A CYTOGENETIC INVESTIGATION OF MULTIPOLAR CELL DIVISION AND CYTOMIXIS IN DIPLOID CRESTED WHEATGRASS, *AGROPYRON CRISTATUM*

By

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ABSTRACT

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Multipolar cell division and cytomixis are abnormal cytological phenomena occurring during meiotic or mitotic process. They can occur spontaneously, or can be induced by chemicals and/or physical means. They play very important roles in chromosome number evolution. *Agropyron cristatum*, #CC-37-119, was obtained from a seed treated with 0.1% colchicine solution for 12 hours. Different types of cytological irregularities were observed in the clones of CC-37-119, i.e., multipolar cell division, cytomixis, chromatin bridges, lagging chromosomes, unequal segregation, precocious division, early segregation and chromosomal fragments. Colchicine-induced irregularities were found to be inheritable. Multipolar cell division appears to be the main cause of gametes with reduced chromosome numbers. Cytomixis seems to give rise to microspores with an increase or decrease in chromosome number. All the observed irregularities appear to reduce the pollen viability. They appear to provide possible mechanisms for the production of aneuploid gametes which in turn may lead to aneuploid evolution.

DEDICATION

I want to dedicate this thesis to my beloved parents, Mr. and Mrs. Wang Sen-Dean. Without their love, care, encouragement and support, this work would not have been possible. I also wish to express my deep appreciation to my advisor, Dr. William Tai, for his assistance and direction in the preparation of this thesis. Last, but not least, I wish to thank my dear husband, Clovis.

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INTRODUCTION

Multipolar cell division (MPD) has occurred spontaneously and can be induced artifically in many plant and animal species. Tai (1970) observed that the chromosome complement became subdivided into several groups in the meiotic division of colchicine-treated diploid *Agropyron cristatum*. The grouping seemed to be based on the origin of the genomes in hybrids and polyploids. He proposed that multipolar cell division provided a possible mechanism for changes in chromosome numbers, which evolutionarily led to aneuploidy or polyhaploidy. Tai (1970, 1971) also suggested that in plant breeding, induction of haploidy by MPD and doubling of the chromosome complement in the haploids could produce an individual homozygous for every gene locus.

Cytomixis appears to be another mechanism for change in chromosome number, which in turn results in aneuploid evolution (Romanov and Orlova 1971; Tai and Vickery 1972; Cheng 1974). The migration of chromosomal materials from the donor cell to the recipient one may have the consequence of an increase or decrease of chromosome numbers. It has been observed in many plants including diploid (Kosova 1973), hybrid and polyploid (Kihara and Lilienfeld 1934; Katterman 1933; Tai and Vickery 1972) and mutants (Kamra 1960) during mitotic or meiotic processes.

The present investigation was designed to make further and detailed observations of colchicine-treated *Agropyron cristatum*. The objectives of this investigation are:

1. To observe the chromosome behavior of a colchicine-treated diploid *Agropyron cristatum* plant which has shown MPD and cytomixis.
2. To examine the inheritance of these induced irregularities.
3. To analyze the significance of multipolar cell division in chromosome number evolution.
4. To evaluate the significance of cytomixis in chromosome number evolution.

LITERATURE REVIEW

Multipolar Cell Division

Multipolar cell division is a phenomenon in which the chromosome complement is separated into two or more groups in either meiosis or mitosis. Each group functions more or less independently and has its own chromosome movement. Multipolar cell division results in variable chromosome numbers in the daughter cells (Tai 1970). The same phenomenon has been described in various materials by different terms, such as "incompact spindle" (Darlington and Thomas 1937), "double plate metaphase" (Upcott 1939; Vaarama 1949), somatic meiosis (Huskins 1948), reductional grouping (Wilson 1950), multipolar spindle (Therman and Timonen 1950; Walters 1958), split spindle (Neilsen and Nath 1961) and complement fractionation (Thompson 1962).

Multipolar cell division has been found to occur spontaneously in animal species and tissue culture. After analyzing multipolar mitosis in tumors, Boveri (1888) concluded that the phenomenon was pathological. Baltzer (1909) observed that tri-polar mitosis in the sea-urchin eggs which may produce daughter cells with a reduced number of chromosomes. In the analysis of rat liver cells, Gläss (1956, 1957) found that multipolar mitosis occurred in all haploid, diploid, triploid and tetraploid clones of rat liver cells. The haploid and triploid chromosome groups existed simultaneously within the same tetraploid cells. Haploids observed to have 10+11 chromosome grouping and triploids had 2n-1n and 1n-1n-1n distinct genome groups. Therefore, he suggested that multipolar cell division is very important to genome separation and to lower the ploidy level. Knudson (1958) discovered that the formation of multipolar spindles during the first meiotic division caused the majority of sterile bulls. Rizzoni _et_ _al_. (1974) pointed out that in mammalian cell culture, euploid segregation occurred through multipolar cell division, and that the frequency of multipolarity increased linearly with the age of culture. Also the human cancer cells are often characterized by the occurrence of multipolar cell divisions and multinucleated cells (Therman and Timonen 1950, 1954; Timonen and Therman 1950; Ofterbo and Wolf 1967).

More reports about spontaneous multipolar cell division come from plants including haploid, diploid, polyploid, hybrids and tissue culture. In haploid _Ulva_ _mutabilis_, the chromosomes segregated randomly following multipolar meiosis forming viable zoospores (Hoxmark and Nordby 1974). Vasek (1962) found that multipolar cell division occurred in the microsporogenesis of diploid _Clarkia_ _exilis_. He suggested a recessive gene for the occurrence and persistence of multipolar cell division.

The presence of multipolar cell division in polyploid plants is very common and frequent, and has been described by many authors (Upcott 1939; Vaarama 1949; Weimarck 1973). In the study of _Rubus_ hybrids, Thompson (1962) and Bammi (1965) proposed that complement fraction resulted in chromosome grouping in meiotic as well as mitotic cells. Dewey (1974) observed chromosome grouping and multiple metaphase plates in hybrids and induced amphiplcids of Elymus conadensis x Agropyron libanoticum. Based on the study of Daucus carata culture Bayliss (1973) suggested that multipolar cell division might result from lack of physical organization of the tissues cr the chemical constituents cf the medium.

Multipolar cell division can also be induced artificially by different means. Induced multipolar cell division is always asscciated with lagging chromcsomes, chromatin bridge, chromosomal fragments, unequal segregaticn, micronueclei, supernumerary cells, etc.

Many incrganic and organic chemicals which are dangerous to human health have the ability to induce multipolar cell division. Colchicine is one of the many excellent inducing agents. From the observations on onicn cells, Östergreen (1950) suggested that different concentraticns of colchicine wculd give different degrees cf mitotic spindle abnormalities, and that low concentration cf colchicine will induce multipclar cell divisicn. Radiation, e.g., X-rays and gamma-rays, has also been reported tc induce multipolar cell division (Rustard 1959; Levis and Martin 1963; Amer and Farah 1974). The percentage of multipclar cell division and asscciated abnormalities appears to increase with the dosage and duration of radiation. Both extremely high and low temperatures will suppress the start cf cell division and increase the frequency of multipclar cell division (Huskins and Cheng 1950; Mazia 1961).

Cytological irregularities can also be induced by microorganisms. Nimnoi (1974) reported that potato spindle tube virus induced multipolar cell division in potato pollen mother cells. Abraham (1974) found that spindle abnormalities and lagging chromosomes in the stamen hairs of *Tradescantia* clone O_2 growing in cowdung sand.

The significance of multipolar cell division in chromosome evolution has been discussed by many authors. It appears to be a source of aneuploidy. Thompson (1962) and Tai (1970, 1971, 1972) suggested multipolar cell division will produce gametes with extremely variable chromosome numbers. If too many chromosomes are gained or lost, the gametes may not live and function. However, if only a small number of chromosomes are involved, the gametes may survive and be functional as well. They also pointed out that multipolar cell division seems to provide a mechanism to reduce the ploidy level from polyploids to polyhaploids. Multipolar cell division is also responsible for genome separation (Tai 1970; Cheng 1976), and thus can be used as a tool for plant breeding (Thompson 1962; Tai 1970, 1971).

Cytomixis

At the beginning of the 19th century, the phenomenon of the migration of nuclei or chromatin from one cell to the neighboring one was discovered in plant somatic and reproductive cells (Miehe 1901; in Cheng Kuo-chang 1974; Kornike 1901). Later it was called chromatin extrusion or "cytomixis" (Gates 1911). Currently the term "cytomixis" is used in a much broader rather than the narrower sense. In the present study the term "cytomixis" is used to describe the migration of chromatin or parts of a nucleus from one cell to another, and the presence of cells with additional chromosomal materials.

Cytomixis is fairly widespread. It has been described in many species including diploids (Kosova 1973; Syemyarykhina abd Kuptsow 1974; Cheng 1974), polyploid hybrids (Katterman 1933; Kihara and Lilienfeld 1934; Romanov and Orlova 1971; Tai and Vickary 1972; Shkutina and Kozlovskaya 1974; Syemyarykhina and Kuptsow 1974), and mutants (Sarvella 1958; Kamra 1960). It was observed in somatic tissues (Tarkowska 1960) but more frequently in pollen mother cells (Kihara and Lilienfeld 1934; Katterman 1933; Romanov and Orlova 1971; Tai and Vickery 1972; Shkutina and Kozlovskaya 1974).

