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A Cytological Study of the Induced Octoploid of an Agropyron-Hordeum Hybrid

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1955

UTAH STATE AGRICULTURAL COLLEGE

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A CYTOLOGICAL STUDY OF THE INDUCED OCTOPLOID OF AN *AGROPYRON-HORDEUM* HYBRID

by

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The writer wishes to express his thanks to Dr. W. S. Boyle for his guidance during the course of this study.

R. Bruce Ashman

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INTRODUCTION

This study is concerned with the meiotic chromosome behavior of a colchicine induced octoploid derived from a tetraploid hybrid between Agropyron trachycaulum and Hordeum jubatum.

The occurrence of hybrids, both interspecific and intergeneric, is frequent in the Gramineae generally (Stebbins, 1949), and is especially common in the tribe Hordeae of which both A. trachycaulum and H. jubatum are members. Intergeneric hybrids in this tribe have been studied by Stebbins et al. (1946a, and 1946b), Stebbins and Walters (1949), and Stebbins and Singh (1950) in an effort to determine true phylogenetic relationships on which to base a taxonomic classification, and results of these studies have indicated that the current taxonomic treatment of this tribe is highly artificial. Attempts to produce a perennial wheat and improve forage grasses has lent additional stimulus to the study of crosses in the Hordeae. An excellent discussion and literature review on these studies is given by Myers (1947).

Polyploidy has been found to occur in all plant groups to a greater or lesser extent (Stebbins, 1950). Stebbins (1949) estimates the frequency of polyploid species in the Gramineae to be about twice as high as in the Angiosperms as a whole. It is evident from this that in cytogenetic studies of hybrids in the Gramineae the frequent and complicating factor of polyploidy must be a constant consideration.

Cytological studies relating fertility to chromosome behavior in polyploids and polyploid hybrids have thus far been inconclusive. As Sears (1941) has shown from his study of hybrids and amphidiploids in

the Triticinae, meiotic irregularities where polyploidy is involved can not always be correlated with the degree of fertility. Similiar results were reported by Stebbins (1949), and led to his conclusion that accurate predictions as to fertility or sterility of a certain hybrid combination can not be made.

The particular value of the present study lies in the fact that the origin of the polyploid hybrid is known and the cytology of its progenitors is reasonably well understood. Such studies can furnish basic information regarding chromosome behavior, fertility problems, and phylogenetic relationships in polyploids in general and particularly in the Hordeae.

MATERIALS AND METHODS

Origin of experimental material

The hybrid grass was found occurring naturally in the field by Mr. Wesley Keller. Its hybrid origin was suspected when no seed was found after examination of a large number of spikes. On the basis of its taxonomic characteristics, and the plants associated with it in the field the parents of the hybrid were tentatively identified by W. S. Boyle and A. H. Holmgren as Agropyron trachycaulum and Hordeum jubatum. Synthesis of the hybrid by Boyle and Holmgren later confirmed the above parentage.

The 2n chromosome number of both A. trachycaulum and H. jubatum as determined by root tip smears is 28 (Stebbins and Love, 1941). Pollen mother cell smears of both species by Boyle (oral communication) revealed the constant occurrence of 14 bivalents. Since a majority of species in the Hordeae have chromosome numbers in multiples of 7 (Myers, 1947), A. trachycaulum and H. jubatum are probably allotetraploids.

The tetraploid hybrid resulting from the cross between these two plants was highly sterile. Examination of hundreds of spikes has failed to produce a single seed. Chromosome association in this plant during meiosis in the pollen mother cells commonly shows 7 bivalents plus 14 univalents (Boyle, oral communication). The univalents behave as laggards at both anaphase I and II, and as a result of these the tetrads contain numerous micronuclei. No viable pollen was found in pollen grain smears.

This sterile hybrid was treated with colchicine by Boyle and some culms with the chromosome number doubled (octoploid) were formed. These culms produced viable seed, and the resulting F_2 colchicine generation

furnished the material for this study.

Methods

During the summer of 1953 spikes from approximately 30 colchicine induced octoploid plants were collected and fixed in 3:1 absolute alcohol and glacial acetic acid. After 24 hours the spikes were transferred to 70 percent alcohol. Later the same year pollen mother cell smears were made from this material using the acetocarmine smear technique. The smears prepared from this material were generally poor and it was extremely difficult to prepare smears that could be interpreted without question.

Early in December the same plants were placed under 14 hour a day illumination to induce flowering. In February spikes were collected and fixed in Newcomer's fixative (Newcomer 1953). This fixative was definitely superior. Good acetocarmine smears were prepared with relative ease considering the high chromosome number.

Pollen grain smears were made in acetocarmine from material fixed as above. Good pollen stained dark and appeared round and full; sterile pollen appeared shrunken and empty (Plate 10).

Microscopic examination and interpretation of the slides was made with a Bausch & Lomb microscope using a 90X apochromatic objective (N.A. 1.30), an acromatic-aplanatic condenser (N.A. 1.40), and compensating oculars. Photographs were made with a similiar microscope, equipped with the same type objective, condenser, and oculars. The plates included in this paper are contact prints of 5 x 7 negatives, and the degree of magnification is indicated in the legend for each plate.

