MULTIPOLAR CELL DIVISION IN *AGROPYRON CRISTATUM* (L.) GAERTN. (GRAMINEAE)

A Dissertation
for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY

Yung-reui Chen

1975

This is to certify that the

thesis entitled

MULTIPOLAR CELL DIVISION IN AGROPYRON CRISTATUM(L.) GAERTN. (GRAMINEAE)

presented by

Yung-reui Chen

has been accepted towards fulfillment
of the requirements for

Ph. D. degree in Botany

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Date July 30, 1975

O-7639

ABSTRACT

MULTIPOLAR CELL DIVISION IN AGROPYRON CRISTATUM(L.) GAERTN. (GRAMINEAE)

By

Yung-reui Chen

Multipolar cell division occurs both in plants and animals. It has been observed in human cancer cells and in hybrids of animals, but most observations have come from haploid, diploid, polyploid, and hybrid plants. Multipolar cell division can occur spontaneously, or it can be induced artifically by chemicals, radiation, microbial infection, extremes in temperature and pressure, and other means. Recently more information about this phenomenon has become available, but the mechanism of multipolar cell division is still disputed.

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 seeds were harvested and planted the following year. Five spikes from each F_1 plant were collected and fixed in Newcomer's solution for cytological observation.

The effects of colchicine-treatment were found to be inheritable. All F_1 progeny of CB-9-85 showed multipolar cell division irrespective of their ploidy, 7 II or 7 II + 1 I. Multipolar cell division was associated with the occurrence of univalents, unequal disjunction, precocious division, chromosome fragments, chromosome bridges, and micronuclei. Multipolar cell division was found to be the main source of multinucleated and aneuploid pollen. The furrowing process, which

normally occurs during both meiosis I and meiosis II and results in the formation of a quartet was studied. Supernumerary cytoplasmic cleavage occurred at all stages after metaphase I and led to the formation of supernumerary cells in the pollen quartet (pollen fertility was related to the size of the grain, with small grains displaying low fertility). In plants which failed to form quartets, the dyads separated into two independent cells, which went through meiosis II and formed pollen grains containing from 1 to 13 X-shaped chromosomes. Seed fertility in plants which do 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 loss in natural populations. A scheme explaining the role of multipolar cell division in genome segregation and chromosome elimination was presented.

The furrowing process in microsporogenesis of the plant was compared to cytokinesis in animals. The interpretation of furrowing as a shearing force upon the cell was presented based on evidence from both microscopic observation and from a review of the literature.

MULTIPOLAR CELL DIVISION IN AGROPYRON CRISTATUM(L.) GAERTN.(GRAMINEAE)

By

Yung-reui Chen

A DISSERTATION

Submitted to

Michigan State University

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1975

ACKNOWLEDGEMENT

I wish to express my appreciation to Dr. Irving W. Knobloch, my major professor, for his assistance and encouragement during the course of this work. I am grateful to have been a student under the guidance of Drs. Gary R. Hooper, Clifford J. Pollard and William Tai and for their kindness in allowing me to use their equipment and in the critical evaluation of my work. I am indebted to Mr. Gilbert D. Starks and Ms. Lynn E. Murry for their advice and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Multipolar cell division is a spindle apparatus abnormality which 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; Bosemark, 1967; Tai, 1970 and Burk et al, 1972) 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; Nicklas, 1959 and Benazzi-Lentati, 1970) as well as in tumor cells (Boveri, 1888; Therman and Timemen, 1950 and Heneen et al, 1970) and in tissue culture (Fenter and Porter, 1965 and Bayliss, 1973). Multipolar cell divison produces daughter cells with different chromosome numbers. Generally it is thought that in multipolar division, the chromosome complement is subdivided into two or more groups that function more or less independently within the cell (Tai, 1971). Other terms, such as incomplete spindle (Darlington and Thomas, 1937), double-plate metaphase (Upcott, 1939; Vaarama, 1949), reductional grouping (Wilson, 1950), somatic meiosis (Huskin, 1948), multipolar spindles (Therman and Timemen, 1950; Knudson, 1958; Walters, 1958), split spindles (Neilson and Nath, 1961) and complement fractionation (Thompson, 1962; Vasek, 1962), have been used to describe the same phenomenon found in different materials.

Multipolar division has been found to occur spontaneously, or to be induced by radiation (Martini and Bozzini, 1966), cold treatment (Huskins and Cheng, 1950; Langridge, 1967), chemicals (Ostergreen, 1950; Eigsti

and Dustin, 1955; Kilman, 1966), virus (Levan and Hauschka, 1953; Halkka and Halkka, 1969) and other treatment (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; Levis, 1962; Brinkley et al, 1967; Martin and Sprague, 1970), but most frequently in polyploids and hybrids of both plants and animals. Success in MCD (Multipolar Cell Division) induction is related to the strength of a given treatment (Östergreen, 1950; Levis and Martin, 1963). Following the correlation between the concentration of colchicine and mitotic spindle abnormalities. Östergreen presented three categories of mitosis. First, a strong concentration of colchicine gave complete spindle inactivation and apolar mitosis (c-mitosis or stathmokinesis). Second, a medium one showed partial inactivation of spindle and multipolar mitosis (mesostathmokinesis). Third, a weak treatment of colchicine resulted in abnormal orientation of spindle and unipolar mitosis (tropokinesis). Both spontaneous and induced multipolar division can be transmitted from generation to generation (Vasek, 1962).

The significance of multipolar cell divison 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 (the pole determinants existed as diffuse particles which coalesced and congregated at the moment when spindle poles were normally formed and existed separately at other times). Similiarly, Swanson and Nelson (1942) considered extra-pole determinants to be a prerequisite for multipolar meiosis. The spindle organizer and split described by Walters (1958, 1960) 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 stage. 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 (culture number CB-9-85), Tai (1970) presented a new model for the spindle organizer. He suggested that the spindle organizer was a cell organelle whose function is 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 multipolar cell division has since been observed by many authors (Pera, 1970a; Avivi, et al, 1970; Palitti and Rizzoni, 1972; Pera and Rainer, 1973; Rizzone, et al, 1974).

In the present study, microsporogenesis in the F_1 progeny of CB-9-85 will be examined. The objectives of this investigation were:

1. To observe the chromosome behavior of the F_1 progeny of CB-9-85.

2. To determine the relationship between nuclear division and cytokinesis.
3. To correlate multipolar cell division with pollent fertility, and seed fertility.
4. To evaluate the significance of the change in chromosome number brought about by multipolar cell division.

LITERATURE REVIEW

1. Spontaneous multipolar cell divisions

The occurrence of multipolar cell division was described by Mayzel (1875) in animal tumor tissue and by Strasburger (1880) in plant cells. After analyzing multipolar mitosis in tumors, Boveri (1888) came to the conclusion that the distribtuion of chromosomes in multipolar mitosis was a matter of chance and that all multipolar configurations should be regarded as pathological phenomena. This opinion dominated the thought of the later investigators who thought that multipolar cell division could only take place rarely and that its significance was negligible (Stern, 1958; Thompson, 1962). Recently it has been found that multipolar cell division may be very closely related to genome segregation and the evolution of chromosome numbers (Gläss, 1956; Lewis, 1967; Teplitz, 1968; Tai, 1970; Benazzi-Lentati, 1970; Brown, 1972 and Pera, 1972).

A. Multipolar cell division in animals

Baltzer (1909) observed that after tripolar mitosis of dispermic triploid sea-urchin eggs, normal larvae developed. This may have been the first indication that multipolar mitosis produces daughter nuclei with a reduced number of chromosomes. After studying the development of claret mutant of Drosophila simulus, Wald (1936) found that mutated claret gene gave rise to certain spindle abnormalities. In the absence of the second and third chromosomes, formation of the spindle was

completely stopped. Both in spontaneously occurring multipolar cell divisions (Telplitz et al, 1968; Pera, 1970a, 1970b) and in those induced artificially (Halkka and Halkka, 1969; Heneen et al, 1970). These studies may shed some light for treating cancer tissues in human beings (Heneen, 1970).