The cause of cytomixis still remains unsolved. Some believed it is an abnormal phenomenon. Tarkowska (1960, 1965, 1966) suggested that some mechanical stimuli creates sufficiently large pressure differences between cells and induces cytomixis from cells with higher internal pressure to those with lower. Katterman (1933) and Takats (1959) believed that it was caused by methods of fixation, and that different types of fixative would have different frequencies of cytomixis. Some thought that it is the results of abnormal environmental conditions, i.e., a change of temperature, rainfall, drought and radiation (Katterman 1933). Cytomixis also had been discovered in diseased plants, therefore it was proposed that extrusion of chromatin was caused by pathogens (Tischler 1934) or by the weakening of plants (Fraser 1914). Woodworth (1929) and Kamra (1960) suggested that it is the result of hybridization. Based on the experiments and observations of lily and *Lysium chinense*, Cheng (1974) strongly proposed that cytomixis is a normal physiological condition and may be enhanced by external environmental factors, e.g., temperature fixation, mechanical changes, etc. (Gates 1911; Kihara and Lilienfeld 1934).

Migration of chromosomal materials from the donor cell into the adjacent recipient cell has been observed most frequently in the early meiotic prophase I (Katterman 1933; Kihara and Lilienfeld 1934; Bopp-Hassenkamp 1959; Romanov and Orlova 1971; Tai and Vickery 1972; Shkutina and Kozlovskaya 1974). It also occurred rarely at metaphase I and anaphase I (Romanov and Orlova 1971). In somatic cells, it usually occurs during interphase but not other mitotic stages (Cheng 1974). Romanov and Orlova (1971) suggested that chromatin migrates from the donor cell through the cytomictic channels into the cytoplasm of the recipient cell. Usually it migrates in multiple directions, but sometimes only in a single direction (Cheng 1974; Heslop-Harrison 1966; Weiling 1965).

The significance of cytomixis has been analyzed by a number of authors. Sinoto (1922) suggested that cytomixis is an artifact, there is no need to consider its significance in genetics. Some authors proposed that cytomixis may result in an increase or decrease of the chromosome numbers in pollen mother cells, and provides a mechanism for aneuploidy evolution (Katterman 1933; Kihara and Lilienfeld 1934; Kamra 1960; Sadasivaiah and Magoon 1965; Romanov and Orlova 1971; Tai 1967; Tai and Vickery 1972; Cheng 1974; Kosova 1974). Cheng (1974) discovered double and multinucleated protoplast in culture, and suggested that cytomixis could cause the formation of multinucleated cells, which in turn would produce polyploids (Romanov and Orlova 1971).

MATERIALS AND METHODS

Origin of Materials

During a study of colchicine-treated diploid (2n=14) Fairway crested wheatgrass (*Agropyron cristatum*), Tai (1964) discovered a plant (CC-37-119) to have different types of cytological irregularities. Plant CC-37-119 was obtained from a seed treated with 0.1% aqueous solution of colchicine for 12 hours. The plant has been maintained at Evans Farm, Utah State University by Dr. Douglas R. Dewey. Clones were sent to Dr. Tai at Michigan State University. These materials were used in this current research.

Cytological Methods

For mitotic studies, root tips from newly transplanted CC-37-119 plants were excised and pretreated in 0.01% colchicine solution for four hours in the spring of 1978, followed by fixation in Farmer's solution (3 parts absolute ethanol: 1 part glacial acetic acid) overnight. Root tips were stored in the same solution in the refrigerator (at 3°C). The aceto-carmine smear technique was used to prepare slides for observation. If the Feulgen stain technique was used, the root tips were hydrolyzed in 1 N HCl at 60°C for 10-11 minutes.

In the spring of 1978, young spikes were collected for meiotic analysis from plants maintained in the greenhouse and the open field. When spikes were collected from 4–8 a.m., the pollen mother cells showed the best separation of chromosomes and therefore best for cytological observations. The spikes were fixed immediately in Farmer's fixative and stored in the same solution under refrigeration (3^{o}C). The aceto-carmine smear technique was used again to prepare slides. Premeiotic Mitosis, all stages of the meiotic division and pollen mitosis were examined for the study of chromosome behavior.

All cytological observations were made with a Zeiss photomicroscope II under phase contrast illumination. Photomicrographs were taken with 35mm Kodak Panatomic-X film or with 4x5" Kodak Contrast Process Ortho film.

Pollen viability was determined on the basis of the staining reaction with the I_2-KI solution. Pollen grains were considered viable if they were round and stained darkly under bright field observation. Shrunken and lightly stained pollen grains were recorded as non-viable.

RESULTS

Mitosis

All cells examined for mitosis appear to be fully normal with no abnormalities observed at any mitotic stage. The chromosomes behave as normally as any typical diploid species during the entire mitotic process. Plant CC-37-119 exhibits a normal diploid chromosome number of 2n=14 in its root tip cells (Fig. 1). Its karyotype shows a pair of acrocentric chromosomes with satellites. Schulz-Schaeffer and Jurasits (1962) observed no satellite chromosome in diploid Fairway crested wheatgrass. Perhaps, the staining and squash technique that they used resulted in chromosomes too condensed to determine fine details. The rest of the complement have similar morphology as was described by them with two pairs of metacentric chromosomes and four pairs of acrocentric chromosomes.

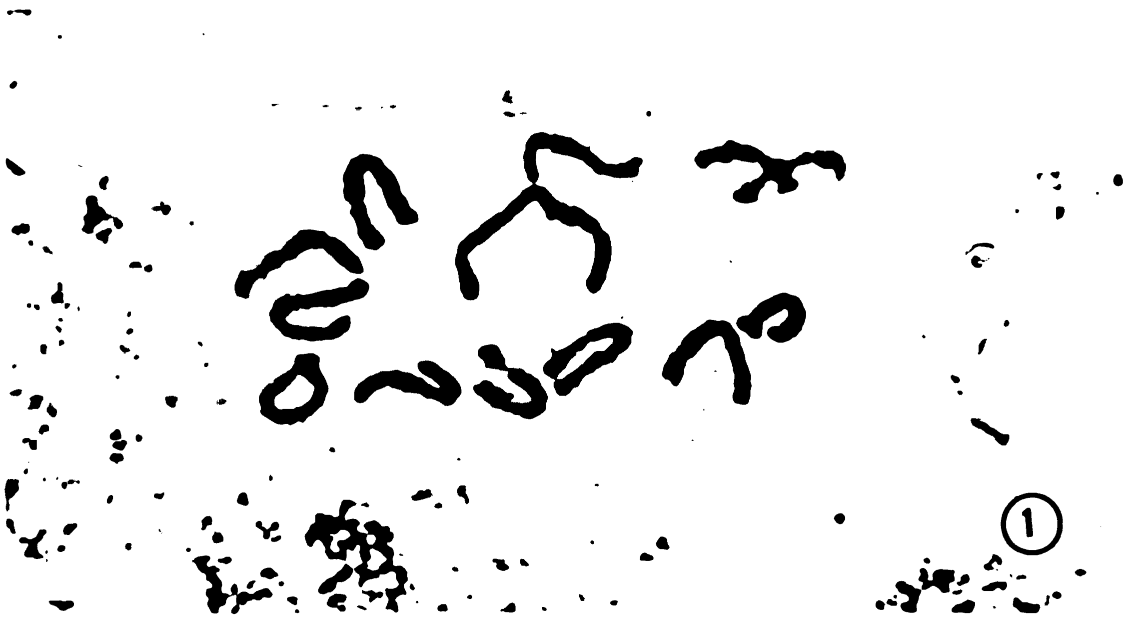
Premeiotic Mitosis

Chromosome behavior of premeiotic mitosis appeared to be normal. There are 14 chromosomes at metaphase and a 14-14 equal disjunction at anaphase. No chromosome pairing was observed at premeiotic mitosis.

Meiosis

With a total of 5800 pollen mother cells and another 1587 observed at the quartet stage, 62.48% and 60.24% appeared to be cytologically

Figure 1. Metaphase of mitosis with a normal chromsome number 2n=4 in clones of CC-37-119 (2415x)



normal, respectively. The rest of the cells showed different types of cytological irregularities e.g., congregation of chromosomes into groups (multipolar cell division), lagging chromosomes, chromatin bridges, unequal segregation, early segregation, chromosome fragments, cytomixis, micronuclei, etc.

Normal Meiotic Behavior

Prophase I Leptotene: The chromosomes are not tightly packed together. They are visible as very thin, long and uncoiled threads, single stranded and unpaired.

Zygotene: The chromosomes start to shorten and thicken. Homologous chromosomes come slowly together, align side by side and begin to pair. The sister chromatids of each homologue are no longer visible.