MEIOTIC CHROMOSOME BEHAVIOR

Prophase

It was not possible to prepare smears of stages earlier than diakinesis that could be interpreted accurately. The high chromosome number of this plant, in addition to the usual difficulties encountered in smears of early prophase stages, made it impossible to obtain definite information on initial chiasmata frequency and the nature of prophase pairing.

Diakinesis and metaphase I

Eighty cells at metaphase I or diakinesis were examined (Plates 1-5), and the results are shown in Table 1.

Univalents occurred in 58 percent of the cells, and averaged 1.5 per cell. Their frequency ranged from 0 to 6.

Bivalents were present in all cells examined, and averaged 25.2 per cell. The majority of the bivalents (80.5 percent) were closed or ring bivalents. Chiasmata frequency could not be determined with any degree of assurance because of the difficulties in preparing satisfactory prophase smears (see above), and the general completion of terminalization by metaphase.

Trivalents were found in 20 percent of the cells. Only one cell was found however that contained more than one trivalent, and that one contained 3.

Quadrivalents occurred with greater frequency than either univalents or trivalents: 64 percent of the cells examined. Their number per cell varied from 0 to 3, and averaged 0.8 per cell. Both chain and ring quadrivalents were found (Plates 1 and 3).

Twenty different chromosome associations were found at metaphase I and diakinesis. These are shown in Table 1 with the number of cells in each. It will be noted that 44 cells (55 percent) fell into 3 particular associations.

Anaphase I

Thirty-three cells at anaphase I were examined (Table 2, and Plate 6). Twenty-two of the cells (66.7 percent) showed at least one laggard. The number per cell varied from 0 to 4 with an average of 1.4 per cell. No bridge-fragments were found in this stage. With the exception of the laggards the divisions were regular, though in several instances precocious division of dyads was observed.

Anaphase II

Forty-two cells at anaphase II were examined (Table 2). Laggards were found in this stage in a considerably greater number than in anaphase I; 90.5 percent of the cells contained at least one laggard, with an average of 3.1 per cell (Plates 7 and 8).

Tetrads

One hundred and twenty-seven tetrads were examined, and 92.9 percent contained at least one micronucleus (Table 3, and Plate 9). Micronuclei averaged 5.8 per tetrad.

Pollen grains

Of the 10,254 pollen grains examined 44 percent were abortive. Among the good pollen grains a variation in size was noted (Plate 10).

Table 1. Chromosome associations at diakinesis and metaphase I

Associations				Number of Cells
I	II	III	IV	
0	26	0	1	17
0	28	0	0	14
2	25	0	1	13
1	26	1	0	5
3	25	1	0	5
2	23	0	2	4
4	26	0	0	4
1	24	1	1	3
0	24	0	2	2
2	21	0	3	2
4	22	0	2	2
0	22	0	3	1
2	27	0	0	1
3	23	1	1	1
3	21	1	2	1
4	24	0	1	1
5	19	3	1	1
5	20	1	2	1
6	23	0	1	1
6	25	0	0	1
Averages 1.5	25.3	0.2	0.8	

Table 2. Frequency of lagging chromosomes at anaphase I and II

	No. Cells	Percent with Laggards	Average Number Laggards
Anaphase I	33	66.7	1.4
Anaphase II	42	90.5	3.1

Table 3. Frequency of micronuclei in tetrads

	No.	Percent with Micro-nuclei	Average Number Micro-nuclei
Tetrads	127	92.9	5.8

DISCUSSION

Since both of the parents are probably allotetraploids, and the sterile F_1 hybrid commonly shows 7 bivalents and 14 univalents in the pollen mother cell division (Boyle, oral communication), the two parents in all probability contain one genome in common. Based on this assumption, if the genomic formulas for the parents are represented as AABB and AACC, the genomic formula of the sterile F_1 hybrid could be represented AABC. Assuming the above to be true the colchicine induced octoploid would have a genomic formula of AAAABBCC.

In the light of the above assumptions, it becomes possible to speculate on the chromosome associations that can be expected to occur in this plant. Fourteen chromosomes should have only one identical homologue, and thus constantly show 14 bivalents at metaphase I. Seven chromosomes should have 3 identical homologues, thus making possible a variety of pairing associations ranging from 14 bivalents to 7 quadrivalents, and including all possible combinations within this range. Thus, the possible associations considering all 56 chromosomes could vary from 28 bivalents to 7 quadrivalents plus 14 bivalents.

