ABNORMAL CHROMOSOME SEGREGATION DURING MICROSPOROGENESIS IN *AGROPYRON CRISTATUM*

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Abstract: *Agropyron cristatum* CB-9-85, was obtained from a seed treated with a 0.1% colchicine solution. The plant which displayed multipolar cell division was grown in the field to permit open pollination. The effects of colchicine-treatment were found to be inheritable. All F_1 progeny of CB-9-85 showed abnormal chromosome segregation irrespective of their chromosome number, 7 II or 7 II+1 I. Multipolar cell division, univalents, unequal disjunction, precocious division, chromosome fragments, chromosome bridges and micronuclei were found related to the formation of multinucleated and aneuploid pollen. Pollen fertility was related to the size of the grain, with small grains displaying low fertility. Seed fertility in plants which did not form quartets was zero. Multipolar cell division is the only mechanism which can be demonstrated by experimental data that can explain genome segregation and chromosome elimination in natural populations and a scheme is presented.

INTRODUCTION

The abnormality of spindle apparatus results in the appearance of two or more metaphase plates within a single cell in the process of either mitosis or meiosis. It can be found in a great number of plant species (Davis, 1901; Aver, 1957; Bammi, 1958) and has been reported to occur in plant tissue culture (Inoue, 1952). In animals it has been seen in various tissues (Bower, 1922; Koller, 1934; Benazzi-Lentati, 1970) as well as in tumor cells (Heneen *et al.*, 1970) and tissue cultures (Fenter and Porter, 1965; Bayliss, 1973). Split spindles produced daughter cells with different chromosome numbers.

Spindle abnormalities have been found to occur spontaneously or to be induced by radiation (Martini and Bozzini, 1966), cold treatment (Huskins and Cheng, 1950), chemicals (Eigsti and Dustin, 1955; Kihlman, 1966), virus (Levan and Hauschka, 1953; Halkka and Halkka, 1969) and other treatments (Abraham, 1974). Its spontaneous occurrence has been reported in haploid, diploid, polyploid and hybrid plants (Martini and Bozzini, 1966; Gottschalk and Miletinovig, 1973; Amer and Farah, 1974; Srivastava, 1974), and in animals (Mazia *et al.*, 1960; Martin and Sprague, 1970), but most frequently in polyploids and hybrids of both plants and animals. Success in multipolar cell division induction is related to the strength of a given treatment, following the correlation between the concentration of colchicine and mitotic spindle abnormalities (Östergreen, 1950). Both spontaneous and induced multipolar division can be transmitted from generation to generation (Vasek, 1962).

The significance of multipolar cell division has been discussed by a number of authors. Darlington and Thomas (1937) proposed that the spindles were developed through cooperation between centromere and pole determinants. Similiarly, Swanson and Nelson (1942) considered extra-polar determinants to be a prerequisite for multipolar meiosis. The spindle organizer and

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split spindles described by Walters (1958) are comparable with pole determinants. She indicated that two spindle organizers behaved independently in each of many spindles and the number of chromosomes in each group was somewhat proportional to the size of the spindle, which in turn determined the size of the cells in a quartet. From the study of meiotic behavior in polyploid *Rubus* hybrids, Thompson (1962) observed that each group had various numbers of chromosomes and that they operated independently within meiotic and mitotic cells. She proposed that the significance of complement fractionation was related to unusual breeding results and that it played an important role in the evolutionary process. After careful observation of microsporogenesis of colchicine-treated diploid *Agropyron cristatum*, Tai (1970) presented a new model for the sindle organizer. He suggested that the spindle organizer was a cell organelle whose function was to guide the migration of chromosomes, that the spindle organizers were genome specific, and that they attracted their own chromosomes in a hybrid and separated different genomes into different daughter cells. Moreover, he postulated that evolution of chromosome number is reversible and that multipolar cell division provided a means by which chromosome number could be reduced to a lower ploidy level, the formation of a polyhaploid. Tai (1971) also suggested that multipolar cell division and subsequent doubling of the chromosome number could obtain an individual homozygous for every gene locus. Genome separation via abnormalities of the spindle has since been observed by many authors (Palitti and Rizzoni, 1972; Pera and Rainer, 1973; Rizzoni *et al.*, 1974).