It has been known for many years that multipolar mitosis appears predominantly in polyploids. In the tetraploid cells of rat liver, Gläss (1956) found that three chromosome sets might separate from a fourth one. Therefore, haploid and triploid chromosome groups existed simultaneously within the same cell. Benazzi-Lentati (1970) reported that during gametogenesis in the unfertilized oocytes of planarians, 32 univalents were distributed randomly along the fibers of a multipolar spindle. Entrance of the sperm stimulated the egg to maturation division and converted the multipolar spindle into a bipolar one with both poles equally active. In paedogenesis of the gall midge, Mycophila speyeri, Camenzind (1971) observed that germ-line nuclei kept the high chromosome number (2n = 29) and chromosome elimination in cleavage V and VI lead to somatic nuclei with only 3 chromosomes (2n = 6). Difference in chromosome numbers between germ line and somatic cells is well known in many species (Stern. 1958).

In heterotypic mitosis, the first meiotic division occurs with low frequency. However, in triploid or tetraploid Chlorodhora uhleii, the numbers of chromosomes and spindle poles are abnormal (Bower, 1922). In Drosophila melanogaster (Huttner, 1924), two haploid nuclei of the fertilized egg lie side by side at prophase of the first mitotic division. They form adjacent and parallel spindles, and the metaphase plates lie in the same plane. The two parallel spindles fuse but the two sets of chromosome remain separate at anaphase. Finally the two sets of

chromosomes at each pole are enclosed in one diploid nucleus at telophase. After a series of observations, Gläss (1956, 1957) found that multipolar cell division occurred in diploid cell clones of rat liver cells. He suggested that multipolar cell division was essential for genome segregation to lower the chromosome number from triploid to diploid and from other higher to lower ploidy levels. Palitti and Rizzoni (1972) observed tripolar spindles in diploid kidney cells of Macaca mulatta. After studying the quantitative distribution of DNA to the poles, they found that a diploid tripolar mitosis may produce only one distribution of value (C) i. e., 2:1:1, which reflects chromatid segregation.

Multipolar division has also been found in haploid tissues. Gläss (1956, 1957) found the chromosome grouping (11 + 10) in haploid rat liver cells (n = 21), and Rizzoni et al (1974) discovered multipolar division in XO haploid kidney cells of Rhesus.

In Drosophila pseudoobscura, interracial hybrids between two physiological species (A and B) have been genetically investigated for successive generations (Koller, 1934). The hybrids showed different chromosome behavior in reciprocal crosses. In the cross in which A was used as the female parent and B, as the male, giant spermatids were formed immediately after the first spermatocytes. Multipolar spindles and multinucleated spermatogonial and spermatocytes were found in males derived from the reciprocal cross. Knudson (1958) found that the majority of the sterile bulls had partial testicular hypoplasia (arrested spermiogenesis) and that the cause was multipolar spindle formation during the first meiotic division. Teplitz et al. (1968) studied in vitro, specific somatic hybrid cells between mink and cattle cells and found that recombination took place 24 hours after tetrapolar mitosis. However, after

72 hours, the hybrid cells gradually regressed to parental lines. In Microtus agrestics, 29% of all tetraploids showed multipolar spindle formation in mitosis (Pera, 1970a, 1970b), and haploid, diploid, triploid and tetraploid daughter nuclei were observed. Pera pointed out that multipolar cell division was the only mechanism of somatic reduction which could be demonstrated. Volpe and Earleg (1970) experimentally simulated natural twining in cattle by joining two frog embryos in parabiosis. After 2 months, donor type cells showed selective disadvantage, but some haploid cells occurred. They concluded that an orderly movement of an intact haploid set into daughter cells rather than an uncontrolled random separation of homologues was occurring.

Cells in culture offer many advantages for studies relating to internal or external effects of chromosomes. The cell population is relatively homogeneous, the cell cycle can be effectively controlled, and excellent cytological preparations can be made from the culture. Schmid (1965) observed that multipolar spindles formed after endoreduplication in several kinds of cultured cells and that several micronuclei existed in one cell. Sinha (1967) found that in short-term peripheral blood culture, many cells were tetraploid or near-haploid among the majority of the diploid ones. He suggested that the diploid proliferating bone marrow nuclei fused to form tetraploid hybrid cells which subsequently gave rise to near-haploid daughter cells by a process of double reduction division. He also found tetrapolar anaphase in tetraploid cells. Palitti and Rizzoni (1972) indicated that haploid sets of chromatids behave as functional entities of segregation in multipolar cell division and that the presence of preferential distribution cannot be attributed to the heterogeneity of the cells producing multipolar cell division. Pera and

Rainer (1973) determined the ploidy in cells with multipolar cell division and their daughter nuclei by measuring the relative Feulgen stained DNA content and by counting the predominantly constitutive heterochromatin in sex chromosomes. They found that the chromosomes undergoing multipolar cell division separated into complete genomes, and the daughter nuclei were haploid, diploid, triploid and tetraploid. Transformation of multipolar cell division into bipolar mitosis in living cell was also reported. 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 the culture.

Cancer cells are often characterized by the occurrence of multinucleated cells and cells with lobated nuclei. After a series of cytological investigations, from normal tissue to highly malignant tumors, Therman and Timenem (1950, 1954) reached two conclusions. First, the most constant feature of human cancer cells consists of a change in the ratio of metaphase to prophase cells (Prophase Index), which, in normal utterine epithelium, is very constant, less than 1.5, whereas in cancer cells, it was found to be much greater. Ofterbo and Wolf (1967) reported that one type of binucleated cell forms an H-shaped tetrapolar metaphase, producing four uninucleated cells in telophase while the other type forms a tripolar metaphase producing three daughter cells, two of which sometimes fuse.

B. Multipolar cell divison in plants

Most information about multipolar cell division comes from dividing plant cells, meristem cells and pollen mother cells, whose developmental patterns are easily traced. Contemporary animal cytogenetists invariably

use a hypotonic solution to disperse the chromosomes of colchicine-treated cells. It is well known that colchicine disrupts spindle formation, thereby preventing anaphase movement and that hypotonic treatments change the spatial relationship among metaphase chromosomes. Multipolar cell division is so closely related to chromosome arrangement, spindle abnormalities, and anaphase movement that it cannot be observed using these techniques.

Multipolar cell division occurs in a wide range of plants, from algae to flowering plants. In the Hepaticae, Farmer (1894) stated that the quadripolar spindle is an ephemeral structure which breaks down when the chromosomes become delimited and individualized, and it is replaced by two independent spindles that separate the chromosomes into groups. Davis (1901) argued that the four-poled spindle does not exist, and that Farmer's observations came from prophase rather than metaphase cell. In cells of Chlamydomonas eugametos, treated with colchicine, Buffulose (1959) found that polyploids were not stable and that giant polyploid cells gave rise to haploids by multipolar cell division. In haploid Ulva mutabilis (Hoxmark and Nordby, 1974) the chromosomes segregated randomly following multipolar meiosis forming viable zoospores.

Polyploidy is very common in plants, and the earliest reports of multipolar cell division came from polyploids. In the tetraploid, Primula kewensis, an extra metaphase plate, presumably derived from a split spindle, contained 1 to 9 chromosomes (Upcott, 1939). In polyploid Ribes nigrum, the presence of a multipolar spindle was correlated with asynchrony (Vaarama, 1949). Suto (1955), working on polyploid maize, found that multipolar cell division is always associated with high pollen sterility. In hexaploid Paspalum commesenii, tripolar spindles forming post-reconstitution cells were found (Pi and Chao, 1974). Studies of

octapolid Medicago showed normal, haploid, and aneuploid chromosome numbers in the cells of the same root tipe following multipolar mitosis (Sadasivaiah and Lesins, 1974). Weimarck (1973) observed multipolar cell division while studying the meiosis of octaploid Triticales.

Cornell scientists (Beadle and McClintock, 1928; and Beadle, 1931) first related multipolar cell division to pollen degeneration in their studies of diploid Zea mays. Clark (1940) and Suto (1955) stated that a recessive gene caused abnormal spindle formation and resulted in pollen abortion in corn. In the microsporogenesis of Clarkia exilis, Vasek (1962) found that multipolar cell division occurred during the first meiotic division and that the nucleus divided regularly during the second meiotic division, regardless of the number of chromosomes it contained. Moreover, he indicated that a recessive gene for multipolar cell division occurs and persists in natural Clarkia populations. Sosniklina (1973) reported both sticky chromosomes and multipolar cell division in the meiosis of inbred, diploid rye. In desynaptic mutants of Hordeum vulgare, Sharma and Reinberg (1974) noted the failure of chiasma formation and the presence of multipolar cell division.

