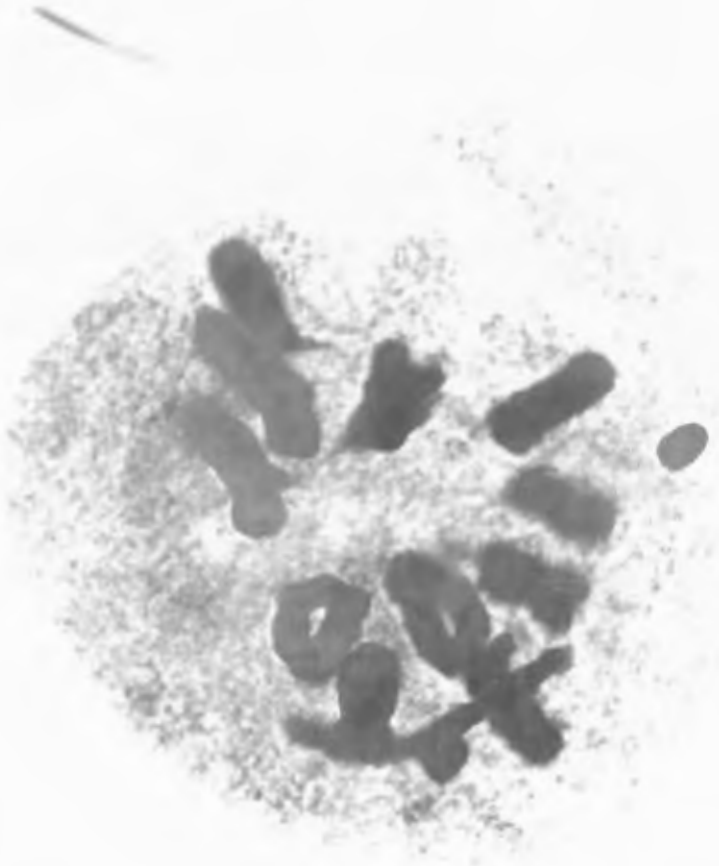

Figure 20. Metaphase I in a PMC, showing one B-chromosome. 1500X

Figure 21. Metaphase I in a PMC showing one B-chromosome. 1500X

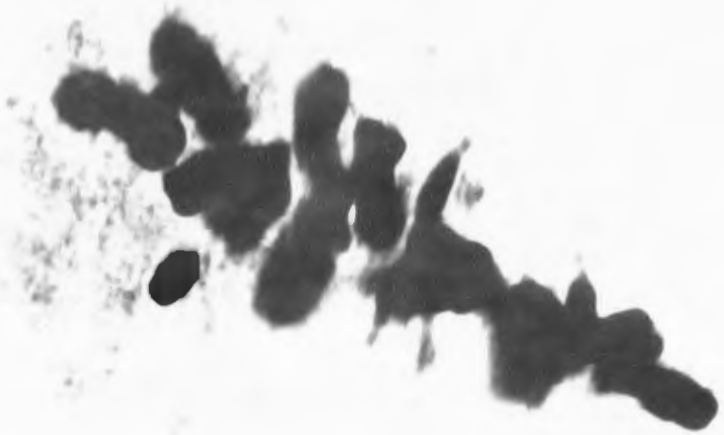

Figure 22. Lateral view of metaphase I in a PMC, showing one B-chromosome. 1500X

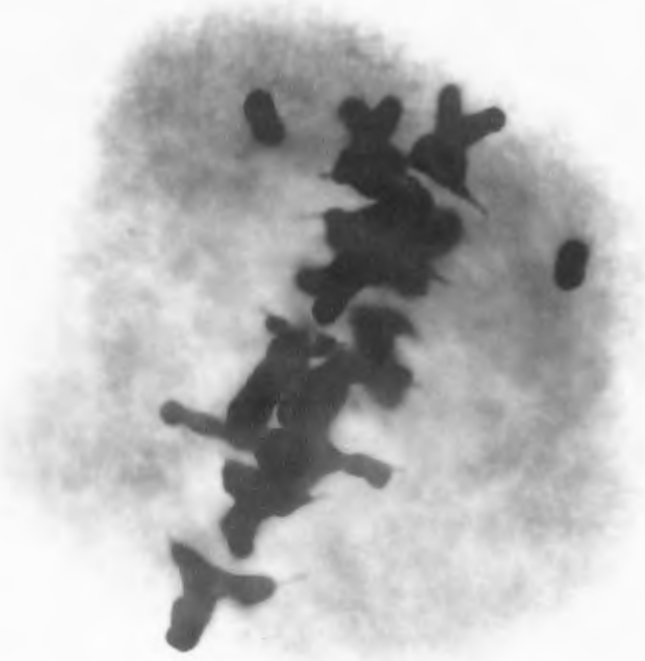

Figure 23. Lateral view of metaphase I in a PMC, showing two B-chromosomes. 1500X