In the present study, microsporogenesis in the F_1 progeny of CB-9-85 has been examined. The objectives of this investigation are: to observe the chromosome behavior of the F_1 progeny of CB-9-85, to correlate multipolar meiosis with pollen fertility, and seed fertility, and to evaluate the significance of the change in chromosome number brought about by multipolar cell division.

MATERIALS AND METHODS

Fairway crested wheatgrass, and inbreeding important plant, has been identified as *Agropyron cristatum* (L.) Gaertn, *A. cristatiforme* Sarkar, *A. dagnae* Grass, and *A. pictiniforme* Roem. *et* Schult (Taylor and McCog, 1973). Diploid ($2n=14$) *A. cristatum* was treated with colchicine and the progeny produced were called CB-9-85, which was found to have multipolar cell division (Tai, 1970). Seeds harvested from CB-9-85 were grown in open field nurseries of the Department of Botany and Plant Pathology, Michigan State University. Some of the seeds produced vigorous plants; some weak plants; and some failed to germinate. These plants are probably the result of outcrossing for the fertility of selfed CB-9-85 is less than 5%. Young spikes for cytological observation were collected around 6 a.m. and fixed immediately in Newcomer's solution. Root tips for chromosome counts were fixed in Farmer's solution. For cytological observation, the materials were squashed and stained with acetocarmine. Pictures were taken with a Zeiss microscope.

In the field, the developmental stages of the spikes were asynchronous and the stage of development often influenced the success of pollination. The best material (spikes) for cytology came from the middle of the flowering period. Spikes from early or late blooming plants were often nonfertile. During the blooming period, two spikes were collected from each plant to check pollen viability. Pollen viability was examined with an iodine solution. Pollen viability was examined with an iodine solution. Pollen grains, which were fully expanded and darkly stained at 100×, were rated viable. All counts were made on the first days of flowering. For fertility studies, five spikes were collected from each plant after the seed was well-developed but before the spike shattered. Fertility was measured by counting the number of seedlings which germinated in a petri dish on wet filter paper.

RESULTS

1. Multipolar cell division

Forty-five progeny of CB-9-85 were observed. Most of the plants were diploid with seven bivalents. Only five plants were trisomic with 7 II + 1 I or 6 II + 1 III. Regardless of chromosome configuration, all of the plants display multipolar cell division during microsporogenesis. The percentage of multipolar cell division occurring in each individual plant was different, and the frequency of multipolar cell division observed in meiosis also varied from one developmental stage to another. Multipolar cell division affected the seed and pollen fertility to a certain degree. But other factors, such as chromosome fragments, chromosome bridges, lagging chromosomes, and precocious division also affected those processes (Table 1). Detailed observations of multipolar cell division for different stages of microsporogenesis is described in the following sections·

Table 1. Chromosome behavior at anaphase I in F_1 progeny of CB-9-85

F_1 progeny	Normal	MCD*	Chromosome fragments	Laggards	Chromosome bridges	Precocious division	Unequal disjunction
Normal	295(39.1)†	364(48.2)	14(1.9)	35(4.6)	23(3.0)	11(1.5)	13(1.7)
Trisomic	38(22.7)	93(55.7)	4(2.4)	20(12.0)	9(5.4)‡	3(1.8)	—

* denotes multipolar cell division.

† denotes % in parenthesis.

‡ three of them are chromosome bridge and fragment presented in the same cells.

A. Metaphase I

Multipolarity first becomes evident during the first meiotic metaphase of the seven bivalent pollen mother cell. Instead of the seven bivalents being oriented on one spindle and one metaphase plate, the chromosomes are attached to two or more spindles and metaphase plates. A high percentage of the metaphase cell have 2 and 3 spindles (Fig. 1) and sometimes as many as 4 were observed (Table 2).