Gaine, et al (1926, cited in Ivanov, 1938) in haploid Triticum compactum and Chizaki (1934) in haploid T. monococcum found abnormal spindles curving around the periphery of rare pollen mother cells with the chromosomes randomly scattered. After a series of experiments with Nicotiana rustica, Ivanov (1938) pointed out that interspecific hybridization, low temperatures, and pollination with abnormal pollen could induce the formation of haploids. He also observed several chromosomes lying on the heterotypic spindle in pollen mother cells of the haploid plants. In Hordeum distichum, Tometorp (1939) showed that chromosomes

grouped in a 6-1 distribution occurred more frequently than 5-2 or 4-3, and that quartets, triads and dyads were found in the anther. In haploid barley, produced by crossing H. bulbosum (2X) and H. vulgare (2X), chromosomes are grouped both in mitosis and meiosis (Symko, 1969). Majumdar and Sarkar (1974) observed that univalents were scattered over the entire area of the pollen mother cells when two or more spindle appeared in haploid Zea mays.

Interspecific and intraspecific hybrids are found in various plants, especially soft-fruit crops and ornamentals. Grass hybrids are listed in Knobloch (1968). Hybrid vigor is a common phenomenon, but hybrids are often sterile. Those which are fertile may return to their original state in one to several generations. Of the various internal and external factors involved in this regression, multipolar cell division plays a demonstrable and important role in the process.

Darlington and Thomas's (1937) observation on cell division in Festuca arundinacea (2n = 42) X Lolium perenne (2n = 14), showed that meiotic irregularity in the hybrid was reflected by lack of compactness at one or both poles and by lack of orientation of one or more bivalents. Similar phenomena were reported in hybrid Zea mays by Clark (1948) and in hybrid Mentha by Swanson and Nelson (1942). A fertile hybrid (n = 17) between Aster cordifolius (n = 9) and A. turbinellus (n = 50) found by Aver (1957) was interpreted by Tai (1970) to represent genome segregation by multipolar cell division. Walters (1958, 1960) reported that multipolar cell division and furrowing occurred in the meiosis of Bromus hybrids. Thompson (1962) and Bammi (1965) studied chromosome grouping in meiosis of Rubus hybrids and suggested that complement fractionation caused chromosome grouping in meiotic as well as mitotic cells.

Chromosome grouping and spindle abnormalities were also reported in the hybrids of Agropyron (Nielson and Nath, 1961), Rosa (Klastersha and Narajan, 1974), and Allium (McCollum, 1974). In hybrids and induced amphiploids of Elymus canadensis X Agropyron libanotium, chromosome grouping and three metaphase plates were observed (Dewey, 1974). An extreme example of multipolar cell division comes from completely sterile hybrids of Triticum-Agropyron-Secale, which showed chromosome number ranging from 1 to 19 in metaphase I (Lyumbimova, 1973).

In tissue culture, polyploidy is a more common nuclear variation than aneuploidy or haploidy (Partanen, 1963). The older a culture is, the more nuclear variations are found. In Zea mays endosperm culture, multipolar spindles, polyploidy, and hypoploidy often occur in the mature cells (Straus, 1954). After studying cultured Daucus carota, Bayliss (1973) pointed out that the plant cells, under normal conditions, tended to promote abnormalities of the mitotic spindle. This is supported by data showing increased frequency of multipolar separation and lagging chromosomes. Moreover, Bayliss indicated that the activity of normal mitotic spindles could be affected either by lack of physical organization of the tissues or by the chemical consitituents of the medium. In studying cultured meiocytes of Trillium erectum and Lilium longiflorum, Ito and Stern (1967) concluded that the spindle mechanism appeared to be the source of abnormalities such as aberrant segregation and failure to complete the second meiotic division.

2. Induced multipolar cell division

Spontaneous multipolar cell division occurs both in animals and plants, and its frequency is extremely low compared to induced multipolar cell

division. Inducing agents can be physical, chemical or biological. The strength of the inducing agent and duration of treatment often determine the irreversibility of spindle abnormalities (Brinkley, et al, 1967). Induced multipolar cell division is associated with lagging chromosome, chromosome fragmentation, chromosome bridges, micronuclei and supernumerary cells in the quartet.

In a wide survey of various chemicals considered to be hazardous to human health, many have the ability to induce multipolar cell division. Colchicine, a well known C-mitosis inducing agent, and such narcotic substances as ethylene, acetone and glycol have been reported to induce multipolar cell division as well as chromosome doubling (Eigsti and Dustin, 1955; and Kabatity, 1966). From his work on onion cells, Östergreen (1950) suggested the creation of cytological standards for quantifying the effects of various concentrations of colchicine on spindle distribution. Eigsti and Dustin (1955) stated that spindle regeneration cells is related to the duration of colchicine treatment, that a high specificity exists between colchicine and spindle abnormalities, and that late prophase is the stage most sensitive to low colchicine concentration.

Multipolar cell division can be induced by radiation, and multipolarity increases with time (Levis and Martin, 1963). Virus infection of mammalian cell lines has also resulted in multipolar cell division.

A. Chemicals

Spindle abnormalities induced by chemicals cause secondary, even tertiary, effects in biological systems. For example, colchicine treatment of dividing cells causes: 1) inhibition of spindle formation; 2) pairing aberrations, desynchronization of the mitotic process, and delayed

separation of chromosomes; and 3) the necessity for restitution nuclei (Eigsti and Dustin, 1955). The chemical action of colchicine on the spindle is still unknown. Simple observations on the physiological and developmental effects of colchicine (Nooden, 1971) have brought more confusion. Approaching the problem at the molecular level may elucidate the action mechanism (Hotta and Shepard, 1973).

In Agropyron cristatum (Tai) and Triticum aestivum (Dover and Riley, 1973), colchicine-induced multipolar cell division was observed in the microspore mother cells. Colchicine and its derivations affected spindle function in tissue culture of Chinese hamster cells (Brindley, et al, 1967; and Puck and Cox, 1969).

Many inorganic and organic compounds can induce multipolar cell division. Nitrous oxide induced multipolar cell division occurs in wheat and barley embryos (Dvorak, et al, 1973). Hydroquinone disrupts the spindle resulting in the formation of three metaphase plates in the intestine of the mouse (Parmentier and Dustin, 1948). Ethylene oxide, ethylene glycol, polyethylene glycol and glycol have been reported to induce spindle and chromosome abnormalities (Maguire, 1974). Mercaptoethanol, a famous reducing agent, was responsible for mitotic multipolarity in sea urchin eggs (Mazia, et al, 1960). Its role in splitting disulfide linkages (-S-S-) in subunits of the microtubules was discussed by Kihlman (1966) and DuPraw (1970). Several drugs, including ceresan (Sass, 1938), sodium nucleate (Huskin, 1948), antibiotics (Wilson, 1950), Gamma-hexa florocyclohexane (Hervas and Gimenez-Martin, 1974), vinblastine and aesculin (Martin and Sprague, 1970), are reported to cause multipolar cell division. Rotenone directly hinders tubulin synthesis causing the microtubules to fail to assemble and inhibiting mitosis (Brinkley, et al, 1967).

Inhibitors of RNA and protein synthesis have been reported to induce multipolar cell division in Trillium erectum (Kemp, 1964). Multipolar cell division induced by formulated insecticides is much higher than that induced by pure compounds (Amer and Farah, 1974).

B. Physical-radiation

X-rays and gamma-rays have high penetrating power and can cause deep-seated radiation effects. In general, cell division seems to be more readily inhibited by radiation than is cell enlargement (Dertinger and Jung, 1970), and the mechanism of radiation induced mitotic inhibition is still unknown.

In the spermatogenesis of sea urchin, multipolar cell division occurred following X-irradiation (Rustard, 1959). Rustard pointed out that the percentage of multipolar cell division increased with dose. In the guinea-pig, mitotic depression and mitotic delay are proportional to the damage after X-ray treatment, and multipolar cell division and micronuclei are the consequences of giant cell formation (Levis, 1962). Levis and Martin (1963) showed a shift in relative proportions of multipolar spindles formed after high dosages of radiation and in the proportion of spindle multipolarity which increased steadily with time. In X-irradiated KB human cell lines, there was a high, positive linear correlation ($r = 0.9988$) between the frequency of multipolar anaphase and radiation dosage, and this was interpreted to represent a single target phenomenon (Fenter and Porter, 1965). In X-ray induced mutants of Triticum durum, Martini and Bozzini (1966) found random distribution of chromosomes, lagging chromosomes, and multipolar spindles in anaphase. Gottachalk and Milietinovig (1973) reported desynaptic chromosomes and

multipolar cell division in radiation-induced trisomic Pisum sativum. In regular irradiated peas, Kalloo (1972) found lagging chromosomes, chromosome bridges, and chromosome grouping in both root tip cells and meiocytes. Similar results were reported in Vicia rosea by Sudhakaram (1972), and in Hordeum vulgare by Srivastava (1974).