Figure 24. Anaphase I in a PMC, showing two B-chromosomes. 1500X

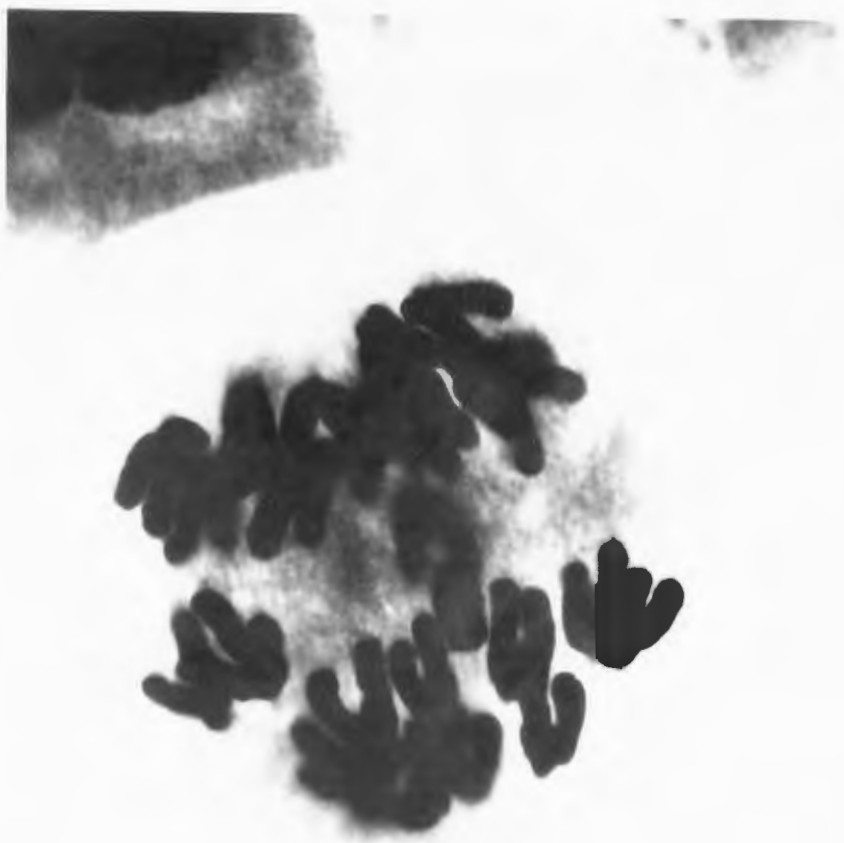

Figure 25. Anaphase I in a PMC. 1500X

Figure 26. Anaphase I in a PMC, showing one B-chromosome. 1360X

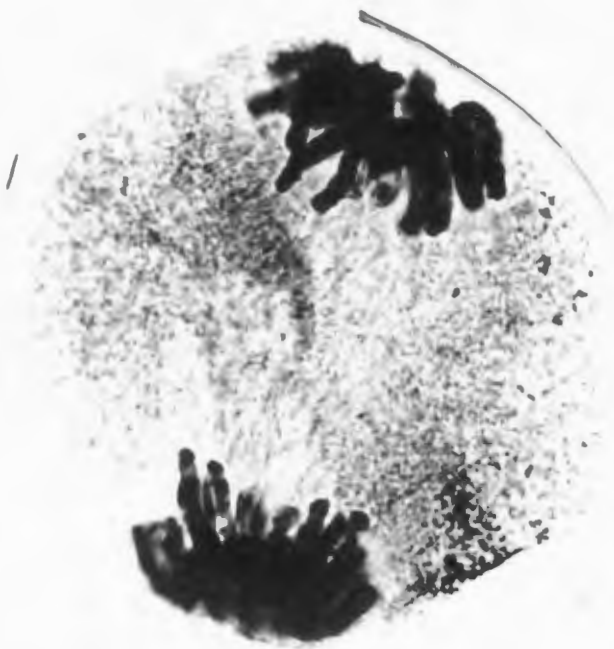