Pachytene: The paired homologous chromosomes continue to shorten, condense and remain tightly twisted around each other (Fig. 2).

Diplotene: Terminalization of chiasmata begins at this stage. Therefore, homologous chromosomes start to pull apart from each other and holes appear in bivalent configurations. The bivalents are usually randomly distributed. In some cells, the nucleolus remains visible and the nuclear envelope remains intact. In other cells, they both may disintegrate and disappear.

Diakinesis: The chiasmata continue to terminalize and still hold the chromosomes together at the end. The chromosomes reach maximum shortening and condensation. The nucleolus disappears and the nuclar envelope is

Normal Meictic Behavicr

Figure 2. Pachytene (785x)

Figure 3. Diakinesis (661x)

Figure 4. Metaphase I (661x)

Figure 5. Anaphase I (537x)

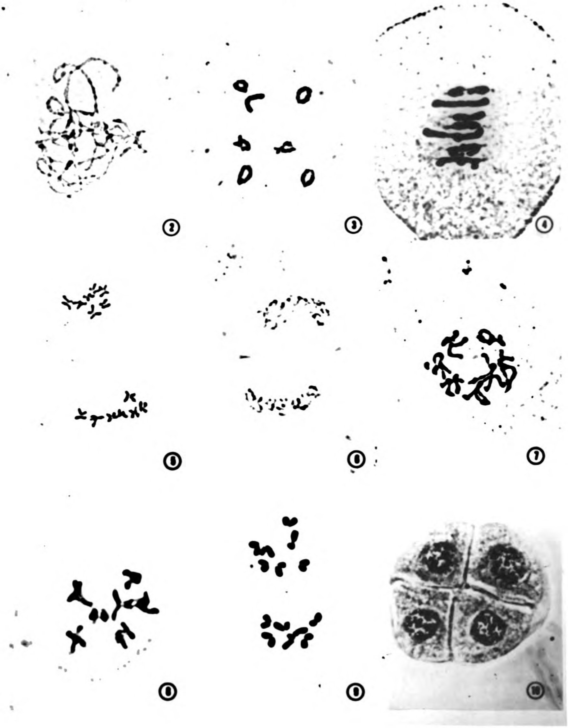
Figure 6. Telophase I (827x)

Figure 7. Prophase II (1042x)

Figure 8. Metaphase II (1419x)

Figure 9. Anaphase II (1273x)

Figure 10. Quartet (661x)



disintegrated completely. Seven bivalents are randomly distributed all over the cytoplasm. The chromosomes show typical morphology with no overlapping. Usually, this is the best stage at which one can analyze the pairing behavior of chromosomes (Fig. 3).

<u>Metaphase I</u> Chromosomes reach the maximum degree of condensation. Seven bivalents move towards the equatorial position and align themselves to form a single metaphase plate (Fig. 4).

<u>Anaphase I and Telophase I</u> At anaphase I, the homologous chromosomes start to separate from each other with seven dyad chromosomes moving towards each pole (Fig. 5). After the chromosomes reach the poles, a new nuclear envelope is formed at each pole to form a new nucleus. Cleavage of cytoplasm at the equator separates the primary microsporocyte into two daughter cells. Each has a haploid chromosome number (Fig. 6).

<u>Meiosis II</u> Second meiosis takes place independently in two secondary microsporocytes. Once again the chromosomes start to condense at prophase II and each dyad shows a typical X shape (Fig. 7). The seven X-shaped dyads align themselves at the equator during metaphase II (Fig. 8). At anaphase II, the chromatids separate from each other (Fig. 9). After separation, each chromatid is termed as a single-stranded chromosome and seven of them migrate towards the opposite poles. Eventually, cytokinesis takes place and a pollen mother cell gives rise to four daughter cells, a quartet. Each daughter cell has seven chromosomes (Fig. 10).

Abnormal Meiotic Behavior: Cytomixis

Figure 29. Part of the nucleus (arrow) of donor cell migrated into the adjacent recipient cell at early prophase I. (1195x)

Figure 30. Cells were connected by several spiralized chromosomal threads (arrows) at early prophase I. (1017x)

Figure 31. Additional chromosomal material (arrow) in the cell at early prophase I. (759x)

Figure 32. Additional chromosomal materials (arrow) in the cell at early prophase I. (759x)

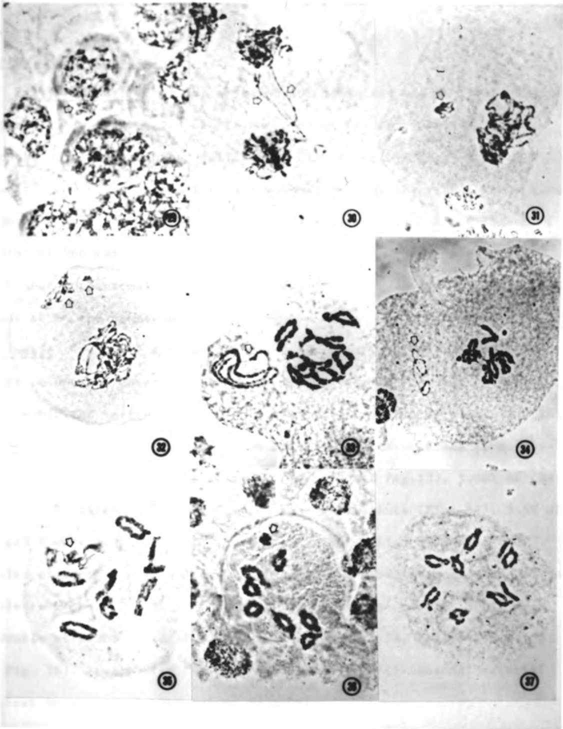
Figure 33. Cell had 7 bivalents with one extra coil of prophase chromosome (arrow) at diakinesis. (825x)

Figure 34. Cell had 7 bivalents with one extra coil of prophase chromosome (arrow) at diakinesis. (458x)

Figure 35. Cell had 7 bivalents with one extra coil of prophase chromosome (arrow) at diakinesis. (1017x)

Figure 36. Cell had 7 bivalents with a big mass of double-stranded thick chromosomal threads (arrow) at diakinesis. (903x)

Figure 37. Cell had 7 bivalents plus 2 univalents at diakinesis. (788x)



Abnormal Meiotic Behavior

Early Prophase I The occurence of cytomixis was observed at early prophase I, in which parts of the nucleus of some donor cells migrated into the adjacent recipient cells (Fig. 29). Some cells were seen to be connected by several spiralized chromosomal threads (Fig. 30). Donor chromosomes were present in the plasma of the adjacent cell. About 1% out of a total of 800 early prophase I cells observed were found to have additional chromosomal materials in the cells (Fig. 31 and Fig. 32). They appear to be the consequence of cytomixis.

Diakinesis Out of a total of 1114 cells observed, 66.97% (746 cells) showed no unusual chromosome behavior at diakinesis. The rest of the cells exhibited various types of cytological abnormalities (Table 1). 28% of the total 1114 cells showed a tendency for the chromosomes to congregate into two or more groups (Fig. 11, Fig. 12 and Fig.13). 1.08% of the cells had 8 bivalents or 7 bivalents plus 2 univalents (Fig. 37), 3.5% of the cells were observed to have 6 or 5 bivalents (Fig. 38). Also the results of cytomixis were observed in which the recipient cells had seven bivalents with one extra coil of prophase chromosome or with a big mass of double stranded thick chromosomal threads (Fig. 33, Fig. 34, Fig. 35 and Fig. 36). It appeared that a certain amount of chromosomal material was lost by some cells and gained by others.

Metaphase I At metaphase I, multipolar divisions first became conclusively evident. Instead of aligning themselves to form a single

Abnormal Meiotic Behavior: Multipolar Cell Division (MPD)

Figure 11. Chromosomes congregated into 2 groups; one group with 4 bi-valents, the other with 3 bivalents (4-3) at diakinesis. (751x)

Figure 12. Chromosomes congregated into 2 groups: one group with 6 bivalents, the other with 1 bivalent (6-1) at diakinesis. (821x)

Figure 13. Chromosomes congregated into 4 groups with 4-1-1-1 at diakinesis. (765x)

Figure 14. Cell with 7 bivalents separated into 2 groups (4-3) to form two "micrometaphase" plates at metaphase I. (748x)

Figure 15. Cell with 3 groups (4-2-1) of bivalents at metaphase I. (680x)

Figure 16. Cell with 4 groups (3-2-1-1) at metaphase I. (680x)

Figure 17. Abnormal groupings of dyad chromosomes (4-3) at anaphase I. (595x)

Figure 18. Abnormal groupings of dyad chromosomes (3-2-2) at anaphase I. (776x)

Figure 19. Chromosomes separated into 2 groups (6-1) at prophase II. (850x)