Table 2. Chromosome grouping in F_1 progeny of CB-9-85 with seven bivalents

Meiotic stage	7	6-1	5-2	4-3	5-1-1	4-2-1	3-3-1	3-2-2	Other
Meta I	929 (48.1)*	102 (5.3)	71 (3.7)	128 (6.6)	58 (3.0)	168 (8.7)	132 (6.8)	96 (4.9)	249 (12.9)
Ana I	295 (44.8)	53 (8.0)	49 (7.4)	40 (6.1)	24 (3.6)	45 (6.8)	38 (5.8)	36 (5.5)	79 (12.0)
Meta II	353 (46.5)	70 (9.0)	70 (9.0)	82 (10.6)	6 (0.7)	46 (5.4)	29 (3.7)	38 (4.9)	79 (10.2)
Ana II	233 (58.7)	19 (4.8)	29 (7.3)	28 (4.5)	12 (3.0)	26 (6.6)	12 (3.0)	71 (4.3)	31 (7.8)

* denotes % in parenthesis.

In the seven bivalents, three categories of configuration were observed. The categories are: 1) normal cells with bivalents associated with a single spindle, 2) cells with two bivalent groupings (6-1, 5-2 and 4-3) oriented on two spindles, and 3) cells with three bivalent groupings (5-1-4, 4-2-1, 3-3-1 and 3-2-2) arranged on three spindles. Four and five bivalent groupings also may occur in one cell, but their frequencies were very low and extremely variable. The overall frequency of multipolarity as observed in metaphase I is 51.9% (Table2). The

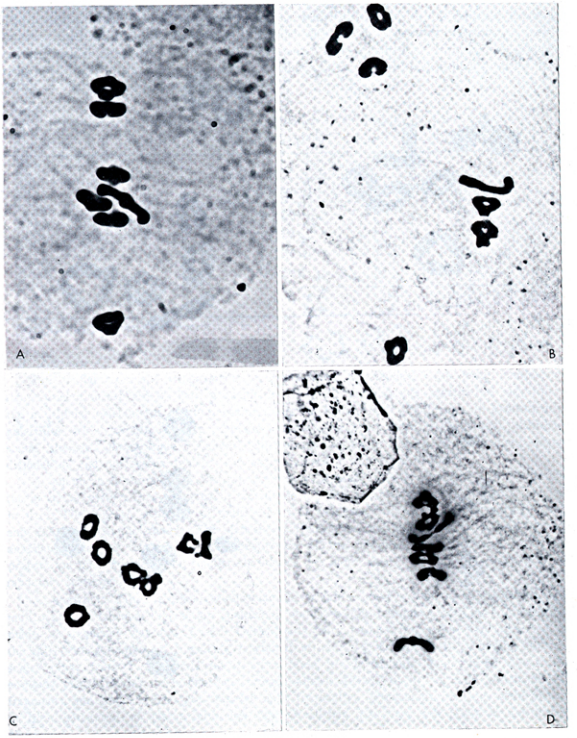

Fig. 1. Chromosome grouping at metaphase I in 7 bivalent plant.
A. 4-2-1 B. 3-3-1 C. 2-2-2-1 D. 6-1