Figure 27. Early telophase in a PMC. 1500X

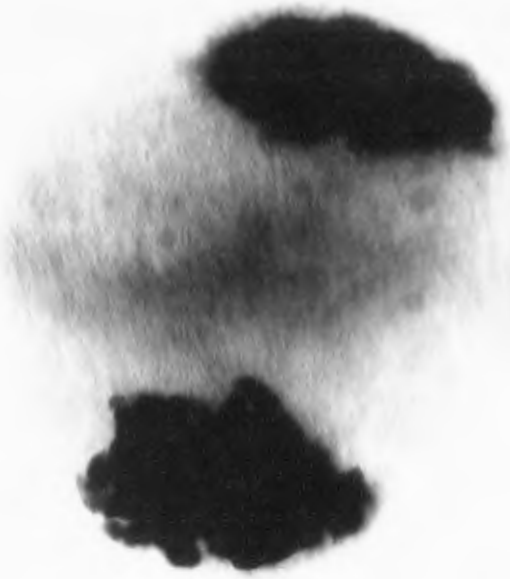

Figure 28. Telophase I in a PMC. 1500X

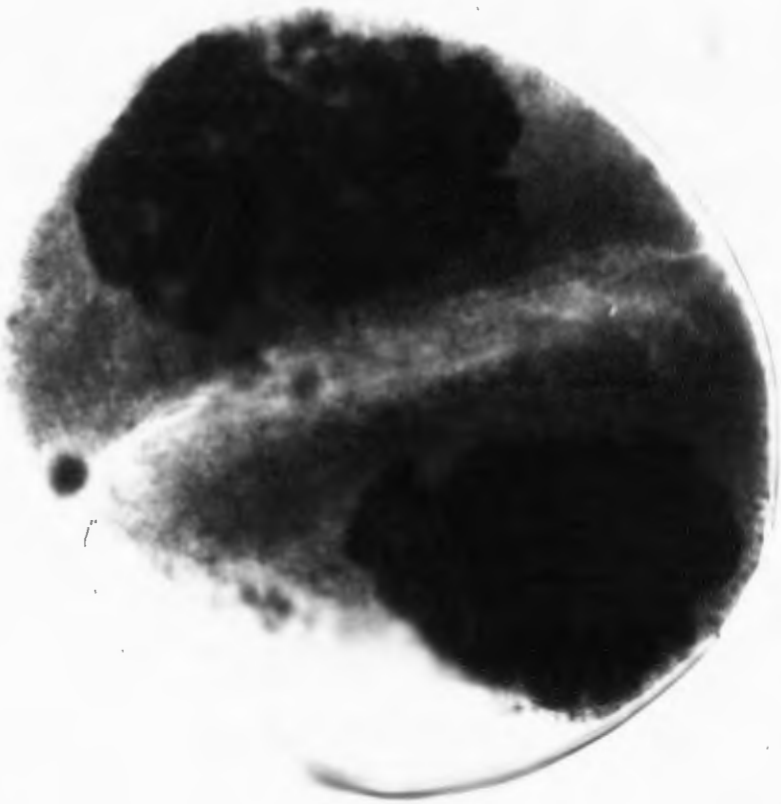

Figure 29. Interphase between meiosis I, II in a PMC. 1500X