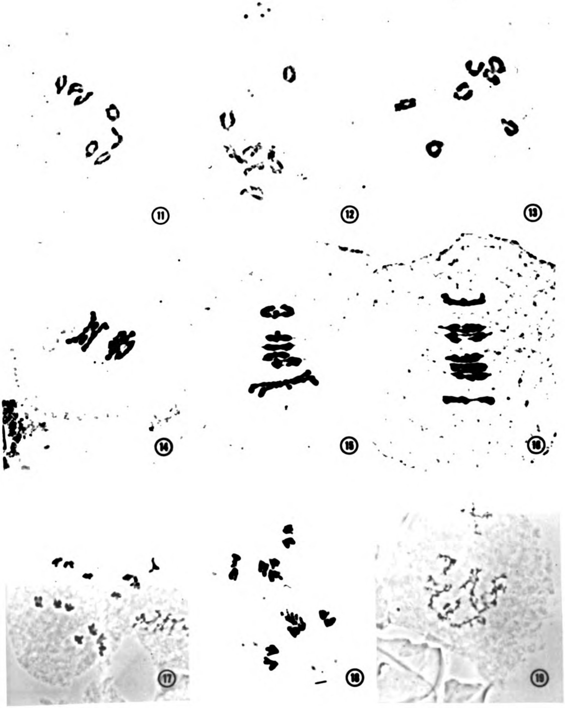

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Table 1 Chromosome Behavior at Diakinesis in Clones of CC-37-119

	Number of Bivalents	Number of Cells	Percentage		
	7	746	66.97%		
2 groups	6-1	101	9.07%	21.36%	28.10% (313 cells)
	5-2	71	6.37%		
	4- 3	66	5.92%		
3 groups	5-1-1	17	1.53%	5.93%	
	4-2-1	27	2.42%		
	3-3-1	15	1.35%		
	3-2-2	7	0.63%		
4 groups	3-2-1-1	6	0.54%	0.81%	
	4-1-1-1	3	0.27%		
	8	12	1.08%		
	6	32	2.87%		
	5	7	0.63%		
	Cytomixis	4	0.36%		

Total Number of Cells 1114

equatorial plate, the seven bivalents were separated into two or more groups and formed more than one metaphase plate (Table 2). Each group contained one to six bivalents.

Grouping of chromosomes at metaphase I and later stages can be divided into four categories: (1.) normal cells with seven bivalents forming a single metaphase plate (Fig. 4); (2.) cells with seven bivalents separated into two groups (6-1, 5-2 or 4-3) to form two "micro-metaphase" plates, (Tai, 1970) (Fig. 14); (3.) cells with three groups (5-1-1, 4-2-1, 3-3-1 or 3-2-2) of bivalents (Fig. 15); (4.) cells with four groups (4-1-1-1, 3-2-1-1 or 2-2-2-1) (Fig. 16). Two-group separations were more frequent than three-group or four-group separations. The overall frequency of multipolar cell division observed at metaphase I is 29.99%, or 413 of 1377 cells observed. Irregular chromosome numbers were observed in 2.48% of the cells which had 8, 6 or 5 bivalents line up on the equator (Table 2) (Fig. 42 and Fig. 43). They oriented on one or more metaphase plates. Occasionally cells with abnormal chromosome numbers exhibit multipolar cell division.

Out of 1377 cells, 0.44% in this stage exhibited cytomixis. In addition to seven bivalents, cells with extra chromosome materials were observed. The seven bivalents may form a single metaphase plate or multiple microplates. (Fig. 39, Fig. 40 and Fig. 41). Early segregation of bivalents into dyads were observed at this stage.

Irregular chromosome behavior which occurred at earlier stages continued at anaphase I (Table 3). Multipolar cell division and the

Abnormal Meiotic Behavior:

Figure 38. Cell had 6 bivalents at diakinesis. (729x)

Figure 39. Cell had 5 bivalents with one prophase chromosomal threads (arrow) at metaphase I. (463x)

Figure 40. Cell with 7 bivalents to form a single metaphase plate had extra chromosomal materials (arrow) at metaphase I. (579x)

Figure 41. Multipolar cell division and cytomixis occurred in the same cell. Cell with 7 bivalents to form multiple microplates (2-2-2-1) with extra chromosomal materials (arrow) at metaphase I (695x)

Figure 42. Cell had 8 bivalents and separated into 2 groups (6-2) at metaphase I. (1028x)

Figure 43. Cell had 6 bivalents and separated into 2 groups (5-1) at metaphase I. (897x)

Figure 44. Cell had extra chromosomal material (arrow) at anaphase I. (440x)

Figure 45. Multipolar cell division and cytomisix occurred in the same cell. Cell separated into 3 groups (3-2-2) had extra chromosomal material (arrow) at anaphase I. (637x)

Figure 46. Cell had a lot of extra chromosomal materials at anaphase I. (672x)

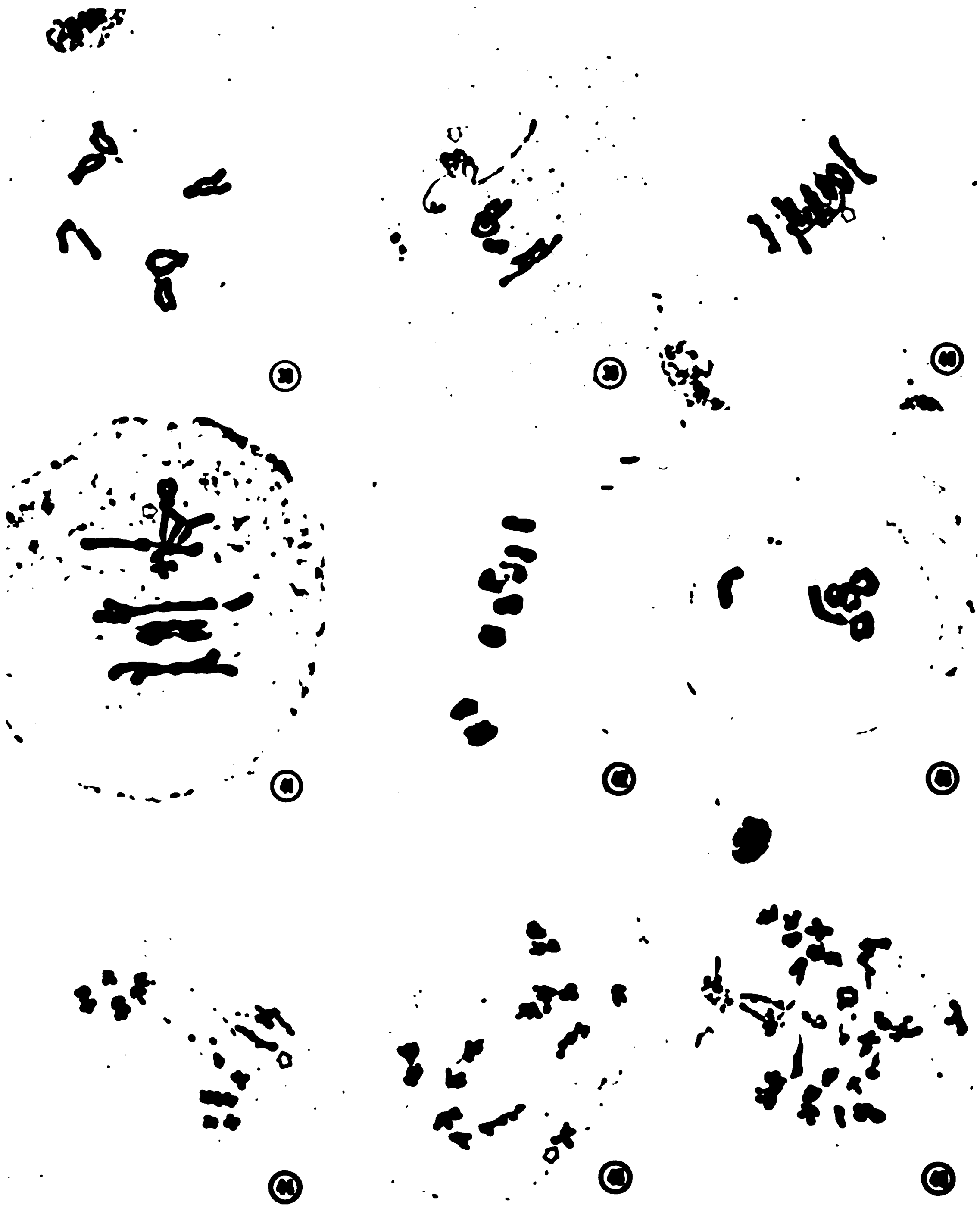

Table 2 Chromosome Behavior at Metaphase I in Clones of CC-37-119

Number of Bivalents		Number of Cells	Percentage		
	7	867	62.96%		
	6-1	160	11.62%		
2 groups	5-2	89	6.46%	24.47%	
	4-3	88	6.39%		29.99%
	5-1-1	15	1.09%		(413
	4-2-1	27	1.96%		cells)
3 groups	3-3-1	7	0.51%	4.65%	
	3-2-2	15	1.09%		
	4-1-1-1	2	0.15%		
4 groups	3-2-1-1	5	0.36%	0.87%	
	2-2-2-1	5	0.36%		
	8	6	0.44%		
	6	25	1.82%		
	5	3	0.22%		
	Cytomixis	6	0.44%		
	Early Separation	57	4.14%		

Total Number of Cells 1377

Table 3 Chromosome Behavior at Anaphase I in Clones of CC-37-119

	Number of Bivalents	Number of Cells	Percentage		
	7	678	60.37%		
	6-1	151	13.45%		
2 groups	5-2	77	6.86%	25.30%	
	4-3	56	4.99%		28.05%
	5-1-1	5	0.45%		(315
	4-2-1	10	0.89%		cells)
3 groups	3-3-1	9	0.80%	2.59%	
	3-2-2	5	0.45%		
4 groups	3-2-1-1	2	0.18%	0.18%	
	8	10	0.89%		
	6	22	1.96%		
	Cytomixis	4	0.36%		
	Unequal Segregation	26	2.32%		
	Precocious Division	12	1.07%		
	Chromatin Bridge	41	3.65%		
	Lagging Chromosome	15	1.34%		

Total Number of Cells 1123

cytomixis were both observed at this stage. Approximately 28.05% of the total 1123 cells examined at anaphase I had abnormal groupings of dyad chromosomes (Fig. 17 and Fig. 18). The grouping patterns seem to follow those observed at metaphase I. Five cells or 0.36% of the total contained extra chromosomal materials which may have resulted from cytomixis at earlier stages (Fig. 44, Fig. 45 and Fig. 46).