The assumed genomic formula was largely substantiated by the chromosome associations found at metaphase I, as can be seen from Table 1. As would be expected bivalents were the most common association, and 14 cells were found in which the chromosome associations consisted entirely of 28 bivalents. The chromosome association at the other end of the range, 14 bivalents plus 7 quadrivalents, was not found in any of the cells examined. This could hardly be considered as unusual however, for even in auto-

polyploids the maximum possible number of multivalents is rarely found in a single cell (Myers, 1940). If 2 or 3 of the 4 homologues pair completely the possibility of quadrivalent formation is eliminated, and the remaining chromosomes if there are 2 may pair to form a bivalent, or if there is only one a univalent results. Of the 28 chromosomes in this plant capable of being associated in multivalents, it is obvious that the probability of this occurring in a single cell is slight. The maximum number of chromosomes found associated in multivalents in any one cell was 13--3 trivalents plus one quadrivalent. This particular association was found in only one cell. However, 3 cells were observed that contained 12 chromosomes associated in multivalents--all as 3 quadrivalents.

The occurrence of univalents, which averaged 1.5 per cell, is difficult to explain when it is considered that each chromosome has at least one identical homologue. However, the occurrence of univalents in amphidiploids has been reported by several other investigators (Love and Suneson, 1945; Sears, 1941, and 1944). Sears (1944) has made the observation that all 56 chromosome wheat amphidiploids reported on have univalents at meiosis and reduced fertility. He further states that the probable cause of the univalent formation is the large numbers of chromosomes which act as mechanical barriers to complete pairing. This explanation may be applicable in some plants, however, as Pope and Love (1952) pointed out, the high chromosome numbers of some plants, such as Agropyron elongatum and Spartina townsendii, do not interrupt the regularity of their meiotic divisions to any great extent.

Armstrong and McLennan (1944) suggested that weak pairing in amphidiploids may occur between chromosomes of the different genomes (allosyndesis), but that the chromosomes are not capable of remaining paired until diakinesis or metaphase, and thus appear as univalents in these stag-

es. The above writers do not attempt to give an explanation as to why the chromosomes do not remain paired, but a plausible one might be that chromosome homology is sufficient for pairing, but is not sufficient to insure the regular formation of chiasmata, which are necessary to preserve a chromosome association after pachytene until metaphase (Darlington, 1937). The above writers' explanation of univalent formation may serve to explain the occurrence of occasional univalents in this plant, but it is doubtfull that it can explain an average frequency of 1.5 per cell. To do this it would necessitate the regular occurrence of allosyndetic chromosome pairing even though identical homologues are present for every chromosome, and as Darlington (1937) has shown allosyndetic pairing under these circumstances rarely occurs.

Sears (1941) stated that multivalent formation can be expected to cause the formation of some univalents. However, as he further points out, in some amphidiploids produced by him univalent occurrence was too frequent to be explained by multivalent occurrence alone, and that these cases could best be explained as the result of some physiological upset. A similiar explanation was favored by Pope and Love (1952). In amphidiploids produced by these writers pairing failure was attributed to disharmonious gene interaction and genetic controlled inhibition of pairing.

In this plant the occurrence of multivalents in 75 percent of the cells is adequate to explain the frequency of univalents, and probably offers the best explanation. Dobzhansky (1951) discussed univalent formation as caused by multivalents, and stated that where chromosome duplication occurs in excess of the diploid number pairing competition arises with the resultant formation of uni-, bi-, and multivalents in inconsistant proportions from cell to cell.

The occurrence of laggards at anaphase I was to be expected on the

basis of univalent occurrence at metaphase I, since unpaired chromosomes generally lag at anaphase. Snyder (1951) in his studies on Elymus reported an increase of laggards at anaphase I over the observed number of univalents at metaphase I. He explained this by noting that following disjunction fragments appeared at anaphase and behaved as laggards. In this plant no fragments were observed in this stage, and the close correlation between the average number of univalents at metaphase I (1.5), and the average number of laggards at anaphase I (1.4) suggests that the origin of the laggards is solely from the univalents.

In several instances in the first anaphase the precocious division of a dyad was noticed similiar to that observed in a Lilium hybrid by Richardson (1936). She explains these precocious divisions as probably having resulted from a bivalent that failed to reach the equator at metaphase, and thus disjunction of this bivalent did not occur until the other chromosomes were well into anaphase. One of the dyads from this late disjunction may separate at the centromere and the 2 sister chromatids move to opposite poles. It is possible that a similiar process is involved in the precocious dyad divisions observed in this plant.

Anaphase of the second division also showed laggards, but in a frequency too high to be explained as a result of first division laggards. Assuming all of the laggards at anaphase I divided equationally this could account for a laggard frequency at anaphase II only as high as that present at anaphase I. Any increase over this number must come from another source. Snyder (1951) obtained similiar data in Elymus hybrids. He explained this increase on the basis of further fragmentation (see above). This explanation is again not applicable to this plant, since no fragments were found in the second division either. In this plant a possible explanation for the increase of laggards at anaphase II is by

the precocious division of dyads noted in anaphase I (see above). The sister chromatids which separate at anaphase I behave as univalents, and thus laggards, through the second division (Richardson, 1936). An additional source of laggards in the second division was the occasional occurrence of dyads that had not separated in the division, and were found occurring as laggards.