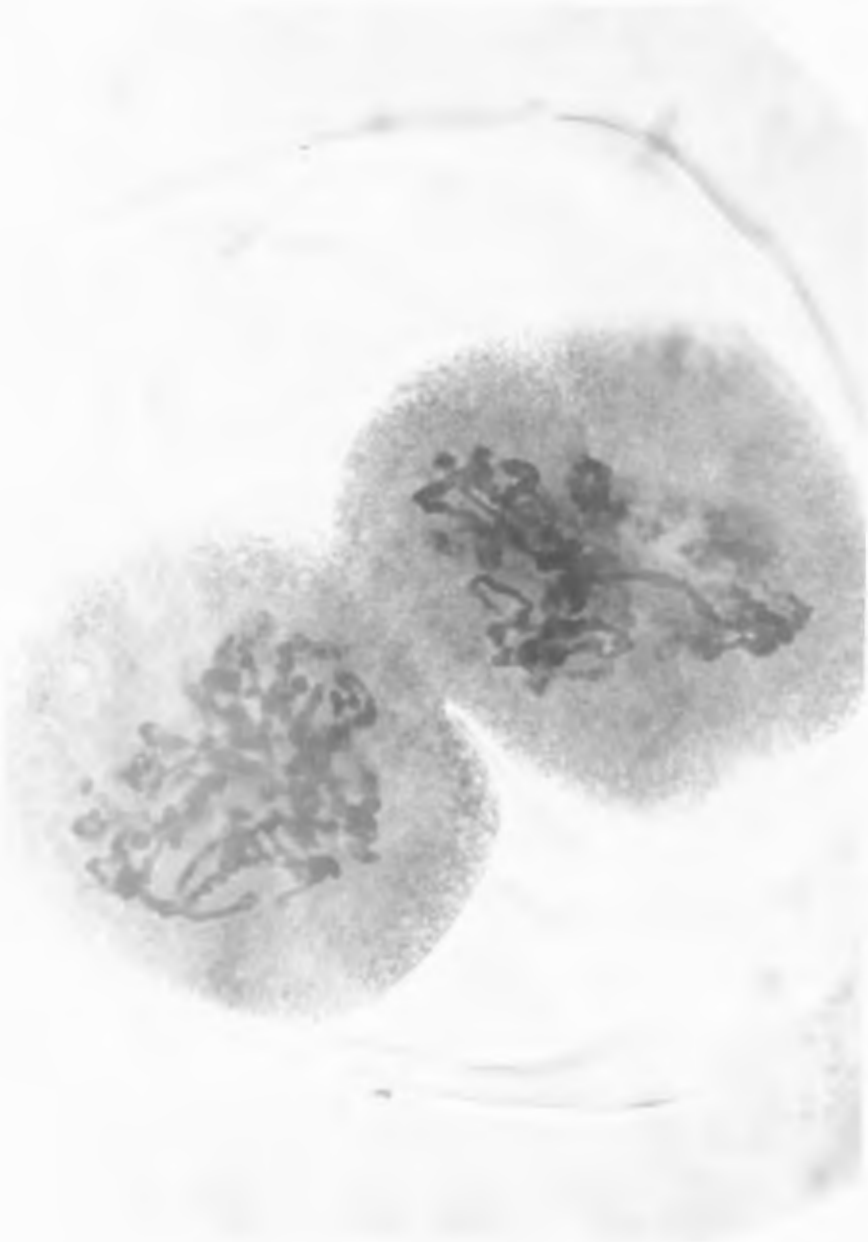

Figure 30. Prophase II in a PMC. 1500 X

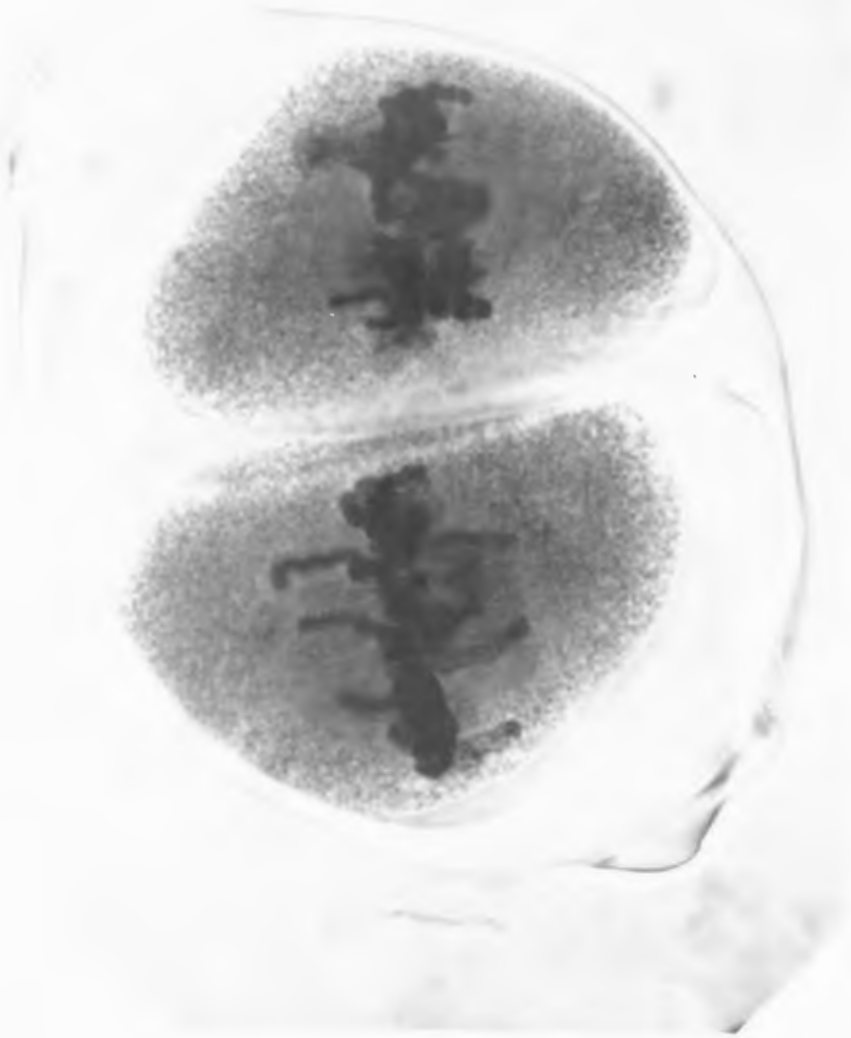

Figure 31. Metaphase II in a PMC. 1500X

Figure 32. Early anaphase II in a PMC. 1500X

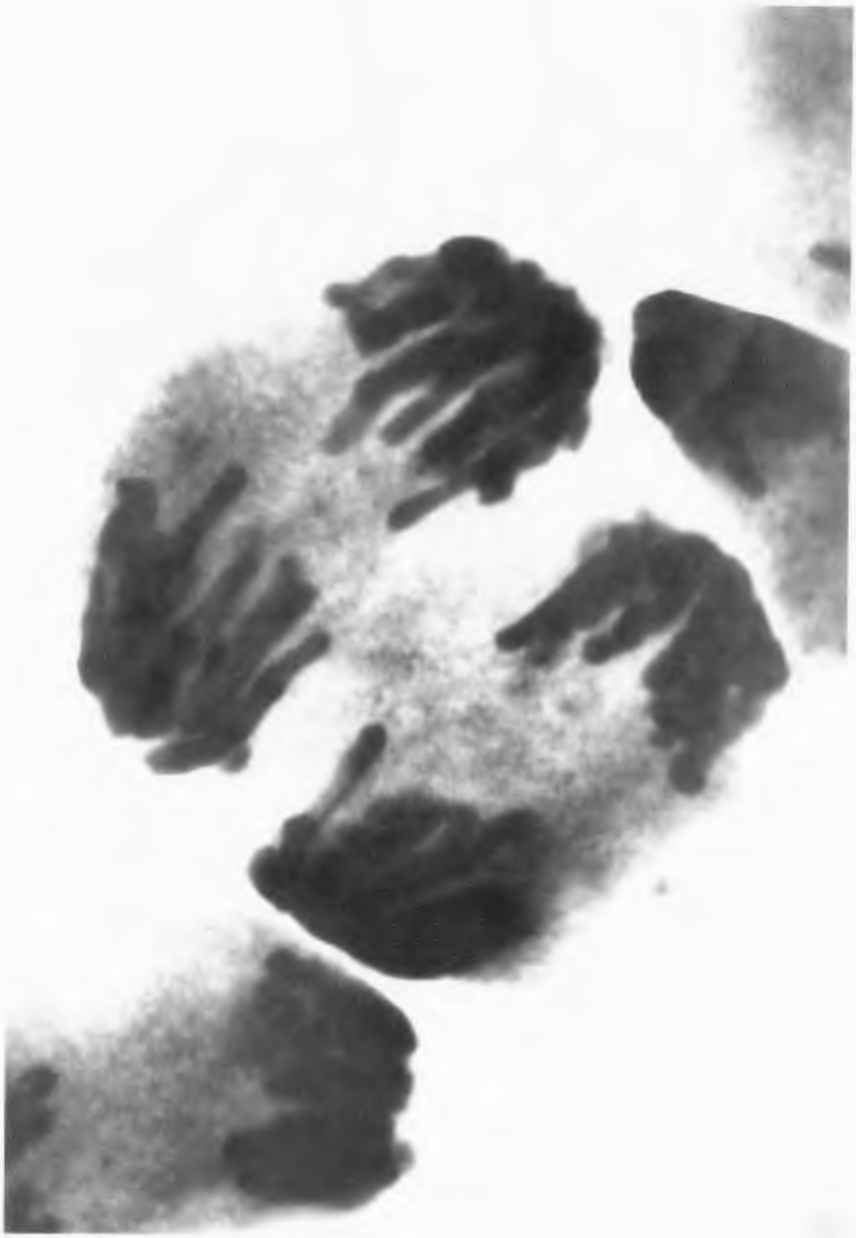