C. Physical-temperature

The temperature coefficient for cell division is between 2 and 3, and different states of mitosis have different sensitivities to temperature. Both high and low temperature extremes suppress the start of cell division (Langridge and McWilliam, 1967). Temperature extremes may also affect the formation and function of the spindle. Growing onion bulbs at 0-5^{o}C for 5-24 days results in an increase in the frequency of multipolar cell division (Huskin and Cheng, 1950). After exposing the cells to abnormally high and low temperature a reduced spindle appears (Mazia, 1961).

D. Biological factors

Cytological abnormalities induced by microorganisms do not appear to be confined to any particular organelle in the cell. The different aberrations reflect the effects of various microorganisms on different hosts. In animal tissue cultures, virus, mycoplasma and rickettsia, were reported to affect cell division most often (Nichols, 1966). In plants only the advanced stages of disease have been studied. The sequential interaction between the cell and the microorganism have been neglected (Schneider, 1973). Nimnoi (1974) first reported the occurrence of multipolar cell division in the pollen mother cells of potato which was infected by potato spindle tube virus.

Abnormal metaphase groupings of chromosomes were found in mouse lymphosarcoma (Levan and Hauschka, 1953). FL human amnion cells infected by PPLO showed a gradual reduction in chromosome number and formed three new varieties (Fogh and Fogh, 1965). Chromosome reduction, endoreduplication, and chromosome aberration were found in leucocytes infected by various viruses (Gripenberg, 1965). Norrby, et al (1966) showed the positive correlation between chromosome pulverization and measles virus which induced cell fusion resulting in mitotic abnormality (Nichols, 1966). Intercellular microbes (virus, mycoplasma, and rickettsia) were reported to cause multipolar cell division in 13 infected species of leaf-hoppers (Halkka and Halkka, 1969).

B chromosomes, which are supernumerary and do not pair with normal chromosomes, occur in both plants and animals. The more B chromosomes an organism has, the more irregular its divisions are. B chromosomes have been called parasites, in the manner of virus, which induce chromosome aberrations (Brown and Bertke, 1974). John (1975) reported that B chromosomes induced spindle abnormalities in plants.

Others

High pressures applied to animal cells have resulted in the formation of multipolar spindles. Within physiological tension limitation normal spindles may be recovered in time (Dietz, 1966; and Nicklas, 1974). Lagging chromosomes, chromosome bridges and spindle abnormalities were found in stamen hairs of Tradescantia Clone 02 growing on cowdung sand (Abraham, 1974).

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MATERIALS AND METHODS

Fairway crested wheatgrass, an economically important plant, has been identified as Agropyron cristatum (L) Gaertn, A. cristatiforme Sarkar, A. dagnae Grass, and A. pectiniforme Roem. et Schult (Taylor and McCoy, 1973). It is characterized by its low, leafy habit and broad spikes. Diploid (2n = 14) A. cristatum was treated with 0.1% colchicine, and the progeny produced were numbered CB-9-85. The original source of the seed was from Utah State University. This plant was found to have multipolar cell division. Seeds harvested from CB-9-85 were grown in the open field nurseries of the Department of Botany and Plant Pathology, Michigan State University, in the summer of 1971. Some of the seeds produced vigorous plants; some, weak plants; and some failed to germinate. These plants are assumed to be the result of outcrossing. Young spikes for cytological observation were collected around 6 AM 6-19 June, 1971, and fixed immediately in Newcomer's solution (6 isopropyl alcohol:3 propionic acid:1 petroleum ether: 1 acetone: 1 dioxane).

In the field, the developmental stages of the spikes are asynchronous and the stage of development often influences the success of pollination. The best material (spikes) for cytology come from the middle of the flowering period. Florets 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 the potassium-iodine (1%) staining reaction. Pollen grains, which were fully expanded and darkly stained at 100X were rated viable. All

counts were made on the first day 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. Normal meiotic behavior in Agropyron cristatum

A. Prophase I

As shown in Figure 1 A, the first stage of meiosis dealt with here is zygotene. At this stage, the chromosomes intertwine or pair in the process of synapsis which ordinarily occurs between homologous chromosomes. The sister chromatids of each homologue are not morphologically detectable, during synapsis. The next stage, pachytene, (Figure 1 B), is characterized by the longitudinal splitting of both homologues into sister chromatids after which the paired chromosome continue to shorten and condense while remaining tightly twisted around each other. Sometimes univalent chromosomes can be observed. At diplotene (Figure 1 C) chromosome contraction continues as well as the gradual separation of the seven homologues. The two chromatids of each homologue are visible, and the sites of genetic exchange can be seen as chiasmata. One or more chiasmata occur in each member of the tetrad. The chiasmata occur more often at the end of the chromosomes, and their frequency decreases with increasing proximity to the centromere. During diakinesis, internal spiralization increases with concomitant contraction of the chromosome, and the major coil is usually completed by the end of this stage (Figure 1 D). The nucleolus fades away, and the seven metacentric bivalents disperse to the nuclear periphery. At this point, prophase I come to an end.

Figure 1. Normal meiotic behavior in diploid Agropyron cristatum.

A. Zygotene (1250x)
B. Pachytene (690x)
C. Late diplotene (850x)
D. Early dikinesis (875x)
E. Metaphase I (1090x)
F. Anaphase I (845x)
G. Early telophase (795x)
H. Furrowing
I. Prophase II (750x)
J. Metaphase II (820x)
K. Anaphase II (860x)
L. Quartet (820)

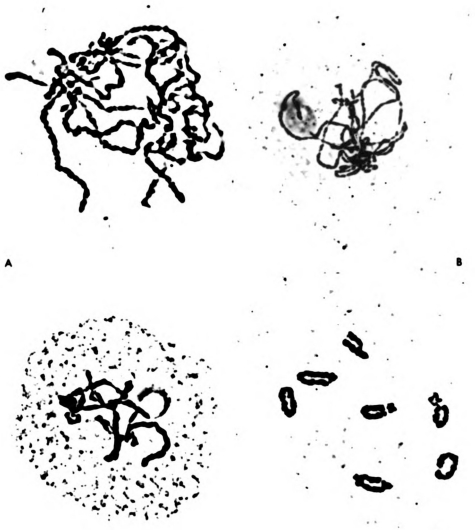

Figure 1

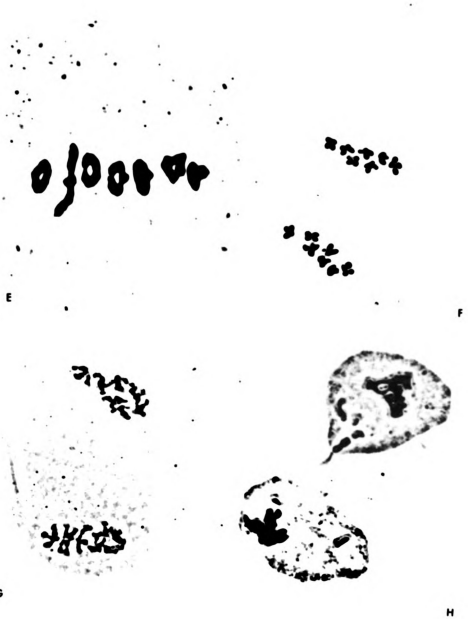

Figure 1

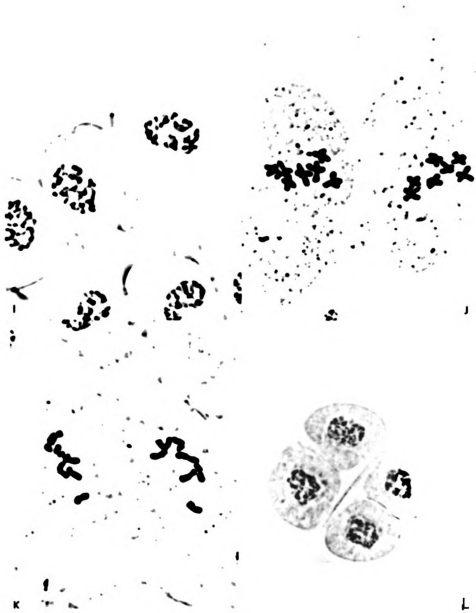

Figure 1

B. Metaphase I

At metaphase I, the nuclear envelope breaks down, and the spindle forms around the chromosomes, which have reached their maximum degree of condensation and appear to be smooth. Then the seven bivalents migrate back toward the equatorial plane with the centromeres of each two homologues oriented towards opposite poles. The two halves of the bivalent are still joined by one chiasma or more, and the shape of the bivalent is determined by the number and positions of chiasmata in each dhromosome arm. They resemble loops or incomplete loops. In general, the arms of the chromosomes adjacent to the centromere appear thin and streatched (Figure 1 E).