Bridge-fragment configurations were found at anaphase II, but not frequently--only in 3 cells. The low frequency of these configurations and their complete absence in the first division suggests that the cause is something other than a cross-over in a heterozygous inversion. It is possible, as shown by Richardson (1936), to have a cross-over in a heterozygous inversion that does not form the typical chromosome bridge until the second division. For this to occur it necessitates heterozygosity for a paracentric inversion in which two cross-overs take place, one in the inverted segment and one between the inverted segment and the centromere. If this occurs the fragment appears at anaphase I, but the bridge does not appear until anaphase II. It is unlikely that cross-overs should only occur in this precise manner or not at all, and that the typical bridge-fragment configuration should not occur at least as often at anaphase I. In addition, in no instance was a fragment found at anaphase I. A more likely explanation is that spontaneous breakage and reunion of sister chromatids occurred, such as that described by Walters (1950) in an interspecific Bromus hybrid, thus causing a bridge to form at anaphase II.

The correlation between the number of micronuclei per tetrad and the number of laggards at anaphase II was good. The average number of laggards per cell at anaphase II was 3.1, and since a tetrad consists of 2

of these cells the average number of micronuclei per tetrad should be approximately 6.2 if the laggards are the source of the micronuclei. The observed frequency of 5.8 micronuclei per tetrad indicates that anaphase II laggards are probably the sole source of the micronuclei. It was noted that the size of the micronuclei was variable, caused perhaps by whether the source of a micronucleus was a chromosome or a dyad, since both occurred as laggards in the second division.

The high frequency of aborted pollen grains is undoubtedly caused to a large extent by the laggards at anaphase II and the resulting micronuclei. It appears however, that other sterility factors are also present. In high polyploids the loss of some chromatin material is not as serious as in a diploid, because, as Stebbins (1950) has pointed out, a high degree of chromosome duplication precludes the necessity of absolute meiotic regularity, and makes possible the formation of viable gametes even with the loss of chromosomes or chromosome segments. Based on the average number of micronuclei per tetrad (5.8), the average number per single pollen grain would be approximately 1.5. If this represents the average number of chromosomes excluded and thus lost from the nucleus in a pollen grain, a 44 percent decrease in fertility of an octoploid plant is hardly explainable on this basis alone.

A contributing factor to the reduced fertility may be unequal distribution of chromosomes in multivalent associations. However, the results of Sears' (1941) experiments tend to show that there is no direct relationship between multivalent frequency and degree of fertility.

In addition another source of reduced fertility may be the presence of unfavorable genetic factors. To what extent each of the above are involved in reducing the fertility of this plant is difficult to say, and

it is entirely possible that all are involved to a greater or lesser extent. As Stebbins (1949) has pointed out the causes of sterility in auto- and allopolyploids are still obscure.

CONCLUSIONS

From the preceding data and discussion it is felt by this writer that the following conclusions can be made:

(1) The results of the studies on chromosome behavior in this plant substantiate the hypothesized genomic formula of AAAABBCC.

(2) The polyploidy present in this plant is of the autoallopolyploid type according to the classification of Stebbins (1950).

(3) The reduced fertility in this plant is the result of a number of causes, but meiotic irregularity is probably the principle one.

(4) While pollen abortion is 44 percent and seed set is only 30 percent (approximately), the possibility is still good that this grass may prove of economic importance because of its perennial habit and vigor. The estimation of the percent seed set was made on plants grown in the greenhouse, and it is probable that seed production on plants grown in the field would be somewhat higher.