Figure 33. Anaphase II in a PMC. 1500X

Figure 34. Telophase II in a PMC. 1500X

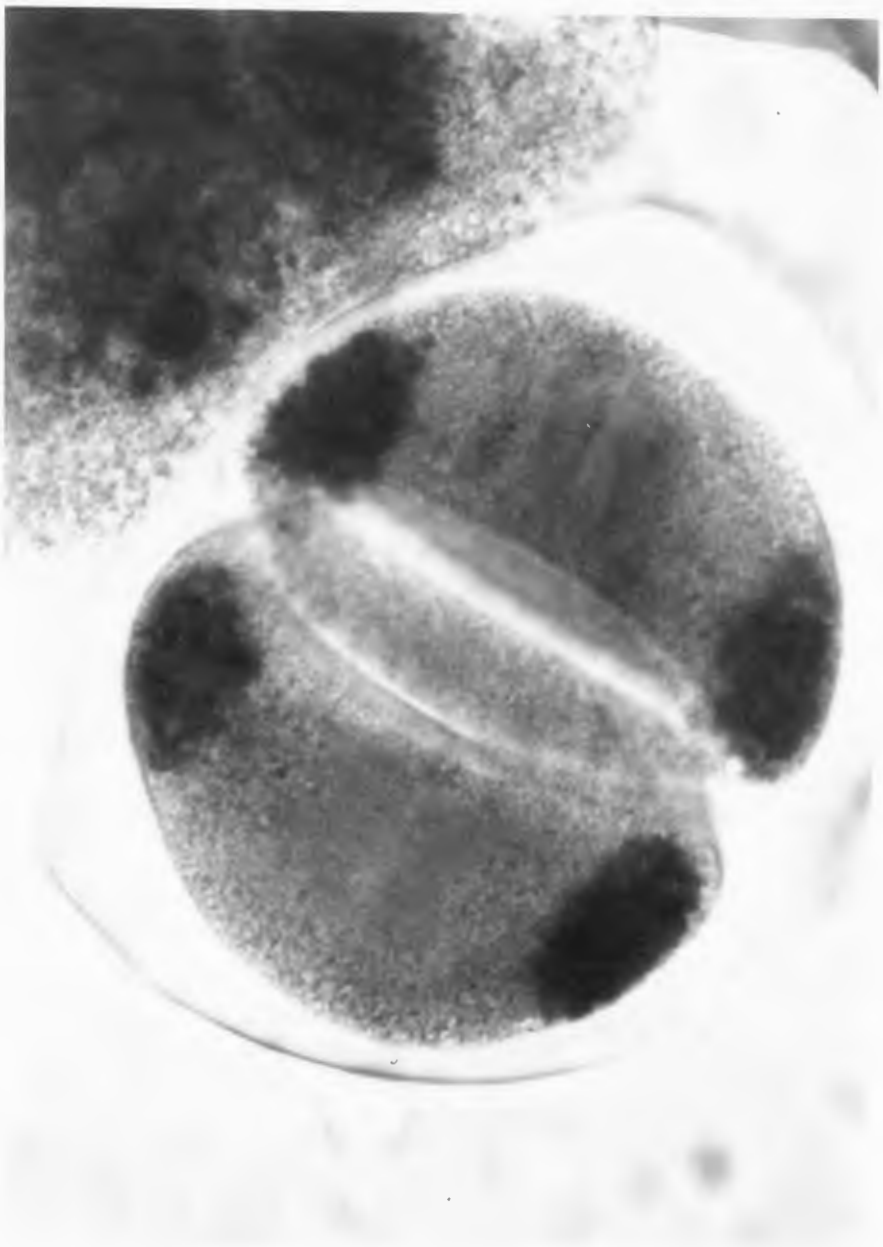

Figure 35. Telophase II in a PMC showing the beginning of cell wall formation in each dyad. 1500X