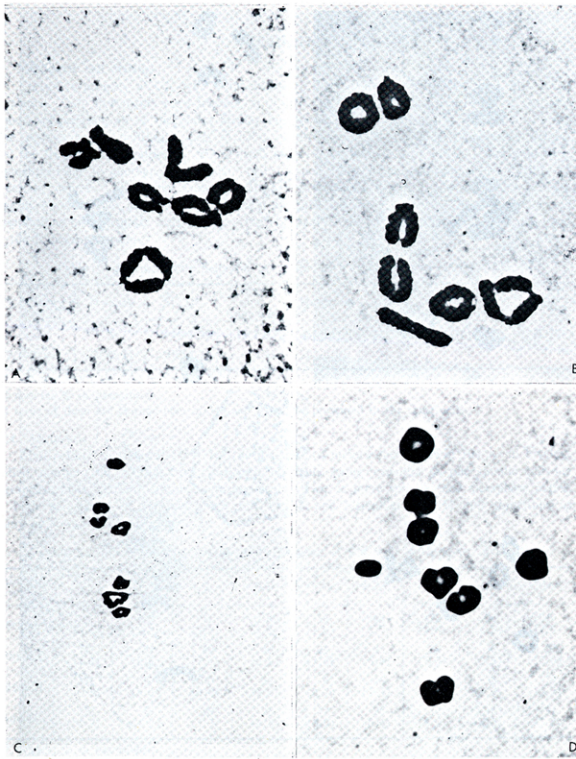

Fig. 2. Chromosome grouping at mataphase I in trisomic plants.
A. 4-2-1.4. (950×) B. 5.5-2 (1060×)
C. 3.5-3-1 (645×) D. 2-2-1-1-1-0.5 (1130×)

cases where multipolar spindles occurred in trisomic plants were more complicated (Fig. 2). The presence of an extra chromosome affects the whole distribution of chromosome groupings. In many cells the extra chromosome is connected with one of the seven bivalents to form a trivalent, but it often lies adjacent to the bivalents. The number of chromosome grouping ranges from one to six. The percentage of multipolarity for individual plants varies from 56.0% to 79.0% and averages 69.7% (Table 3).

Table 3. Abnormal chromosome segregation in F_1 progeny of CB-9-85 with 7 II+1 I or 6 II+1 III chromosomes

Plant number	Abnormality (%)	
	Metaphase I	Anaphase I
A-1-9	200(79.0)*	23(78.3)
A-1-10	207(75.4)	52(71.2)
A-1-27	84(56.0)	20(80.0)
A-1-31	73(68.5)	34(64.7)
Mean (%)	69.7	73.6

* denotes the percentage of abnormality in parenthesis.

B. Anaphase I

The configuration and arrangements of chromosomes at anaphase I are more complicated than those of metaphase I. During anaphase I (Fig. 3), chromosome segregation may follow the multipolar orientations of chromosome pairs at metaphase I (Table 2). In addition irregularilities which occurred during prophase become more pronounced at anaphase I. Chromosomal changes, chromosome fragments, chromosome bridges, lagging chromosomes, unequal disjunction, and precocious division, can be recognized at this stage (Table 1).

At anaphase I, chromosomes at one pole may separate into several groups which follow the grouping pattern that appeared in metaphase I. The separating groups are often unequal in size. The average frequency of multipolarity at anaphase I is 55.2% in the normal plants and 73.6% in trisomic plants. The inconsistent frequencies multipolarity at metaphase I versus anaphase I may be related to the configuration of the chromosomes in the cell during the preparation of the slide. The smaller the space, the more easily the chromosomes may be randomly scattered.

As shown in Table 1, other chromosome abnormalities always seem to be associated with multipolarity. Unequal disjunction completely dominated the trisomic plants, but in the normal plants its frequency was only 1.7%. Occasionally chromosome fragments were observed in the cells with a number of fragments varying from one to several per cell. Lagging chromosomes range from 1 to 3 and occur more often in the trisomic plants (12%) than in the euploidy plants (4.6%). Chromosome bridges almost always occur in cells which display fragments, and single bridges are observed most frequently. Precocious divisions were present in both categories of plants, although their frequency was very low (1.5% in the normal plants and 1.8% in trisomics).

C. Dyad

A cell's chromosome groupings can be followed through to the telophase and may result in the presence of two or more nuclei in the interphase cell. However, the number of nuclei per cell may not be equal to the number of chromosome groupings observed in anaphase I.