C. Anaphase I

At early anaphase I, each of the homologous chromosomes moves toward its respective pole with its daughter chromatids joined at the centromere. The centromeres do not divide at this time. When the active spindle elongates, the seven homologues separate from one another and form 14 dyads which are easily recognized by their shapes (Figure 1 F).

D. Telophase I and Interkinesis

In telophase, the chromatids of each chromosome are more widely separated at their arms than in the previous stages. Sometimes there is some degree of regrouping of the chromosomes as they uncoil at the pole. A new nuclear envelope forms (Figure 1 G).

Cytokinesis

As shown in Figure 1 H, cytoplasmic cleavage (sometimes called furrowing), generally occurs in A. cristatum. The two-celled stage formed indicates that the duration of interkinesis is relatively long and a callose wall is laid down between the two cells. Occasionally the two cells are separated by a thin cytoplasmic connection (Figure 1 H). In the latter case, interkinesis may be of such short duration as to be practically nonexistent, and the chromosomes go through their second division relatively unchanged morphologically.

Prophase II

The X-shaped Prohpase II chromosomes are different from those of anaphase I in that the chromosomes are somewhat despiralized. The daughter chromatids, attached only at their common centromere, begin to repel each other. Second division chromatids are characteristically rather slender. At the end of this stage the nuclear membrane disappears again (Figure 1 I).

Metaphase II

In metaphase II, seven half-chromosomes move to the equatorial plate which is perpendicular to the equatorial plate of first division. Each two chromatids attach by their centromere to opposite spindles (Figure 1 J).

Anaphase II

During anaphase II, the half-chromosomes are functionally separated. Then the separated chromatids, haploid chromosomes, move to opposite

poles (Figure 1 K).

Telophase II

The nuclear membrane reappears, the chromosomes uncoil, and the nuclei are reconstituted during telophase II. After the cell wall is laid down, a four-celled quartet is formed (Figure 1 L).

Pollen mitosis

The haploid number of chromosomes undergoes mitotic division within the thick pollen wall and forms generative nuclei and vegetative nuclei before pollination.

2. Multipolar cell division

Forty-five F_1 progeny of CB-9-85 were observed. Most of the plants are diploid with seven bivalents. Only five plants are trisomic with 7 II + 1 I or 6 II + 1 III (Table 1). Regardless of chromsome configuration, all of the plants display multipolar cell division during microsporogenesis. The percentage of multipolar cell division occuring in each individual plant is different, and the frequency of multipolar cell division observed in meiosis also varies from one developmental stage to another. Multipolar cell division may affect the seed and pollen fertility to a certain degree. But other factors, such as chromosome fragments, chromosome bridges, lagging chromosomes, and precocious division also affect those processes (Tables 2 and 3). Detailed observations of multipolar cell division for different stages of microsporogenesis will be discussed in the following sections.

Table 1. General survey of F_1 progeny of CB-9-85.

Plant number	Chromosome association	Quartet formation	Number of seeds per 5 spikes	Plant size[2]
A-1-1	-	-	135	
A-1-2	7 II	yes	198	
A-1-3	7 II	yes	262	a
A-1-4	7 II	yes	136	
A-1-5	7 II	yes	446	
A-1-6	7 II	yes	199	b
A-1-7	7 II	yes	99	
A-1-8	7 II	yes	286	a, b
A-1-9	7 II + 1 I	yes (weak)	32	
A-1-10	7 II + 1 I or 6 II + 1 III	yes	61	
A-1-11	6 II + 1 III	yes	70	
A-1-12	7 II	-	-	a, b
A-1-13	7 II	yes	204	
A-1-14	7 II	yes	176	
A-1-15	7 II	yes	221	c
A-1-16	-	-	0	a, b
A-1-17	7 II	yes (weak)	23	
A-1-18	7 II	no	0	a, b
A-1-19	7 II	yes	125	a
A-1-20	7 II	no	0	a
A-1-21	7 II	yes	168	
A-1-22	7 II	yes	152	
A-1-23	7 II	yes	7[1]	
A-1-24	7 II	yes	217	
A-1-25	7 II	yes	156	
A-1-26	7 II	yes	221	
A-1-27	6 II + 1 III	yes	0	a, b
A-1-28	7 II	yes	241	
A-1-29	7 II	yes	274	
A-1-30	7 II	no	0	

Table 1. (continued)

A-1-31	7 II + 1 I or 6 II + 1 III	no	0	a, b
A-1-32	7 II	yes	0	
A-1-33	7 II	yes	0	
A-1-34	7 II	yes	233	
A-1-35	7 II	yes	118	
A-1-36	-	-	6	
A-1-37	7 II	yes	150	
A-1-38	7 II	yes	342	
A-1-39	7 II	yes	0	
A-1-40	7 II	yes	350	
A-1-41	7 II	yes (weak)	16	
A-1-42	14 II	yes	20	
A-1-43	7 II	yes	239	
A-1-44	7 II	yes	0	a
A-1-45	7 II	yes (weak)	0	a, b

(1) denotes late blooming tillers.

(2) a: very small plant
b: small number of tillers
c: very vigorous.

Table 2. Chromosome behavior at anaphase I in F_1 progeny of CB-9-85 with seven bivalents

	Normal	MCD*	unequal disjunction (%)	Fragments (%)	Laggards (%)	Bridges (%)	Precocious division (%)	Total
Plant number								
A-1-3	37(61.7)	17(28.3)	0	0	4(6.7)	2(3.3)	0	60
A-1-4	24(30.8)	53(67.9)	0	0	0	1(1.3)	0	78
A-1-13	50(54.9)	37(40.7)	0	0	3(3.3)	1(1.1)	0	91
A-1-16	21(17.9)	74(63.3)	0	7(6.0)	6(5.1)	9(7.7)	0	117
A-1-24	34(59.7)	15(26.3)	0	2(3.5)	5(8.8)	1(1.7)	0	57
A-1-28	19(61.3)	10(32.3)	0	0	1(3.2)	1(3.2)	0	31
A-1-33	38(40.4)	44(46.8)	4(4.3)	2(2.1)	2(2.1)	3(3.2)	1(1.1)	94
A-1-34	25(73.5)	9(26.5)	0	0	0	0	0	34
A-1-37	22(57.9)	11(28.9)	0	2(5.3)	3(7.9)	0	0	38
A-1-44	19(20.0)	52(54.7)	3(3.2)	1(1.0)	11(11.6)	5(5.2)	4(4.2)	95
A-1-45	6(10.0)	42(70.0)	5(10.0)	0	0	0	6(10.0)	60
Total (mean, %)	295(39.1)	364(48.2)	13(1.7)	14(1.9)	35(4.6)	23(3.0)	11(1.5)	755

* MCD: Multipolar Cell Division.

Table 3. Chromosome behavior at anaphase I in F_1 progeny of CB-9-85 with 7 II + 1 I or 6 II + III chromosomes.

	Normal (%)	MCD (%)	Fragments (%)	Laggards (%)	Bridges (%)	Precocious division (%)	Total
Plant number							
A-1-9	7 (25.0)	18 (64.3)	0	3 (10.7)	0	0	28
A-1-10	15 (26.3)	37 (63.9)	1 (1.8)	1 (1.8)	3 (5.2)	0	57
A-1-27	4 (12.1)	16 (48.5)	0	8 (24.2)	3 (9.1)	2 (6.1)	33
A-1-31	12 (24.5)	22 (45.9)	3 (6.1)	8 (16.3)	3*(6.1)	1 (2.1)	49
Total (mean, %)	38 (22.7)	93 (55.7)	4 (2.4)	20 (12.0)	9 (5.4)	3 (1.8)	167

* chromosome bridge and fragment present in the same cell.