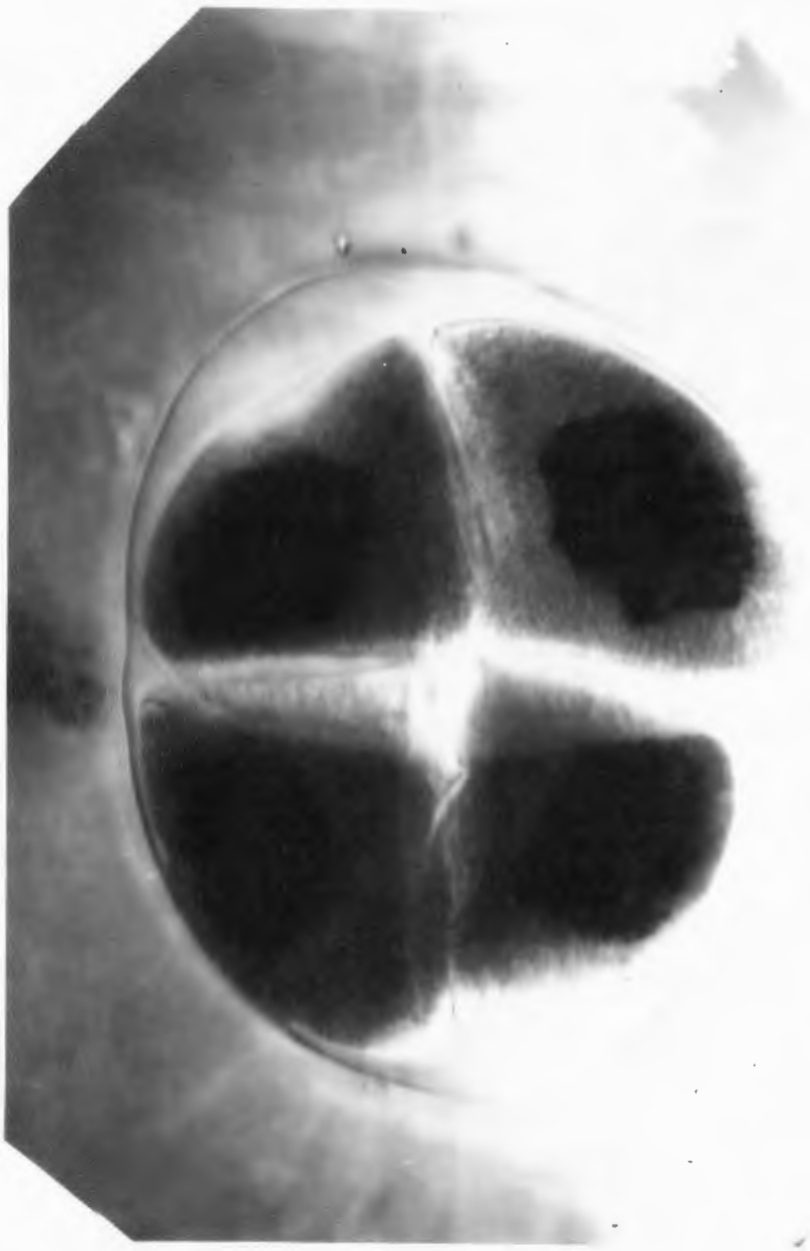

Figure 36. Tetrad stage in a PMC. 1500X

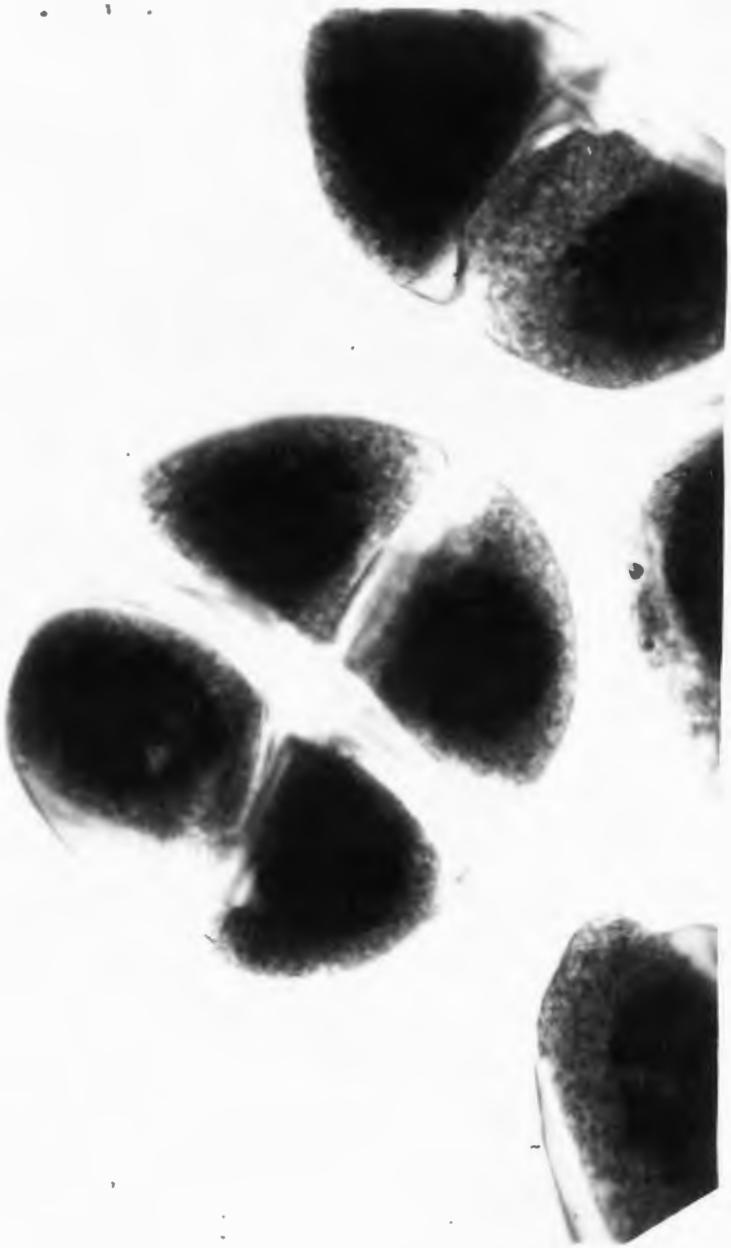