Other types of cytological abnormalities at anaphase I include: chromatin bridge (3.65% out of the total 1123 cells observed) (Fig. 48), lagging chromosomes (1.34%) (Fig. 46), unequal segregation (2.32%) and precocious division (1.07%) (Table 3).

Extra daughter nuclei are the main taype of irregularities observed at telophase I (Table 4). The presence of extra daughter nuclei may be related to earlier irregularities, such as multipolar cell division and cytomixis. Chromatin bridges sometimes remain intact through telophase I.

A prophase II, chromosomes showed a tendency to separate into several groups in some cells (Fig. 19). Chromosomal fragments were also observed.

A total of 957 cells were analyzed at metaphase II (Table 5). Two hundred eighty eight cells or 30.09% exhibited multipolar cell division with supernumerary metaphase plates in pretty much the same patterns as metaphase I (Fig. 20, Fig. 21, Fig. 22 and Fig. 23). Some microcells had one to six chromosomes, but showed typical X-shaped metaphase II chromosome morphology and formed the typical metaphase plates (Fig. 21, Fig. 22 and Fig. 23). The loss of chromosomes may be the results of multipolar

Abnormal Meiotic Behavior: Multipolar Cell Division (MPD)

Figure 20. Cell had two metaphase plates (5-2) at metaphase II. (1012x)

Figure 21. Two microcells at metaphase II, one with 4 dyads, the other with 3 dyads. Arrows showed cytokinesis. (867x)

Figure 22. One cell had normal metaphase II. The other cell had 6-1 grouping and began supernumerary cytokinesis (arrow) at metaphase II. (881x)

Figure 23. One microcell with only one X-shaped chromosomes at metaphase II. (1098x)

Figure 24. Chromosomes separated into 2 groups (4-3) and supernumerary cytokinesis occurred (arrows) at anaphase II. (867x)

Figure 25. Multipolar Cell Division (MPD) at anaphase II (5-2) and supernumerary cytokinesis occurred (arrows). (867x)

Figure 26. Chromosomes separated into 3 groups (3-3-1) at anaphase II. (728x)

Figure 27: Cell with 6 daughter cells at quartet stage. (867x)

Figure 28. Cell with 5 daughter cells and some micronuclei at the quartet stage. (535x)

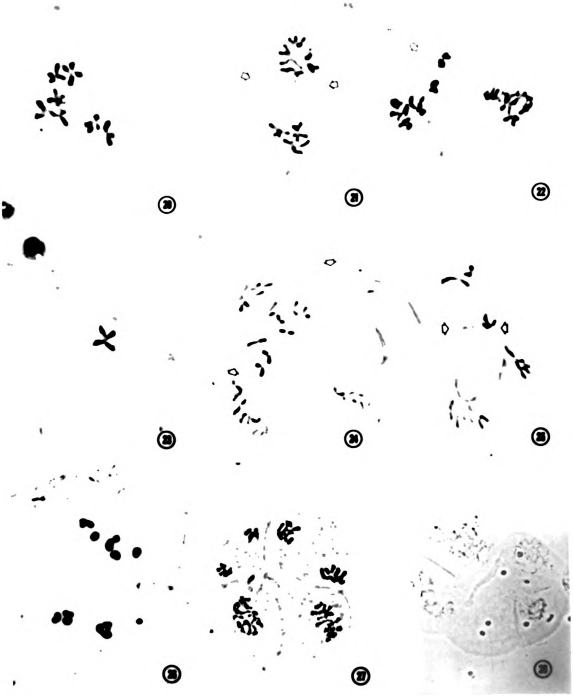

Table 4 The Number of Nuclei at Telophase I in Clones of CC-37-119

Number of Nuclei	Number of Cells	Percentage
2 (Normal)	211	65.73%
3	48	14.95%
4	35	10.90%
5	17	5.30%
Chromatin Bridge	10	3.11%

Total Number of Cells 321

Table 5 Chromosome Behavior at Metaphase II in Clones of CC-37-119

Number of Dyads		Number of Cells	Percentage		
	7	588	61.44%		
2 groups	6-1	119	12.43%	25.70%	30.09% (288 cells)
	5-2	71	7.42%		
	4-3	56	5.85%		
3 groups	5-1-1	7	0.73%	4.18%	
	4-2-1	11	1.15%		
	3-3-1	11	1.15%		
	3-2-2	11	1.15%		
4 groups	4-1-1-1	1	0.10%	0.20%	
	3-2-1-1	1	0.10%		
	8	11	1.15%		
	6	16	1.67%		
	Chromosomal Fragment	9	0.94%		
	Early Segregation	40	4.17%		

Total Number of Cells 957

Table 6 Chromcsome Behavicr at Anaphase II in Clones of CC-37-119

Number of Dyads		Number of Cells	Percentage		
	7	745	60.62%		
2 groups	6-1	180	14.65%	25.96%	27.91% (343 cells)
	5-2	78	6.35%		
	4-3	61	4.96%		
3 groups	5-1-1	9	0.73%	1.87%	
	4-2-1	6	0.49%		
	3-3-1	6	0.49%		
	3-2-2	2	0.16%		
4 groups	4-1-1-1	1	0.08%	0.08%	
	8	11	0.90%		
	6	23	1.87%		
	5	1	0.08%		
	4	1	0.08%		
	Unequal Segregation	10	0.81%		
	Chromatin Bridge	53	4.31%		
	Lagging Chromcsome	34	2.77%		
	Chromosomal Fragment	8	0.65%		

Total Number of Cells 1229

cell division and supernumerary cytokinesis which occurred at metaphase I and/or metaphase II and grouping of chromosomes may proceed into anaphase II. Early segregation of chromatids and chromosomes fragments were also observed at metaphase II.

Multipolar cell division continued at anaphase II (Fig. 24, Fig. 25 and Fig. 26). Chromosomes separated into several groups and migrated toward opposite poles. Out of a total of 1229 cells analyzed at anaphase II, different types of irregularities were recorded as follows: multipolar division 27.91%, chromatin bridge 4.31%, lagging chromosomes 2.77%, fragmentation 0.65% and unequal segregation 0.81% (Table 6). Sometimes more than one type of irregularity may occur in the same cell.

Most telophase II cells had two micronuclei. The remains of chromatin bridges were observed in some cells. A total of 1587 quartets were analyzed. Approximately 39.76% contained either micronuclei or more than four daughter cells (Table 8) (Fig. 27, 28 and Fig. 27). Out of a total of 4802 microspores examined after the quartet stage, 59.18% of them had a normal sized nucleus, 36.26% contained 2 to 10 nuclei of different sizes and another 3.94% carried very small nuclei (Table 9) (Fig. 49).