A. Metaphase I

Multipolarity first becomes evident during the first meiotic metaphase of the pollen mother cell. Instead of the seven bivalents being oriented on one spindle and one metaphase plate, the chromosomes attached to two or more spindles and metaphase plates. A high percentage of the metaphase cells have 2 and 3 spindles (Figure 2 A-D), and sometimes as many as 6 are observed (Tables 4 and 5).

In 7 II plants, three categories of configurations were observed. The categories are: 1) normal cells with 7 bivalents associated with a single spindle, 2) cells with two bivalent groupings (6-1, 5-2 and 4-3) oriented on two spindles each, and 3) cells with three bivalent groupings (5-1-1, 4-2-1, 3-3-1 and 3-2-2) arranged on three spindles each. Four and five bivalent groupings occur in one cell, but their frequencies are very low and extremely variable. The overall frequency of multipolar cell division as observed in metaphase I is 48.1% (Table 3).

The cases where multipolar cell divison occurs in 7 II + 1 I or 6 II + 1 III plants are more complicated (Figure 3). 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. As shown in Table 5, the number of chromosome groupings ranges from one to six. For ease in recording the data, configurations, such as 7.5 II and (6 II + 1 III), where all the chromosomes formed one group, were placed in the same category, and those configurations, such as 6.5 II + 1 II and (5 II + 1 III) + 1 II, were placed in another. This procedure was followed for all groupings. The percentage of multipolar cell division for individual plants varies from 56.0% to 79.0% and

Figure 2. 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

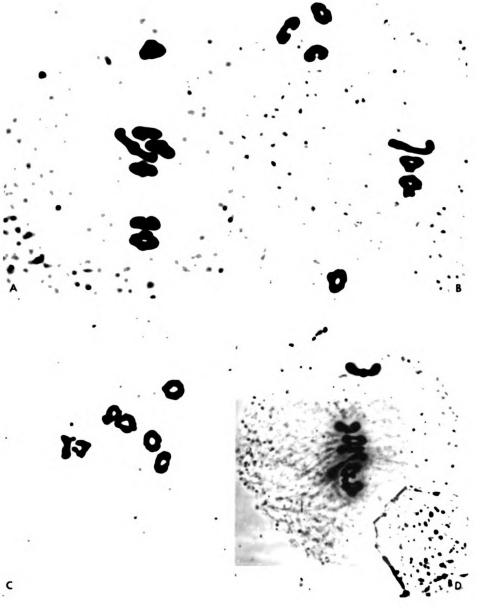

Figure 2

Table 4. Metaphase I chromosome association in some F_1 progeny of CB-9-85 with seven bivalents.

Plant number	Type of grouping													
	normal	6-1	5-2	4-3	5-1-1	4-2-1	3-3-1	3-2-2	4-1-1-1	3-2-1-1	2-2-2-1	other	Total	%
A-1-3	25	8	-	1	-	3	3	-	-	-	1	1	42	40.5
A-1-4	36	3	4	4	-	3	5	5	1	-		5	66	45.5
A-1-13	188	39	25	45	17	59	35	31	14	59	10	27	549	65.8
A-1-16	56	15	19	11	3	13	5	4	-	-	1	-	127	68.4
A-1-24	57	4	5	1	1	4	-	3	-	2	-	-	77	26.0
A-1-28	34	2	-	1	-	-	-	-	-	-	-	-	37	8.1
A-1-33	78	11	7	11	4	25	13	9	1	11	2	2	174	55.2
A-1-34	146	2	3	18	5	9	20	12	-	17	8	10	250	41.6
A-1-37	265	1	2	3	9	20	12	8	-	15	4	1	340	22.1
A-1-44	36	9	6	17	12	27	32	15	4	20	11	10	199	86.9
A-1-45	8	8	-	16	7	5	7	9	1	11	-	-	72	88.9
Total	929	102	71	128	58	168	132	96	21	135	37	56	1933	
%	48.1	5.3	3.7	6.6	3.0	8.7	6.8	4.9	1.1	7.0	1.9	2.9		

Table 5. Metaphase I chromosome association in F_1 progeny of CB-9-85 with 7 II + 1 I or 6 II + 1 III chromosomes.

Type of grouping	Plant number			
	A-1-9	A-1-10	A-1-27	A-1-31
7.5 II*	42	51	37	23
7 II + 1 I	2	8	8	
6.5 II + 1 II*	35	10	2	3
6 II + 1.5 II	12	23	15	1
5.5 II + 2 II	4	1	4	8
5 II + 2.5 II		3	3	9
4.5 II + 3 II		8	1	13
4 II + 3.5 II			4	12
6 II + 3 I	1			
5.5 II + 1 II + 1 II	3	8	2	
5 II + 1.5 II + 1 II	5	1	1	
4.5 II + 2 II + 1 II	23	5		
4 II + 2.5 II + 1 II	11	6		2
3.5 II + 3 II + 1 II	3	3		1
3.5 II + 2 II + 2 II				1
3 II + 3 II + 1.5 II	2			
3 II + 2.5 II + 2 II		10		
5 II + 2 II + 1 I		7		
4 II + 3 II + 1 I	1	1	2	
4.5 II + 1 I + 1 I 1 II	6			
4 II + 1.5 II + 1 I 1 II	4	2		
3.5 II + 2 II + 1 II 1 II	6	8		
3 II + 2.5 II + 1 II 1 II	7	6	3	
3 II + 2 II + 1.5 II 1 II	11	4		
2.5 II + 2 II + 2 II 1 II	9	2		
4 II + 2 II + 1 II + 1 I		3		

Table 5. (continued)

3 II + 3 II + 1 II + 1 I		3		
3 II + 2 II + 2 II + 1 I		7	1	
3.5 II + 1 II + 1 II + 1 II + 1 II	2			
3 II + 1.5 II + 1 II + 1 II + 1 II	1	3		
2 II + 2 II + 1.5 II + 1 II + 1 II	7	5		
3 II + 2 II + 1 II + 1 II + 1 I		1	1	
2 II + 2 II + 2 II + 1 II + 1 I	5	2		
2.5 II + 2 II + 1 II + 1 II + 1 II		7		
2.5 II + 1 II + 1 II + 1 II + 1 II + 1 II		13		
2 II + 2 II + 2 II + 1 II + 1 II + 1 I		1		
Total	200	207	84	73
% of MCD	79.0	75.4	56.0	68.5
Mean (%)				

* See text description.

Figure 3. Chromosome grouping at metaphase I in 7 II + 1 I or 6 II + 1 III plant.

A. 4-2-1.5 (950x)
B. 5.5-2 (1040x)
C. 3.5-3-1 (645x)
D. 2-2-1-1-1-0.5 (1130x)
E. 2-2-1-1-1.5 (890x)
F. 2-2-2-1.5 (600x)
G. 5-1.5-1 (600x)
H. 2-2-2-1-0.5 (680x)