Figure 37. Tetrad stage in a PMC. 1500X

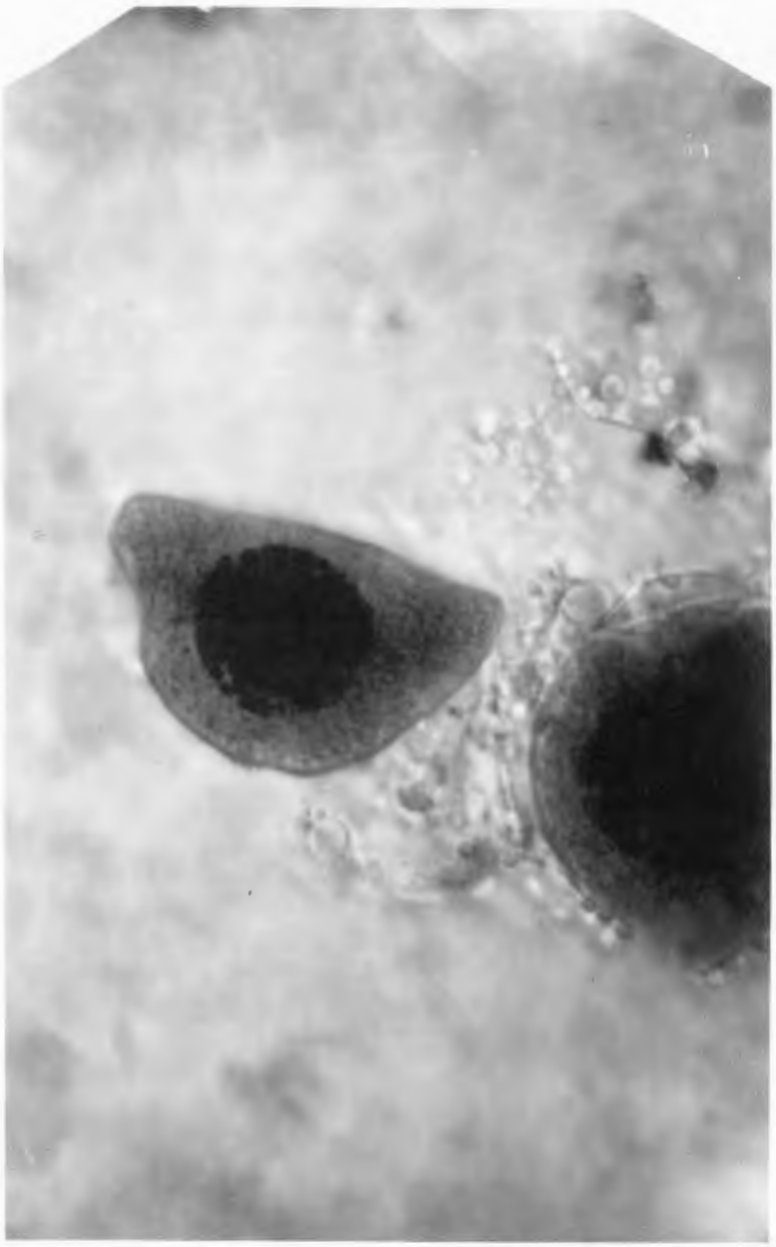

Figure 38. A single microspore