Pollen Mitosis

With a total of 266 metaphase cells and 88 anaphase cells analyzed, 50.00% and 38.64%, respectively, were observed to have normal pollen mitosis. Cells had normal seven X-shaped chromsomes at metaphase (Fig. 50), Abnormal Meictic Behavior

Figure 47. Four daughter cells at quartet stage. One daughter cell had extra chromcsomal materials (661x)

Figure 48. Chromatin bridge at anaphase I. (758x)

Figure 49. One microspore had a normal sized nucleus. The other had three small nuclei (799x)

Normal Pollen Mitosis

Figure 50. One microsporocyte had normal 7 X-shaped chromcsomes at metaphase (1447x)

Figure 51. One microsporocyte had normal 14 single-stranded chromcsomes at anaphase (951x)

Abnormal Pollen Mitosis

Figure 52. One microsporocyte had one X-shaped chromosome (951x)

Figure 53. One microsporocyte had 11 single-stranded chromoscmes (882x)

Figure 54. One microsporocyte had 3 single-stranded chromosomes, twc with 2 single-stranded chromosomes. One had 5 X-stranded chrcmosomes and 5 single-stranded chromcsomes (882x)

Figure 55. One microsporocyte had 8 X-shaped chromcsomes (882x)

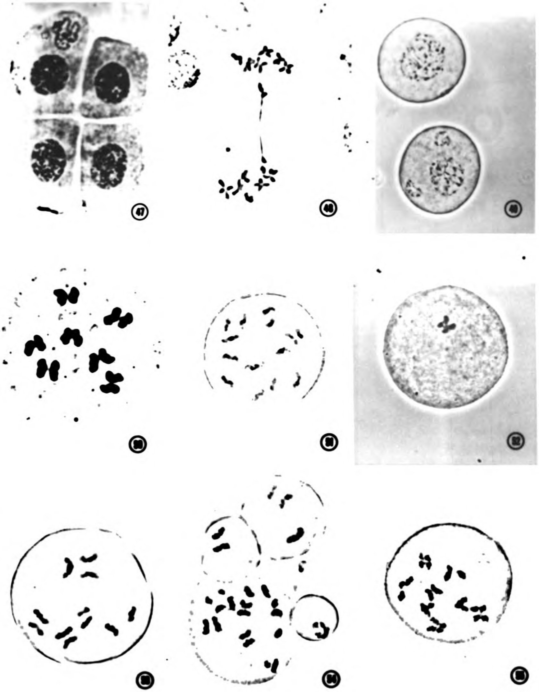

Table 8 The Number of Nuclei at Quartet Stage in Clones of CC-37-119

Number of Nuclei	Number of Cells	Percentage (%)
4	956	60.24
5	122	7.69
6	245	15.44
7	52	3.28
8	163	10.27
9	15	0.95
10	12	0.76
11	7	0.44
12	8	0.50
13	1	0.06
14	4	0.25
15	1	0.06
16	0	0.00
17	1	0.06

Total Number of Cells 1587

Table 9 The Number of Nuclei at Microspore Stage in Clones of CC-37-119

Number of Nuclei	Number of Cells	Percentage (%)
1 (Normal)	2842	59.18
2	1082	22.53
3	344	7.16
4	160	3.33
5	92	1.92
6	37	0.77
7	9	0.19
8	12	0.25
9	2	0.04
10	2	0.04
11	1	0.02
One Small Nuclei	189	3.94
Chromatin Bridge	30	0.62

Total Number of Cells 4802

and normal 14 single-stranded chromosomes at anaphase (Table 10 and Table 11) (Fig. 51). The other cells might have different numbers of chromosomes or chromosome fragments (Table 10 and Table 11) Fig. 52, Fig. 53, Fig. 54, Fig. 55 and Fig. 56). Multipolar mitosis was also observed in pollen grains. (Fig. 54, Fig. 57 and Fig. 58).

Pollen Viability

Pollen viability was examined by the staing reaction with I_2-KI solution. Out of a total of 2783 pollen grains, 58.07% of them were stained darkly and believed to be viable (Table 12) (Fig. 59 and Fig. 60). They were arbitrarily classified into 2 sized groups: the large sized group presumably are those with a normal or more than normal number of chromosomes (Fig. 59); and the small sized group are presumably those to have fewer than the normal number of chromosomes (Fig. 60).

Table 10 Chromosome Behavior at Metaphase of Pollen Mitosis in Clones of CC-37-119

Number of X-Shaped Chromosomes	Number of Cells	Percentage (%)
1	15	5.64
2	5	1.88
3	6	2.26
4	6	2.26
5	7	2.63
6	25	9.40
7 (Normal)	133	50.00
6 X-Shaped + 2 Single-Stranded Chromosomes	24	9.02
5 X-Shaped + 4 Single-Stranded Chromosomes	17	6.39
3 X-Shaped + 8 Single-Stranded Chromosomes	1	0.38
8	12	4.51
6 X-Shaped + 1 Single-Stranded Chromosomes	3	1.13
2 X-Shaped + 1 Single-Stranded Chromosomes	1	0.38
7 X-Shaped + 1 Single-Stranded Chromosomes	1	0.38
7 X-Shaped + 2 Single-Stranded Chromosomes	1	0.38
6 X-Shaped + 3 Single-Stranded Chromosomes	1	0.38
8 X-Shaped + 1 Single-Stranded Chromosomes	2	
7 X-Shaped + Fragment	6	2.26

Total Number of Cells 226

Table 11 Chromosome Behavior at Anaphase of Pollen Mitosis in Clones of CC-37-119

Number of Single-Stranded Chromosomes	Number of Cells	Percentage (%)
1	5	5.68
2	4	4.54
3	4	4.54
4	4	4.54
5	3	3.41
6	2	2.27
7	2	2.27
10	1	1.14
11	2	2.27
12	5	5.68
13	9	10.23
14 (Normal)	34	38.64
15	3	3.41
16	6	6.82
16 + Fragment	2	2.27
14 + Fragment	2	2.27

Total Number of Cells 88

Abnormal Pollen Mitosis

Figure 56. One microsporocyte had 16 single-stranded chromosomes and one chromosome fragment (1122x)

Figure 57. Miltipolar mitosis in microsporocyte (675x)

Figure 58. Multipolar mitosis in microsporocyte (900x)

Viability of pollen grains

Figure 59. Normal sized, viable pollen grain was darkly stained by I_2-KI solution (591x)

Figure 60. Small sized, viable pollen grains were darkly stained by I_2-KI solution (591x)

Figure 61. Non-viable pollen grains were not stained by I_2-KI solution (591x)

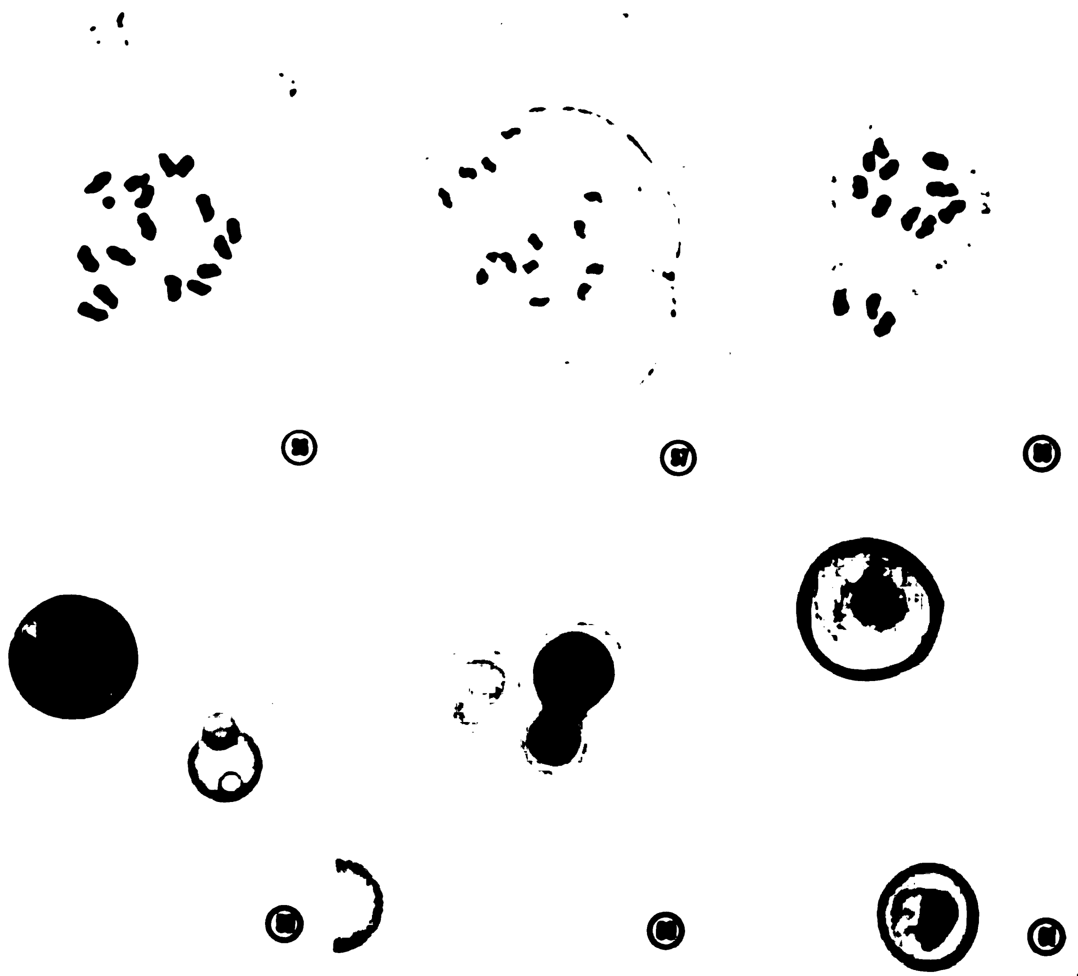

Table 12 Viability of Pollen Grains in Clones of CC-37-119

	Number of Cells	Percentage (%)	
Fertile Pollen Grains			
Normal Size	1314	47.22%	58.07%
Small Size	302	10.82%	
Non-Fertile Pollen Grains			
Normal Size	683	24.54%	41.93%
Small Size	484	17.39%	