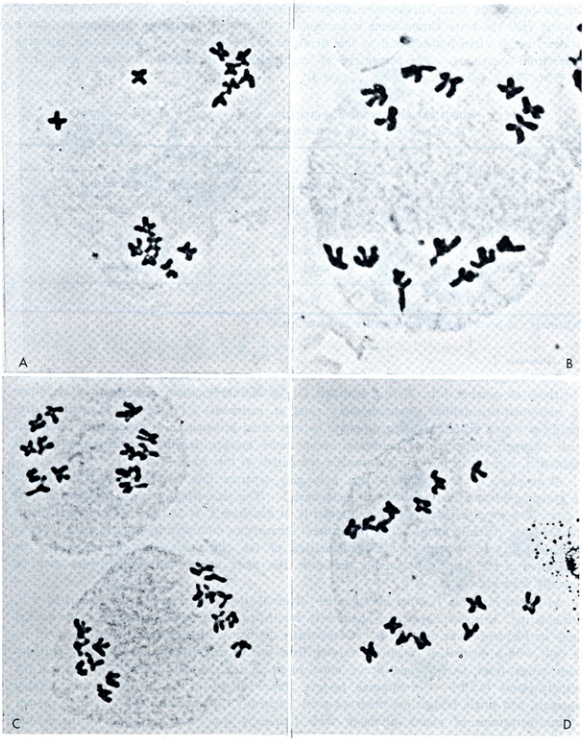

Fig. 3. Chromosome grouping at anaphase I.
A. 6-1 and 6-1 (850×) B. 3-2-2 and 3-2-2 (1000×)
C. 8-7 (950×) D. 4-2-1 and 4-2-1 (800×)

Table 4. The formation of supernumery nuclei at dyad and quartet stages in F_1 progeny of CB-9-85

Plant number	Supernumery nuclei	
	Dyad (3 or more)	Quartet (5 or more)
A-1-13	132(33.5)*	601(66.9)
A-1-17	9(8.7)	27(22.3)
A-1-44	156(65.4)	162(65.1)
A-1-45	58(29.8)	44(55.4)
A-1-9	92(46.8)	303(73.2)
A-1-10	95(51.3)	91(38.9)
A-1-27	41(39.5)	221(57.9)
Mean (%)	39.3	54.2

* denotes the percentage of abnormality in parenthesis.

As shown in Table 4, the presence of micronuclei may be related to multipolar cell division, precocious division, fragments and chromosome bridges.

In general, the first indication of cytoplasmic cleavage in pollen mother cells of *A. cristatum* is found after nuclear division is complete. However, cell furrowing may precede nuclear formation (Chen, 1978). After furrowing, the meiotic cells in most of the plants retain their dyed conformation and continue division, meiosis II. In some plants the meiotic cells divide and separate after cytokinesis with each cell functioning independently. Both normal and multipolar cell division occur in the meiotic cells with non-quartet formation. Seed fertility can be correlated with quartet formation, and it approaches zero in plants with non-quartet formation.

D. Meiosis II

In prophse II, X-shaped chromosomes diperse in groups into the independent sister cells. Chromosome fragments or rod-shaped chromosomes are often present. As shown in Fig. 4 and Table 2, chromosome groupings continue through metaphase II. In 420 of 773 cells, the chromosomes formed two or more groups at the equatorial plate. The frequency of multipolarity observed at this stage ranges from 14.8% to 83.3%. Normally the orientation of dyads at metaphase II can be followed through anaphase II with regular sister chromatid separation. Secondary, supernumerary cytoplasmic cleavage, which gives rise to microcells containing different numbers of dyads, sometimes occurs. This results in supernumerary microcells within the quartet. The frequency of supernumerary cytokinesis is not related to the number of spindles within a cell. It also fails to correlate with supernumerary cytokinesis and the formation of micronuclei. In the quartet stage, cells containing more than one nuclei are often observed (Table 4) and their average frequency is 54.2%.

2. Pollen fertility and seed fertility

Pollen fertility, which is related to chromosome behavior and genetic balance, was examine by staining the pollen with iodine solution. Individual normal plant had a frequency of darkly-stained pollen ranging from 47.2% to 87.9% regardless of pollen size. However, the overall staining frequency of the large cells (79.4%) was much higher than that of the small cells (39.1%, Table 5). The pollen of trisomic plants often shows lower fertility.