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PLATE LEGEND

Plate 1 - Diakinesis, 2 IV and 24 II, 1538X

Plate 2 - Metaphase, 28 II, 1374X

Plate 3 - Metaphase, 1 IV and 25 II and 2 I, 1194X

Plate 4 - Metaphase, 28 II, 1967X

Plate 5 - Metaphase, 1 IV and 26 II, 1997X

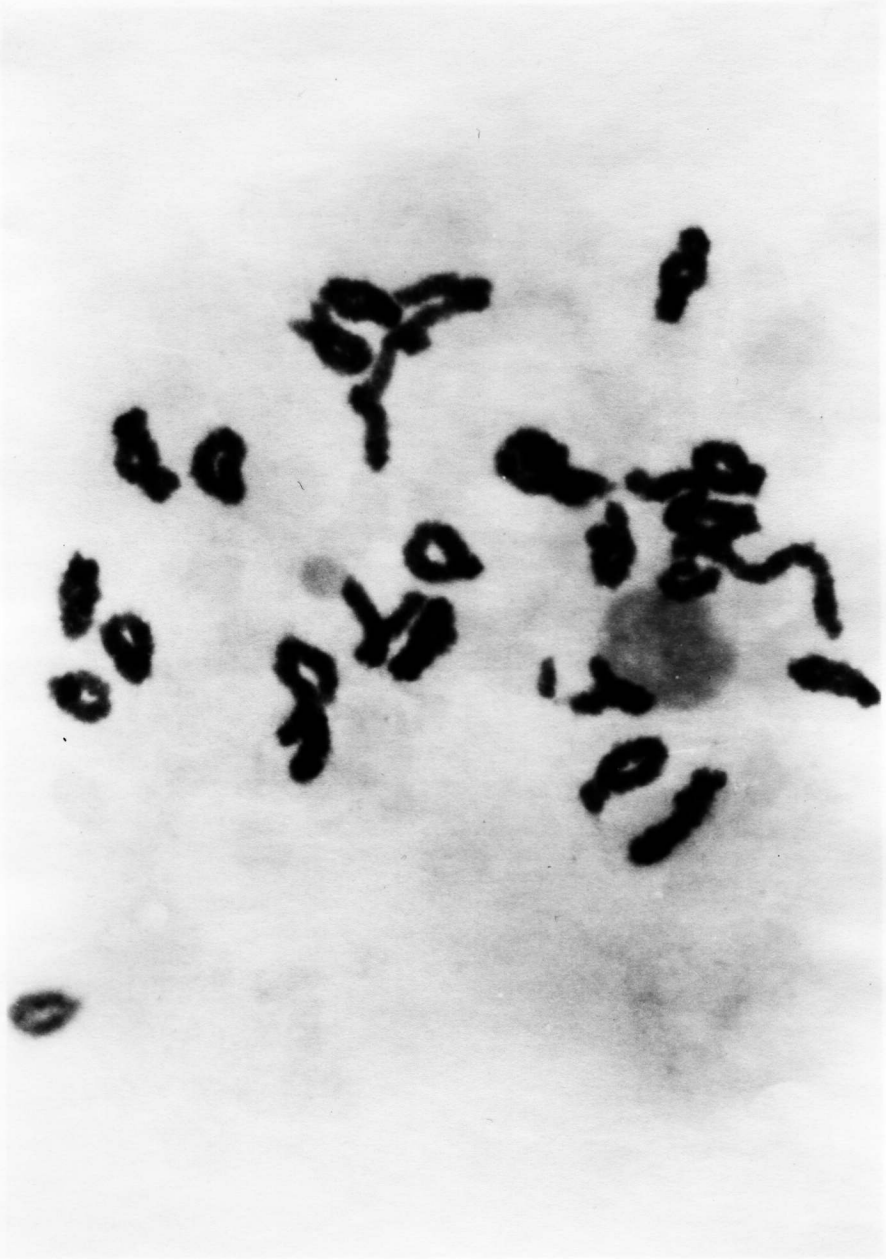
Plate 6 - Anaphase I, precocious dyad division, 1244X

Plate 7 - Anaphase II, laggards at equatorial plate, 897X

Plate 8 - Anaphase II, laggards at equatorial plate, 912X

Plate 9 - Tetrads, micronuclei, 870X

Plate 10 - Pollen grains



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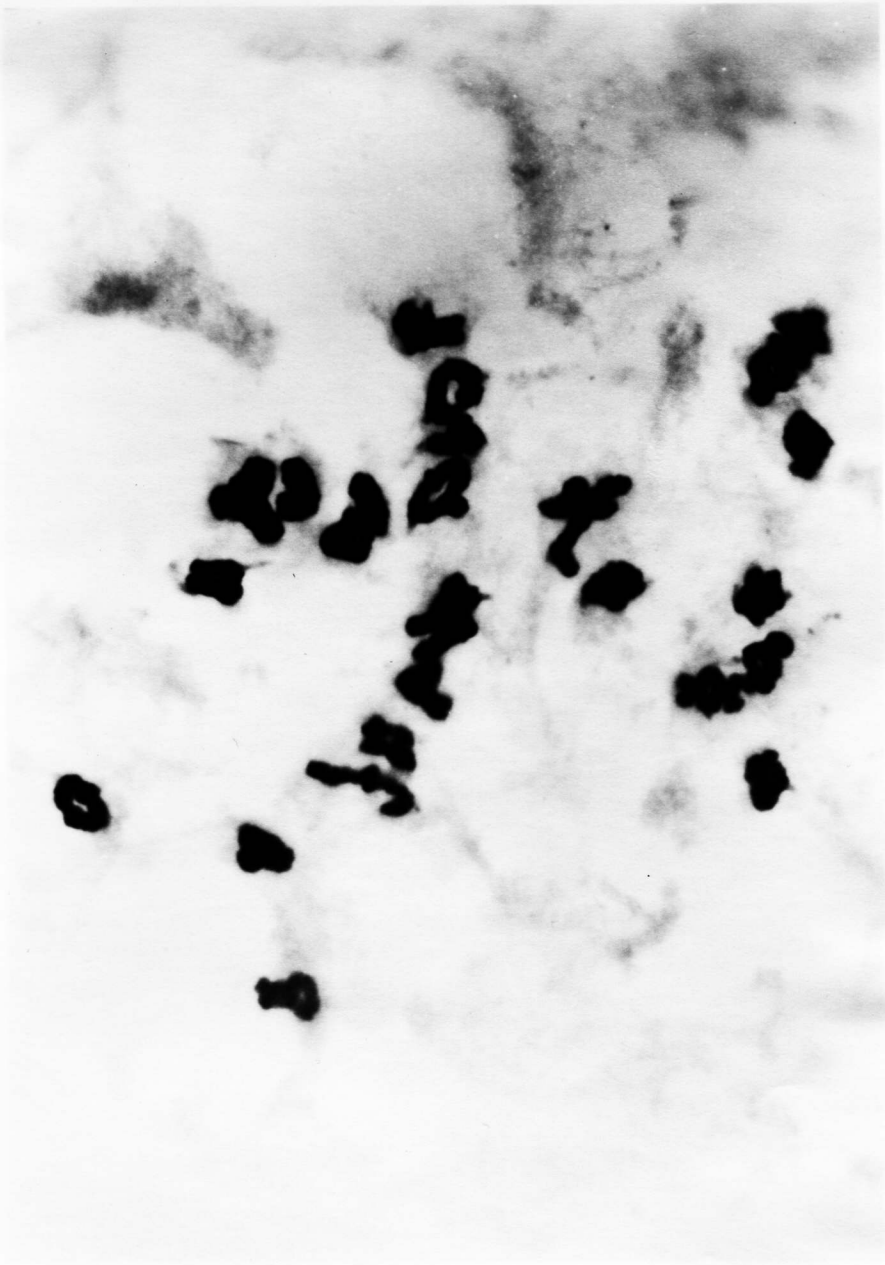