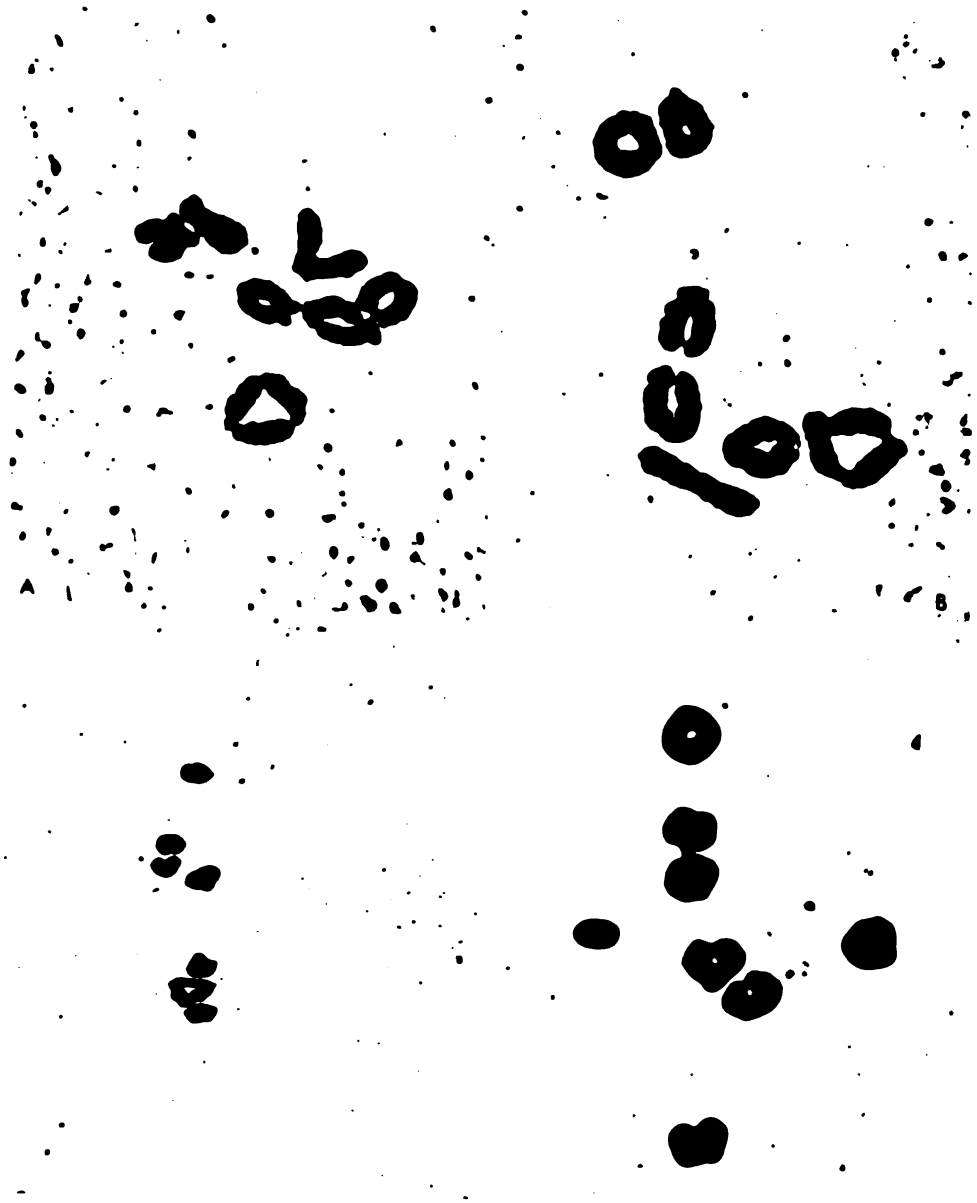

Figure 3

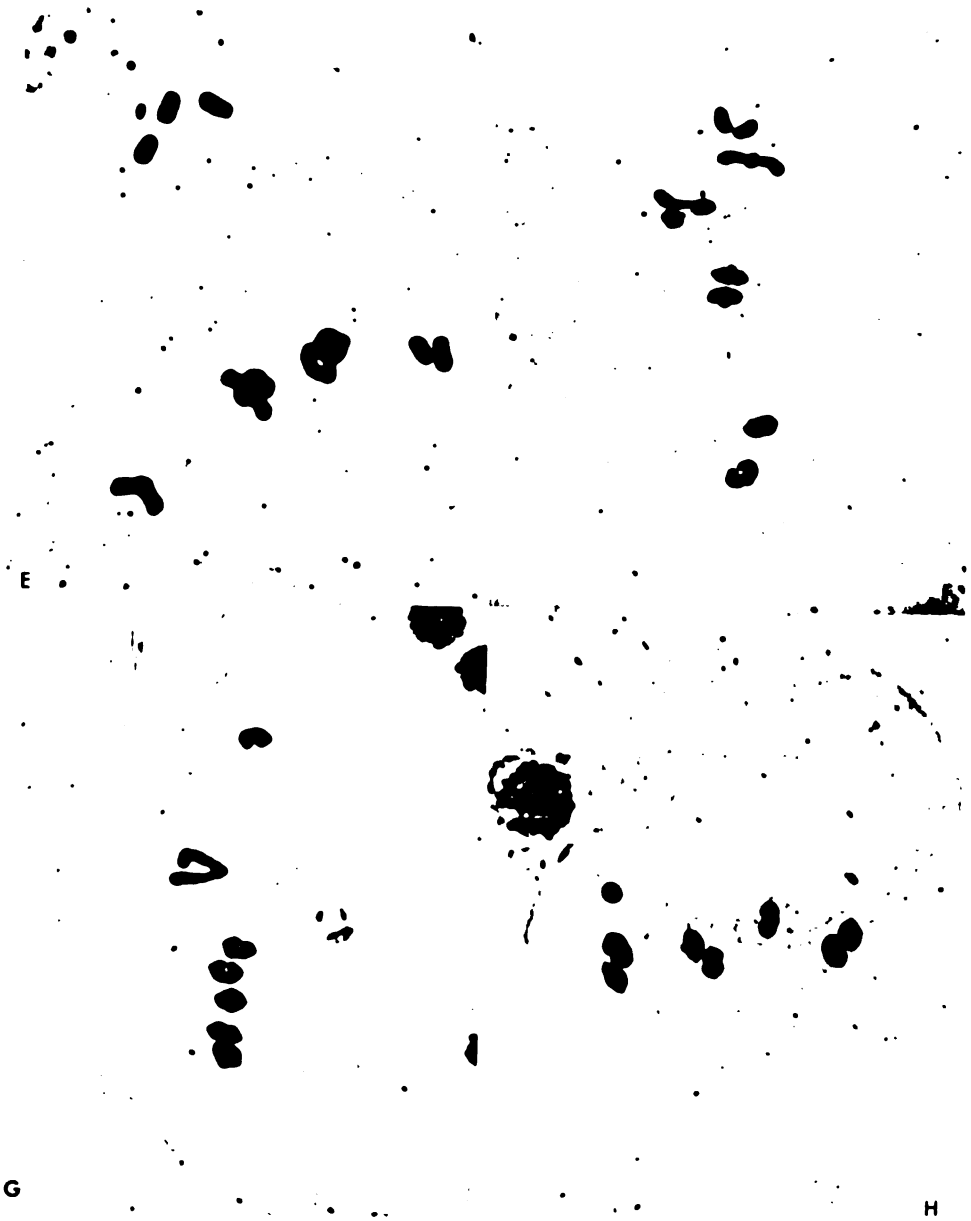

Figure 3

averages 73.6%.

Anaphase I

The configurations and arrangements of chromosomes at anaphase I are more complicated than those of metaphase I. During anaphase I (Figure 4), chromosome segregation may follow the multipolar orientations of chromosome pairs at metaphase I (Table 6). In addition irregularities which occurred during prophase become more pronounced at anaphase I. Chromosomal changes, chromosome fragments (Figures 5 A, B and D), chromosome bridges (Figure 5), lagging chromosomes (Figures 5, A and B) unequal disjunction, and precocious division, can be recognized in this stage.

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 multipolar cell division at this stage is 48.1% in the seven bivalent plants and 69.7% in 7 II + 1 I plants (Table 7). The inconsistant frequencies of multipolar cell division at metaphase I versus anaphase I may be related to the configuration of the chromosomes in the cell during the method of slide preparation. The smaller the space, the more easily the chromosomes may be randomly scattered.

As shown in Tables 2 and 3, other chromosome abnormalities always seem to be associated with multipolar cell division. Unequal disjunction completely dominates the 7 II + 1 I plants, but in the seven bivalent plants, its frequency is only 1.7%. Occasionally chromosome fragments are observed in the cells with the number of fragments varying from one to several per cent. Lagging chromosome (Figure 5), range from 1 to 3

Figure 4. Chromosome grouping at anaphase I.

A. 6-1 and 6-1 (850x)
B. 3-2-2 and 3-2-2 (1000x)
C. 8-7 (950x)
D. 4-2-1 and 4-2-1 (800x)

Figure 4

Table 6. Chromosome segregation at anaphase I in some F_1 progeny of CB-9-85 with 7 II chromosomes

Plant number	Type of grouping									
	7-7	2(6-1)	2(5-2)	2(4-3)	2(5-1-1)	2(4-2-1)	2(3-3-1)	2(3-2-2)	Other*	Total
A-1-3	37	2	4	1	-	5	2	1	2	54
A-1-4	24	1	3	6	1	8	10	4	20	77
A-1-13	50	15	2	6	1	7	2	-	4	87
A-1-16	21	14	11	10	2	9	3	16	9	95
A-1-24	34	3	2	2	5	-	1	2	-	49
A-1-28	19	1	1	2	1	-	1	3	1	29
A-1-33	38	2	5	7	1	9	15	-	5	82
A-1-34	25	3	2	-	-	1	-	1	2	34
A-1-37	22	1	-	2	-	1	-	2	5	33
A-1-44	19	7	11	2	5	3	4	7	13	71
A-1-45	6	4	8	2	8	2	-	-	18	48
Total	295	53	49	40	24	45	38	36	79	659
%	44.8	8.0	7.4	6.1	3.6	6.8	5.8	5.5	12.0	

* denotes other types of chromosome grouping, such as 4-1-1-1, 3-2-1-1, 2-2-2-1, 3-1-1-1 and 2-2-1-1-1.