In the F_1 progeny of CB-9-85, the average number of seed set per spikelet varied among

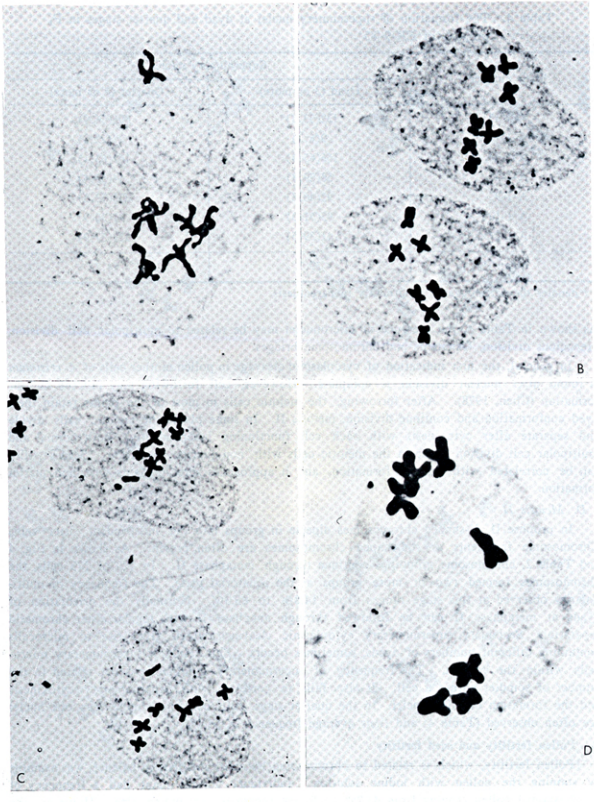

Fig. 4. Chromosome grouping at metaphase II.
A. 6-1 (990×) B. 4-3 (890×)
C. 3-2-1-0.5 and 4-3-0.5 (725×) D. 3-1-3 (1090×)

Table 5. Pollen fertility in F_1 progeny of CB-9-85

F_1 progeny	Large grains		Small grains	
	Stained	Yellowish	Stained	Yellowish
Normal	2604	473	873	834
Trisomic	1174	506	147	752
Total	3778	979	1020	1586
Pollen fertility (%)	79.4		39.1	

individual plants from 0 to 1.72. In healthy, vigorous plants, the spikelets in the middle of the spike produced more seed (3.76) than did the upper and lower spikelets.

DISCUSSION

1. The meaning of multipolar cell division in chromosome evolution

As previously reported, abnormalities of spindles are found in various hybrids of plants of plants and animals and may arise spontaneously or artificially. In the *Triticinae*, Shkutina and Kozlovskaya (1974) indicated that meiotic cells with reduced chromosome number resulted from cytomixis, but they failed to describe multipolar cell division, though their photomicrographs showed this phenomenon in different stages of meiosis. McCollum (1974) described multipolar cell divison, univalents, chromosome bridges, unequal disjunction and micronuclei in the hybrids of common onion, *Allium cepa*×*A. aschanii*. Hybridization of different species is not the only mechanism of polyploidization which frequently produces multipolar cell division. Other manifestations of polyploidization, such as cytomixis (Shkutina and Kozlovskya, 1974), endomitosis (Jensen, 1974), abortive mitosis endoreduplication (Heneen, 1970) and nuclear fusion (Pera, 1970), have also a great influence on the phenomenon of multipolar division. In cultured rat-kangaroo cell, Heneen (1970) indicated that the frequency of abnormalities of spindle and the number of poles per cell increases with the ploidy. Moreover, he showed multipolar configurations may have either a mononucleate or multinucleate origin. Colchicine treatment provides an excellent tool to study the correlation between progressive polyploidization and multipolar cell division both in frequencies and multipolarity (Palitti and Rizzoni, 1972). In addition to arresting cell division, colchicine also increases the frequency of chromosome changes (Sakharov, *et al.*, 1969) and supernumerary cytoplasmic cleavage (Chen, 1978). The present study shows that multipolar cell division resulted from an abnormality of the spindle occurring in the F_1 progeny of CB-9-85 and chromosome numbers of the pollen varied. From the above observations, a scheme (Fig. 5) has been developed to explain the role of multipolar cell division in the change of chromosome number. The role of multipolar cell division in genome segregation was first suggested by Gläss (1956). In his analysis of rat liver chromosomes by length, position of kinetochore, and differentially stained segments, he found that triploid metaphase resulted in distinct genome groupings of the types ($2n$-$1n$ and $1n$-$1n$-$1n$). Similar non-random separation of the genomes occurred in tetraploid and higher polyploid cells. Stern (1958) argued that multipolar cell division is highly inefficient in accomplishing segregations of whole genome because of the random distribution of the chromosomes. Non-random distribution of chromosome sets in cell division occurs spontaneously. In *Sciara*, the intact paternal genome separates from the maternal one during the first spermatocyte division (Crouse, 1943). In *Oenothera* (Cleland, 1936), paternal and maternal homologues display an alternate arrangement in the multivalent rings (Renner's complex) of metaphase I. Then in