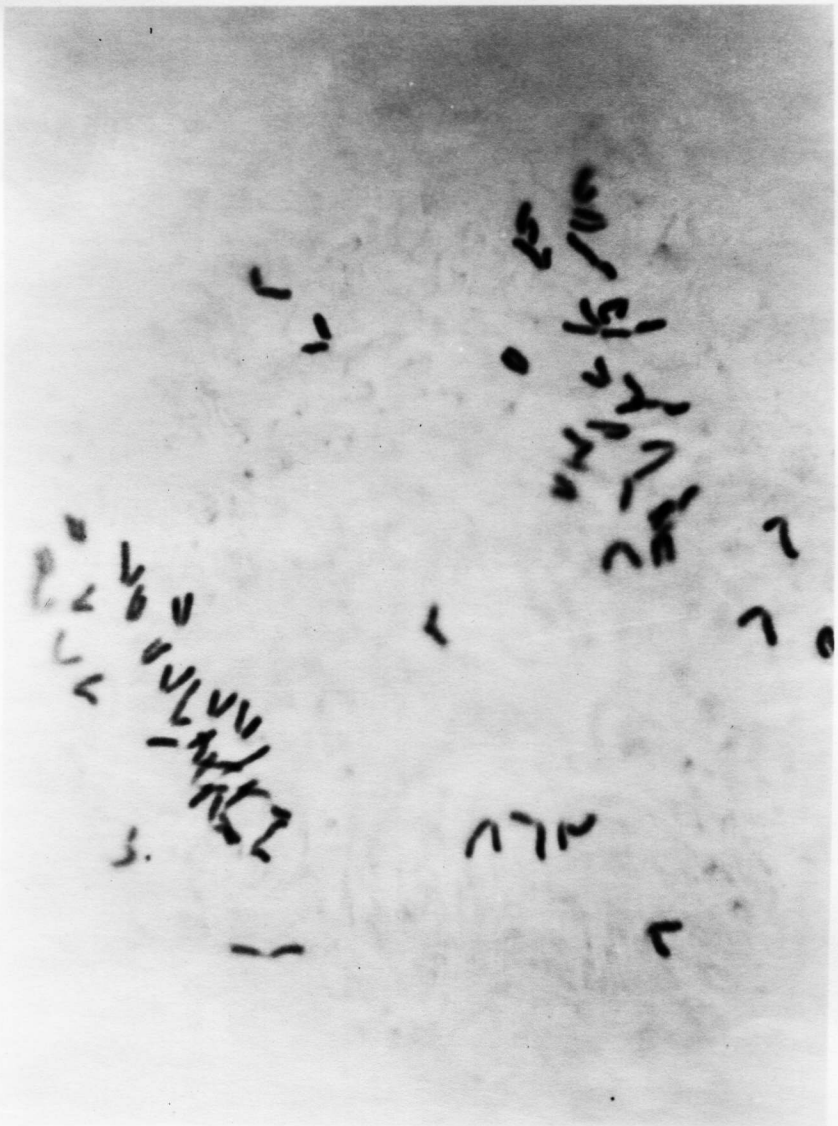
Plate 2

Plate 3

Plate 4

Plate 5

Plate 6



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