after liberation from tetrad. 1500X

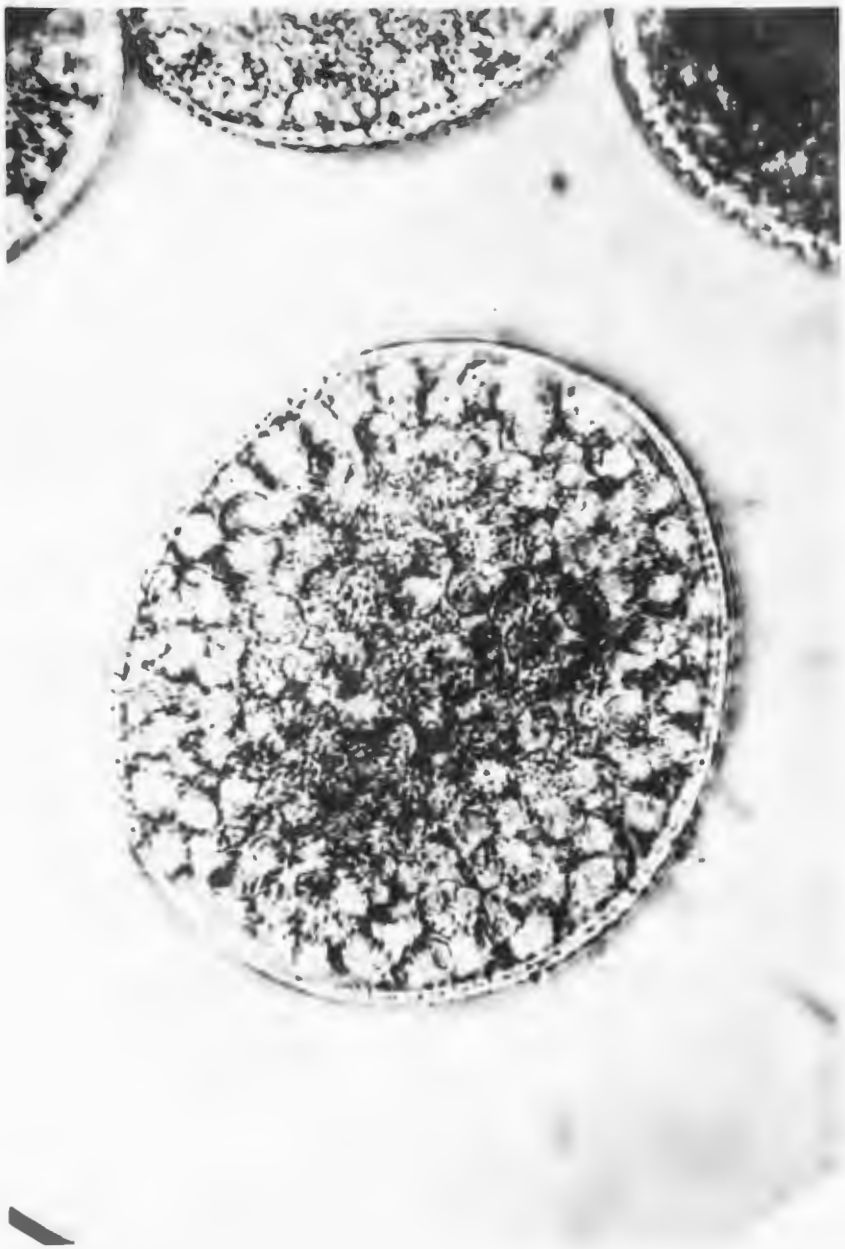

Figure 39. Mature pollen grain. 1500X