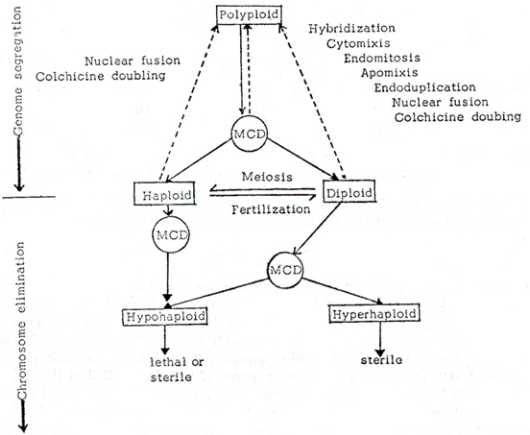

Fig. 5. The role of multipolar cell division (MCD) in the change of chromosome number.

anaphase I, the parental genomes segregate in a very regular manner. In *Daphnia*, the complete set of paternal chromosomes becomes heterochromatic during early embryogeny, and the maternal set remains euchromatic and functioning in the nuclei (Brown and Nur, 1964). Quantitative cytochemical analysis of DNA segregation in multipolar cell division demonstrated that preferential distribution of genome to the poles occurs in cultured mammalian cells (Palitti and Rizzoni, 1972) and in rat kidney epithelial cells (Pera and Rainer, 1973). Rizzoni, *et al.* (1974) studied the Leishman's stain banding pattern of *Rhesus* chromosomes treated with trypsin and found that only haploid and triploid cells were derived from multipolar mitosis. Multipolar cell division seems to be the only mechanism which can explain somatic reduction as a process for the segregation of complete genomes.

Chromosome elimination sometimes occurs in haploid, diploid and polyploid species after multipolar cell division, and it is the main source of aneuploidy. In hybrid cells of mink and cattle, there is a correlation between aneuploidy and neoplasia (Teplitz *et al.*, 1968), although aneuploid cells were of exceptionally low frequency. The aneuploids were not all oncogeneous, but most of the neoplasias were aneuploid. From this point of view, multipolar cell division can be interpreted to be a pathological phenomenon. Spontaneously occurring multipolar cell division in polyploids and hybrids may be necessary to establish genomes in the natural population. Artificially induced multipolar cell division, which always seems to be associated with chromosomal abnormalities (Heneen *et al.*, 1970; Dvorak *et al.*, 1973), has been descibed previously. Both physical and chemical methods for multipolar cell division induction, generally used in agriculture and medicine, seem rather hazardous since the phenotypic expression of induced mutations is not predictable and may be detrimental as often as beneficial. The effects

of colchicine, a well-known chromosome doubling agent with mutagenic properties, were studied in Sakharov *et al.* (1969) in rootlet cells of *Crepis capillaris*. Their work indicated that prolonged treatment with colchicine caused change in ploidy, aneuloidy, fragmentation and other chromosome aberrations. Tai (1970) showed that multipolar cell division resulted in chromosome loss in the colchicine-treated diploid *A. cristatum*. Analysis of the F_1 progeny of CB-9-85 showed that 5 out of 45 plants were hyperdiploid and some of them died before reaching maturity. In microsporogenesis of F_1 plants, multipolar cell shows that the phenomenon of multipolar cell division is inheritable and is related to chromosome elimination.