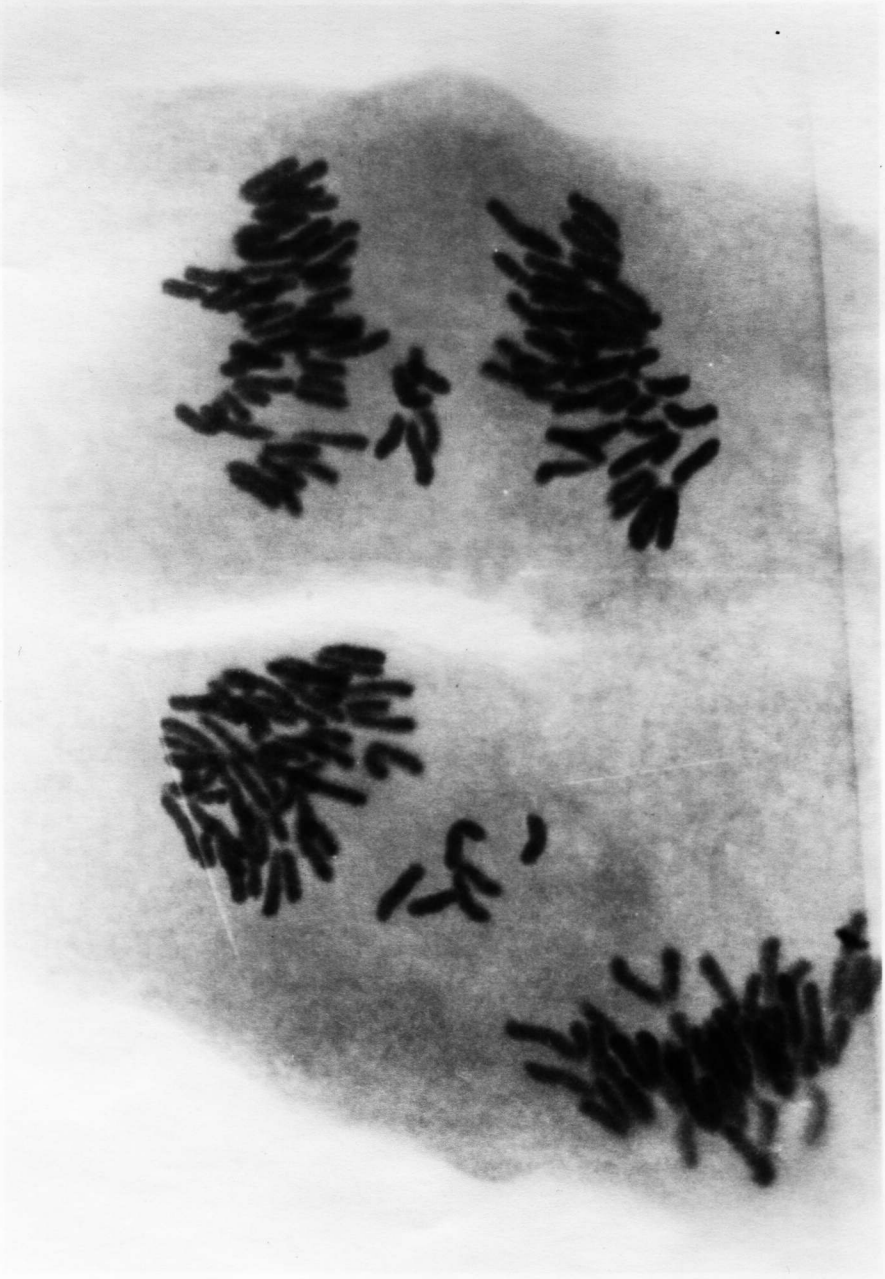

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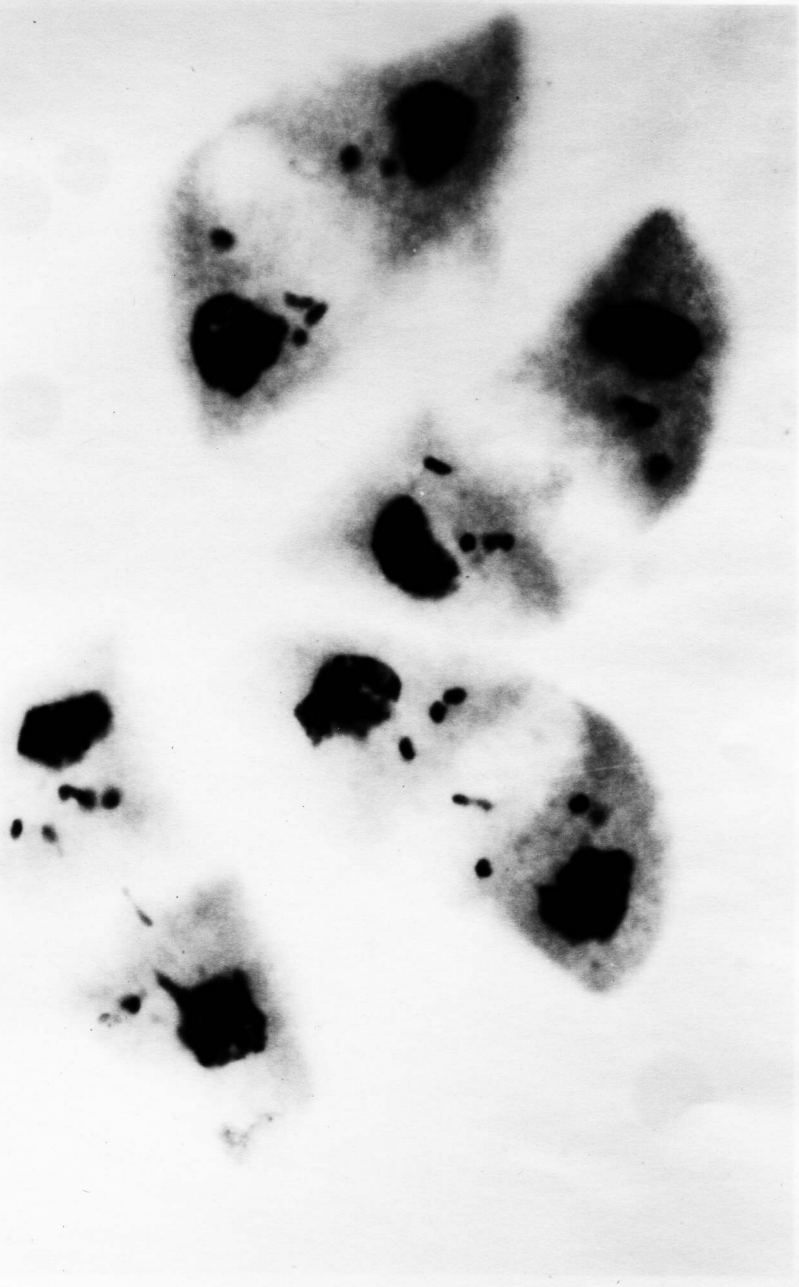