Total Number of Cells 2783

DISCUSSION

Inheritance of Colchicine-Induced Irregularities

During his study of colchicine-treated diploid Fairway crested wheatgrass, Tai (1964, 1970) discovered two plants CC-37-119 and CB-9-85, to have exceptional irregular cytological behavior, e.g. multipolar cell division, supernumerary cytoplasmic cleavage, unequal segregation, chromatin bridges, lagging chromosomes, and micronuclei at different stages of meiosis. Both plants had been treated with 0.1% colchicine solution for 12 hours. Perhaps due to low colchicine concentration and short duration of exposure, polyploidy did not occur; instead both plants exhibited a high degree of spindle abnormalities (Sakharov, et al, 1969). In his study of progeny of CB-9-85, Chen (1976) observed the same types of irregularities. Plant CC-37-119 has been propagated asexually since 1963. Through the years, some types of cytological irregularities persisted in its clones. Effects of colchicine treatment seem to be permanently maintained in the plants without restitution. They are inheritable, transmitted from generation to generation. Tai (1970) proposed that to guide chromosome migration in angiosperm plants, an organelle which he termed "spindle organizer" would move toward opposite sides of the cell and establish themselves as poles. If "spindle organizer" is broken, multipolar cell division (MPD) results. The current study seems to further strengthen this model. Since "spindle organizer" is a physical entity, it can be broken by physical or chemical treatments. Attraction between broken pieces of "spindle organizer" and the chromosomes would result in multipolar cell division.

Multipolar Cell Division

Multipolar cell divisions have been reported to occur spontaneously in both animals or plants or can be induced artificially. In the present study of clones of CC-37-199, multipolar cell division occurred at every meiotic stage from late diakinesis through the quartet stage, and during pollen mitosis, too.

Thompson (1962) suggested that after "complement fractionation", each group of chromosomes is an independent unit. Tai (1970) also emphasized that the behavior of each micrometaphase plate in a cell with multipolar meiosis is an independent event. Their points are further manifested in the present study. All possible numerial combinations of chromosome groupings have been observed in this study. Multipolar cell division (MPD) has been observed at every meiosis stage. From the number of cells exhibited, MPD seems to be dropped from MI and MII to AI and AII. However, a X^2 test showed this difference is not statistically significant (Table 7). This indicates that there has been no restitution of "spindle organizer". Even the chromosomes migrate toward the poles together and become situated close to one another. They remain as a separate group and do not regroup. The chromosome movement of different groups showed certain degrees of asynchronization. One group may remain in metaphase II,

Table 7 Multipolar Cell Division (MPD) at Different Meiotic Stages in Clones of CC-37-119

Stage	Cells with MPD	Cells without MPD	Total Number of Cells
Diakinesis	313	801	1114
Metaphase I	413	964	1377
Anaphase I	315	808	1123
Metaphase II	288	669	957
Anaphase II	343	886	1129
Total	1672	4128	5800

$$\bar{p} = \frac{1672}{5800} = 0.2883$$

$$X^2 = 1.99 < 9.448 \text{ (Non-significant)}$$

while another group proceeds to anaphase II (Fig. 24). One cell had an intact chromosome complement and 2 microcells as results of MPD (Fig. 22). They all came from the same primary microsporocyte, however, multipolar cell division occurred in one secondary microsporocyte without influencing the other (Fig. 22 and Fig. 27). All the above phenomena provide further evidence to substantiate that multipolar cell division in each group is an independent event, and that each micrometaphase plate behaves as an independent unit with its own movement. As the number of groupings increases, the grequency of the occurrence decreases. Two-group separation occurred more frequently than three-group or four-group separation.

The independent behavior of multipolar cell division also suggests the same conclusion as Tai's (1907), namely, an organelle exists which functions analogously to the centriole of animal cells. Darlington and Thomas (1937) proposed that "pole determinants" exist as diffuse particles. They congregate when spindle poles are normally formed, but remain separate at other times. They have the function of centrosomes and possess their continuity as single and coherent bodies. The spindles are developed through the cooperation between centromere and pole determinants. Swanson and Nelson (1942) considered the extra-pole determinants to be a prerequisite for multipolar meiosis. Walters (1958) described the "spindle organizer" that may be a compound structure, usually single and following the regular division cycle. However, it may undergo supernumerary division under extraordinary conditions into substructures. Based on the observations of "complement fractionization", Thompson

(1962) proposed two possible origins of multipolar cell divisions. One is that the chromosomes are guided by a split spindle; the other is that the chromosomes are first grouped and then form their own split spindles. The microtubule-organizer-center described by Pickett-Heaps (1969) is a diffuse, amorphous, osmiophilic and differentiated cytoplasmic region active in microtubule formation. It has the ability to initiate polymerization and depolymerization of microtubules and to determine their orientation.

All above models seem to be in agreement with and can be used to describe our own hypothetical organelle, the spindle organizer (Tai 1970). Tai describes the "spindle organizer" as a single organelle which controls the formation of spindle apparatus and the migration of chromosomes. There is only one spindle organizer in a male or female gamete. The spindle organizer is genome specific. After fertilization, spindle organizers from male and female gametes may either fuse, differentially disintegrate, or remain disjoined (Orton and Tai 1977). If they do not fuse or disintegrate, multipolar cell division arises. Also, the spindle organizer can be broken spontaneously or artificially. When it is broken, multipolar cell division also occurs. It is also a possible mechanism for the occurrence of lagging chromosomes, unequal segregation and early separation which were observed in this investigation and occur frequently in hybrids. Lack of attraction between chromosomes of one species and spindle organizer of a different origin will result in large numbers of laggards.

The significance of multipolar cell division in the evolution of chromosome number has been discussed by several authors. "Split spindle" are known to be a mechanism responsible for variation in chromosome numbers in somatic tissue of many species. (Li and Tu 1947; Huskins 1948; and Vaarama 1949). Thompson (1962) pointed out that "complement fractionation" produces gametes with extremely variable chromosome numbers. If gametes have lost or gained too many chromosomes, they appeared to be aborted. However, some of these gametes will be functional and kept in the population due to a tendency for the formation of balanced or less unbalanced genome. Thompson also proposed that multipolar cell division provides an evolutionary mechanism for decreasing the ploidy level. After careful observation of microsporogenesis of colchicine-treated diploid Agropyron cristatum and inter- and intra-specific hybrids of Mimulus glabratus, Tai (1970), Tai and Vickery (1970, 1972) recognized the same evolutionary significance of multipolar cell division. Tai (1970, 1971) further pointed out the possibilities of using multipolar cell division in the studies of plant genetics and plant breeding. Induction of haploidy by multipolar cell division followed by doubling of the chromosome complement may possibly produce individuals which are homozygous for every gene locus.

The present observations of CC-37-119 clones show that multipolar cell division is the major irregularity which occurred in 1672 of a total of 2176 abnormal cells (76.84%). This indicates that multipolar cell division is the main contribution of variable chromosome numbers during pollen mitosis. Figure 60 shows darkly stained, but smaller sized pollen grains. They represent pollens having fewer chromosomes but which may be viable. All these explain that multipolar cell division appears to provide a possible mechanism for the production of aneupolid microspores.

Cytomixis

Cytomixis is a relatively widespread phenomenon. It has been reported in various plant species by many authors (Katterman 1933; Kihara and Lilienfeld 1934; Sarvella 1958; Kamra 1960; Takowska 1960, 1965, 1966; Romanov and Orlova 1971; Tai 1972; Kosova 1973; Syemyarykhina and Kuptsow 1974; Shkutina and Kozlovskaya 1974; Cheng 1974). In pollen mother cells, cytomixis occurs most frequently in the early prophase I, and rarely in metaphase I and anaphase I (Katterman 1933; Romanov and Orlova 1971; Tai and Vickery 1972; Shkutina and Kozlovskaya 1974). Romanov and Orlova (1971) suggested that chromosomes or parts of nuclei of donor cells migrate through the "cytomictic channel" (Weiling 1965; Heslop-Harrison 1966) into the adjacent recipient cell, and remain in the form of that stage. The migration could be in multiple directions or sometimes in a single direction (Cheng 1974).

In the present study, the actual processes of cytomixis — the migration of chromosomal materials from the donor cell into the adjacent cell — was also observed at early prophase I, usually before pachytene (Fig. 29, Fig. 30, Fig. 31 and Fig. 32). The consequences of cytomixis, i.e., the addition or reduction of chromosome materials were observed at various meiotic stages (Figures 33-46). The extra threads have been proved to be chromosomal materials by their positive reaction toward the Feulgen stain. They usually appear as long threads with different degrees of condensation at early meiosis. As meiosis proceeds in the host cells, these extra materials also assume regular division progress, e.g. further condensation, migration and lining up at the equator. Usually the regular and extra chromosomes move asynchronously within the same protoplast (Fig. 45 and Fig. 46). While with the extra materials move at a slower pace, sometimes, they can catch up and show typical chromosome morphology at metaphase I and/or anaphase I. Subsequently, cells with various chromosome number at metaphase II, anaphase II and pollen mitosis were found. The presence of micronuclei at the quartet stage (Fig. 47) and in microspores was also observed. Darkly stained, different sized pollen grains, representing more or fewer than normal chromosome numbers, may be viable. However, aneuploid gametic chromosome number can be caused by other processes in addition to cytomixis. The importance of cytomixis in the evolution of chromosome number in plants has been recognized by many authors. (Katterman 1933; Kihara and Lilienfeld 1934; Persival 1930; Sarvella 1958; Kamra 1960; Romanov and Orlova 1971; Tai 1967; Tai and Vickery 1972; Cheng 1974).