2. Spindle organizer and multipolar cell division

There are several different explanations for the movement of the chromosomes to the poles (Bajer and Mole'-Bajer, 1972; Nicklas, 1974), but they all concluded that the spindle fibers or microtubules which are oriented two poles are necessary for chromosome movement. Since spindle fibers are so often highly ordered within the cell, it can be expected that an organelle is involved in the assembly and organization of the spindle fiber. Zoologists have long suggested that the centrioles determine polarity and spindle organization during cell division. Favorable evidence come from: 1) the centrioles seem to be involved in the formation of protozoan flagella; 2) centrioles migrate prior to spindle formation; 3) after treatment with mercaptoethanol, the two members of each pair of centrioles formed a tetrapolar spindle in sea urchin embryos (Mazia, *et al.*, 1960). In critical examination of this evidence, another interpretation of the former points may be that the centrioles are appendages attached to the spindle and that their migration is dependent upon the assembly of the microtubules. Interpretation, and acceptance/rejection of the latter point must be based on the knowledge of the chemical action of mercaptoethanol which breaks the disulfite linkage between tubulin dimers, destroys the framework of the spindle, and blocks the migration of the centrioles.

Evidence against these points is: 1) in higher plants, the spindle is formed and operated in the absence of centrioles; 2) microtubules are broken down and repolymerized in many regions of the cell; 3) structural differences exist between the centriole and spindle (DuPraw, 1970); 4) in cultured mammalian cells, the relative proportion of multipolarity of a cell increases steadily with time after X-ray irradiation (Levis and Martin, 1963); 5) the kinetochore equivalent is responsible for spindle formation in the fungus, *Polysticus vesicolor* (Girbardt, 1968); and 6) in the present study, cell furrowing, similiar to that which occurs during spermatogenesis of animal cells, was observed. Based on the above information, centrioles are not necessary for the formation or multipolarity of the spindle.

The spindle organizer, which is described as a cell organelle and which is genome specific, was suggested by Tai in 1970. His theory can be used to explain multipolar cell division as it occurs in amphipolyploids and autopolyploids. The term, microtubule-organizer-center (MTOC), which refers to a diffuse, amorphous, osmophilic, and differentiated cytoplasmic region active in microtubule formation, was adopted by Pickett-Heaps (1969) for her work on meiosis in *Chara*. She suggested that the MTOC had the ability to initiate polymerization and depolymerization of the microtubules and to determine their orientation. This theory lends itself to the explanation of multipolar cell division at the diploid and haploid levels.

3. The significance of quartet formation

One of the major differences between monocotyledons and dicotyledons is in the meiosis of their pollen mother cells (Heslop-Harrison, 1971). The furrowing process occurs in monocotyledons; cell-plate formation in dicotyledons. However, furrowing process presented in numerous dicot genera including *Nicotiana, Primula, Chrysanthenum* and *Helianthus* has been reported (Farr, 1916). In both classes, the quartet stage seems an important one during pollen

formation. Evidence from this study shows that seed fertility is variable in plants that form quartets and zero in plants that do not form quartet may provide for metabolite exchange between the pollens and allow each pollen grain to establish a metabolic balance, even though their genetic constitutions differ. The metabolite exchange can be accomplished by cytoplasmic connections between grains of the quartet as described in *Onagraceae* (Skvarla, *et al.*, 1975). Through such an exchange genetically unbalanced pollen may be capable of successful pollination and fertilization. Quartet metabolite exchange provides a suitable explanation for the survival of pollens which can assure the creation, evolution and survival of hybrid and polyploid plants.

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