Plate 9

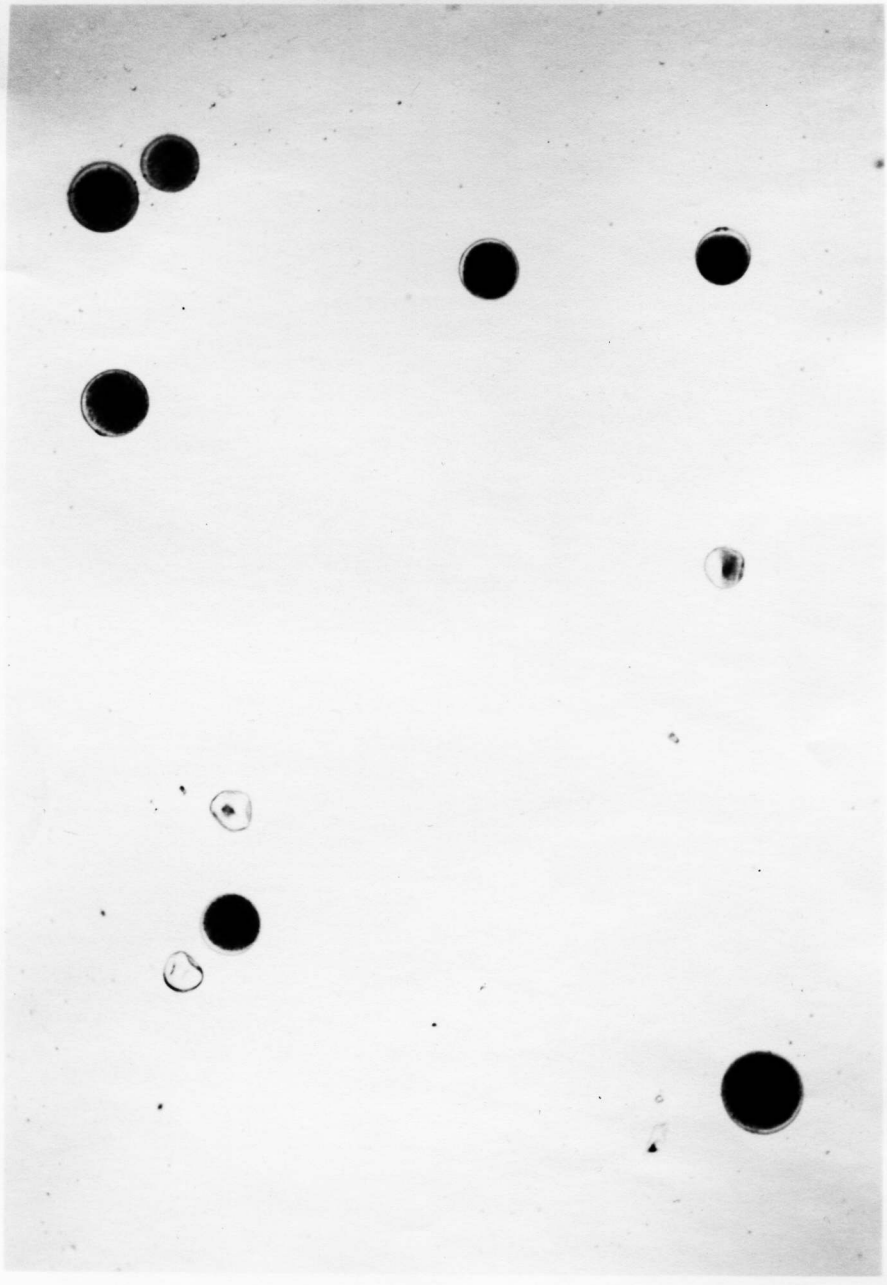

Plate 10