Generally, the chromosomes, transferred through cytomitic channels, revealed severe structural damage, with subsequent disintegration and degeneration (Katterman 1933, Weiling 1965, Tarkowska 1966; Romanov and Orlova 1971; Shkutina and Kozlovskaya 1974). Similar phenomenon was observed in this study. However, it is possible that the chromosomes pass through usually the regularthe cytomitic channels undamaged and intact and are incorporated into the nucleus of the recipient cell (Romanov and Orlova 1971) (Fig. 36, Fig. 37 and Fig. 42). Thus, cytomixis is a possible mechanism that increases and decreases the number of chromosomes of future gametes. Presumably a large number of chromosomes were involved in the donor and recipient cells. They appear to degenerate or form aborted gametes. However, if just a small number of chromosomes are lost, the hypocells still may survive and form unbalanced but functional gametes. Therefore, cytomixis is a possible mechanism for the cause of aneuploidy evolution; i.e. hyperploid and hypoploid (Romanov and Orlova 1971; Tai 1967; Tai and Vickery 1972). Romanov and Orlova (1971) and Cheng (1974) also suggested that cytomixis could be the cause of multinuclear cells which may in turn give rise to polyploids.

The cause of cytomixis remains controversial and unsolved. Some suggested the phenomenon of cytomixis is an abnormal condition which could be caused by mechanical damage or environmental pressure (Takats 1959; Tarkowska 1960, 1965, 1966), by fixing solution (Takats 1959), by abnormal environment conditions (temperature, rainfall, drought, radiation) (Tung, et al 1973 in Cheng 1974), by disease and weakening (Fraser 1914), or by plant hybridization (Woodworth 1929). However, Cheng (1974) strongly suggested that cytomixis is a normal physiological phenomenon; other external factors, e.g., temperature, fixation -- etc., may enhance its occurrence. The results of this study cannot unequivocally explain the cause of cytomixis. Further investigations have to be done.

SUMMARY

The cytology of colchicine-treated, diploid *Agropyron cristatum*, CC-37-119, was studied. The effects of colchicine treatment were found to be inheritable. Different types of cytological irregularities were observed, such as multipolar cell division, cytomixis, chromatin bridges, lagging chromosomes, unequal segregation, precocious division and others. These abnormalities appear to result in the reduction of pollen viability. They may provide mechanisms for the production of aneuploid gametes which could in turn lead to aneuploid plants.

LIST OF REFERENCES

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Abraham, S., Cytologia, 39, 537 (1974).

Amer, S.A., and O.R. Farah, Cytologia, 39, 507 (1974).

Bammi, R.K., Nature, 208, 608 (1965).

Baltzer, F., Aich Zellforsch, 2, 549 (1909).

Bayliss, M.W., Nature, 246, 529 (1973).

Bopp-Hassenkamp, G., Exptl. Cell Res., 18, 182 (1959).

Boveri, T., Zellen-Studien 2 Jena: G. Fisher, (1888).

Cheng, K.C., Acta Genetics Sinica, 1, 117 (1974).

Cheng, Y.R., "Multipolar Cell Division in *Agropyron cristatum* (L.) Gaertn. (*Gramineae*)", Ph. D. Thesis, Michigan State University (1976).

Darlington, C.D., and P.T. Thomas, Ann. Bot., 1, 747 (1937).

Dewey, D.R., Amer. J. Bot., 61, 181 (1974).

Fraser, A.C., Ann. Bot., 28, 633 (1914).

Gates, R.R., Ann. Bot., 25, 909 (1911).

Gläss, E., Chromosoma, 7, 655 (1956).

Gläss, E., Chromosoma, 8, 468 (1957).

Heslop-Harrison, J., Ann. Bot. 30, 221 (1966).

Hoxmark, R.C., and O. Nordby, Hereditas, 76, 239 (1974).

Huskins, C.L., J. Heredity, 39, 311 (1948).

Huskins, C.L., and K.C. Cheng, J. Heredity, 41, 13 (1950).

Kamra, O.M.P., Hereditas, 46, 592 (1960).

Katterman, G., Planta, 18, 751 (1933).

Kihara, H., and F. Lilienfeld, Japan. J. Genet., 10, 1 (1934).

Knudson, O., Ark. Zool., 11, 119 (1958).

Kornike, M., S.-B. Niederrhein. Gesundh. Natur-und Heilk. Zu Bonn, 14 (1901).

Koscva, A.I., Izv. Akad. Nauk. Mold. SSR. Ser. Biol. Khim. Nauk., 5, 9 (1973).

Levis, A.G., and G. Martin, Exptl. Cell Res., 31, 448 (1963).

Li, H.W., and D.S. Tu, Bot. Bull. Acad. Sinica, 1, 173 (1947).

Mazia, D., "Mitosis and the Physiology of Cell Division", Vol. III, Academic Press, New York (1961).

Nielsen, E.L., and J. Nath, Amer. J. Bot., 48, 345 (1961).

Nimnoi, P., "Effect of Potato Spindle Tube Virus on Pollen Function", Ph. D. Thesis, Michigan State University. (1974).

Ofterbo, R., and I. Wolf, Exptl. Cell Res., 48, 39 (1967).

Orton, T.J., and W. Tai, Can. J. Bot., 55, 3023 (1977).

Östergreen, G., Hereditas, 36, 371 (1950).

Percival, J., J. Genetics, 22, 201 (1930).

Pickett-Heaps, J.D., Cytobios., 1, 257 (1969).

Rizzoni, M.F., F. Palitti, and P. Perticone, Chromosoma, 45, 151 (1974).

Romanov, I.D., and I.N. Orlova, Genetika, 7, 5 (1971).

Rustard, R.C., Experientia, 15, 323 (1959).

Sadasivarah, R.S., and Magoon, Genet. Iberica, 17, 103 (1965).

Sakharov, V.V., R.N. Platoniva, E.F. Mekonova, and V.I. Ol'Khovenko, Genetika, 5, 89 (1969).

Sarvella, P., Cytologia, 23, 14 (1958).

Schulz-Schaeffer, J., and P. Jurasits, Amer. J. Bot., 49, 940 (1962).

Shkutina, F.M., and V.F. Kozlovskaya, Genetika, 10, 5 (1974).

Sinota, Y., Tokyo Bot. Mag., 36, 99 (1922).

Swanson, C.P., and R.R. Nelson, Bot. Gaz., 104, 273 (1942).

Syemyarykhina, S.Y., and M.S. Kuptsow, Vyestsi. Akad. Navuk. B. SSSR Syer. Biyal. Navuk., 4, 43 (1974).

Tai, W., "Morphology, Fertility and Cytology of Diploid and Colchicine Induced Tetraploid Fairway Crested Wheatgrass", M.S. Thesis, Utah State University (1964).

Tai, W., "A Cytogenetic Investigation of Diploid Members of the *Mimulus glabratus* Complex", Ph. D. Thesis, University of Utah (1967).

Tai, W., Amer. J. Bot, 57, 1160 (1970).

Tai, W., and R.K. Vickery, Jr., Evolution, 24, 670 (1970).

Tai, W., Genetics, 68, s66 (1971).

Tai, W., and R.K. Vickery, Jr., Amer. J. Bot., 59, 488 (1972).

Takats, S.T., Chromosoma, 10, 430 (1959).

Tarkowska, J., Acta Soc. Bot. Polon., 29, 149 (1960).

Tarkowska, J., Acta Soc, Bot. Polon., 34, 27 (1965).

Tarkowska, J., Acta Soc. Bot. Polon., 35, 25 (1966).

Therman, E., and S. Timonen, Hereditas, 36, 393 (1950).

Therman, E., and S. Timonen, Hereditas, 40, 313 (1954).

Thompson, M.M., Amer. J. Bot., 49, 575 (1962).

Timonen, S., and E. Therman, Cancer Res., 10, 431 (1950).

Tischler, G., "Handbuch der Pflanzennanatomie", Bd. II/2, Berlin: Gebr. Borntrager (1934).

Upcott, M., J. Genet., 39, 79 (1939).

Vaarama, A., Hereditas, 35, 136 (1949).

Vasek, F.C., Amer. J. Bot., 49, 536 (1962).

Walters, M.S., Amer. J. Bot., 45, 271 (1958).

Weiling, F., Planta, 67, 182 (1965).

Weimarck, A., Hereditas, 74, 103 (1973).

Wilson, G.B., J. Heredity, 41, 227 (1950).

Woodworth, R.H., Bot. Gaz., 87, 331 (1929).