Figure 5. Chromosome bridges, chromosome fragments and lagging chromosomes occurring at different stage of meiosis.

A-C. Chromosome bridges at anaphase I (725x)
D-F. Chromosome bridges at two-celled stage (950x)
G. Chromosome bridge at anaphase II (935x)
H. Chromosome bridge at anaphase II (1000x)

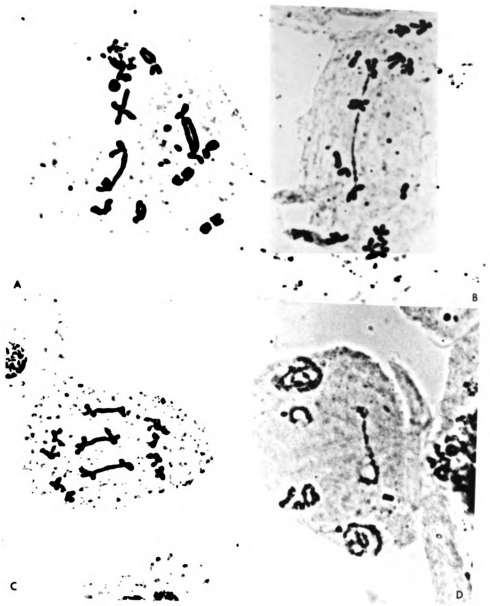

Figure 5

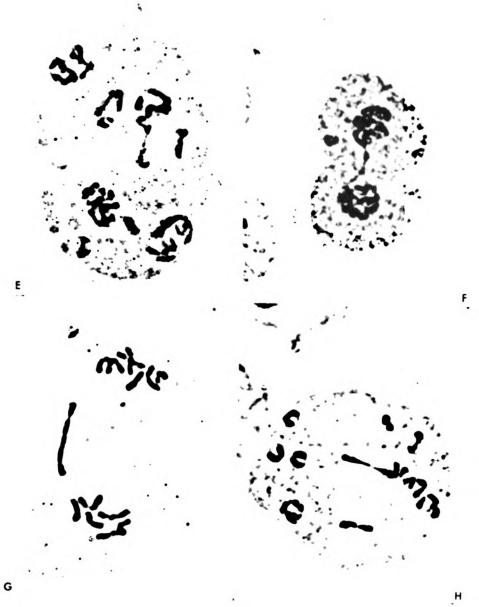

Figure 5

Table 7. Chromosomes segregation at anaphase I in F_1 progeny of CB-9-85 with 7 II + 1 I or 6 II + 1 III chromosomes

Type of grouping	Plant number			
	A-1-9	A-1-10	A-1-27	A-1-31
8 7	7	15	4	12
9 6		1		
8 6-1	1		1	2
7 7-1	2	2	3	4
7-1 6-1	1	3		2
7 6-2		1		1
6-2 6-1		1		
6-2 5-2	3	3		2
5-2-1 4-2-1	2	4	2	4
5-2-0.5 4-2-0.5	1			
4-2-2 4-2-1	1	1	2	1
4-2-2 3-3-1	1	1	1	2
4-2-2 2-2-2-1		1	1	
3-3-2 3-2-2		5	1	1
4-3-1 3-3-1		1		

Table 7. (continued)

4-3-1 4-2-1			1	1
3-2-2 3-2-2-1		1	1	
3-2-2-1 3-2-1-1	1	1		
3-2-2-1 2-2-2-1		3		1
3-2-2-1 2-2-1-1-1		1	2	
2-2-2-2 2-2-2-1		9	1	
2-2-2-1-1 2-2-1-1-1	1			
Total	23	52	20	34
% of MCD	78.3	71.2	80.0	64.7
Mean (%)	73.6			

and occur more often in the 7 II + 1 I plants (12%) than in the 7 bivalent plants (4.6%). Chromosome bridges almost always occur in those cells which display fragments, and single bridges are observed most frequently. Rarely, three chromosome bridges may be seen in one cell (Figure 5C). Precocious divisions occur in both categories of plants, although their frequency is very low (1.5% in 7 bivalent plant and 1.8% in 7 II + 1 I), and result in scattered chromosomes being found in the cell (Figures 5 A and B).

Telophase I and Interkinesis

A cell's chromosome groupings can be followed through telophase and may result in the presence of two or more nuclei in the interphase cell. However, the number of nuclei per one cell may not equal the number of chromosome groupings observed in anaphase I. As shown in Table 8, the presence of micronuclei may be related to multipolar cell division, precocious division, fragments and chromosome bridges. In telophase I, the frequency of multipolar cell division ranges from 28.7% to 65.4%. Sometimes chromosome bridges remain intact through the interpahse or dyad stage (Figures 5 D, E and F).

In general, the first indication of cytoplasmic cleavage in pollen mother cells of A. cristatum is found after nuclear division is complete. However, it may precede nuclear formation. As seen in Figure 6, the process occurs in 3 serial cells whose chromosomes remain separate. The two smaller cells contain 14 chromosomes each, and the larger one contains 30 chromosomes. The process of furrowing involves twisting as well as constriction (a shearing force) instead of a progressive deepening as previously described (Easu, 1965; Brown and Bertke, (1974).

Table 8. The number of nuclei at late-telophase I and interpahse in F_1 progeny of CB-9-85.

Nuclei	Plant number						
	A-1-13	A-1-37	A-1-44	A-1-45	A-1-9	A-1-10	A-1-27
2	263	95	81	137	105	94	63
3	112	8	102	46	52	70	29
4	13	1	44	11	37	21	8
5			3	1	3		3
6	5		7			4	1
7							
8	1						
9							
10	1						
Total	395	104	237	195	197	189	104
Abnormality (%)	33.5	8.7	65.4	29.8	46.8	51.3	39.5

Figure 6. Furrowing process in asynchronized cells (640x).

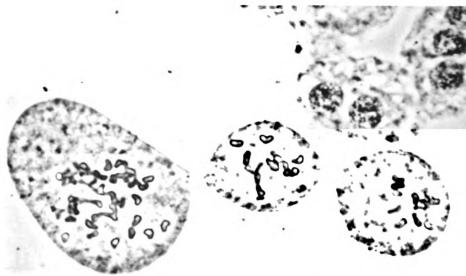

Figure 6

After furrowing, the meiotic cells in most of the plants retain their dyad conformation and continue division, meiosis II. In some plants (A-1-18, A-1-20, A-1-30 and A-1-31), the meiotic cells divide and separate after cytokinesis with each cell functioning independently.

The chromosome behavior during cell division in these cells is similiar to that of haploid pollen. Both normal and multipolar cell division occur in meiotic cells with non-quartet formation. Seed fertility can be correlated with quartet formation, and it approaches zero in plants with non-quartet formation (Table 1).

Meiosis II

In prophase II, X-shaped chromosomes disperse in groups into the independent sister cells. Chromosome fragments or rod-shaped chromosomes are often present. As shown in Figure 7 and Table 9, chromosome groupings continue through metaphase II. In 420 of 773 cells, the chromosomes formed two or more groups on the equatorial plate. The frequency of multipolar cell division observed in 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 (Table 10). Secondary, supernumerary cytoplasmic cleavage, which gives rise to microcells containing different numbers of dyads, (Figure 8), sometimes occurs. This results in supernumerary microcells within the quartet (Figure 9). As shown in Figure 9C, supernumerary microcells containing 8 nuclei originated from one pollen mother cell. 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,

Figure 7. Chromosome grouping at metaphase II.

A. 6-1 (990x)
B. 4-3 (890x)
C. 3-2-1-0.5 and 4-3-0.5 (725x)
D. 3-1-3 (1090x)

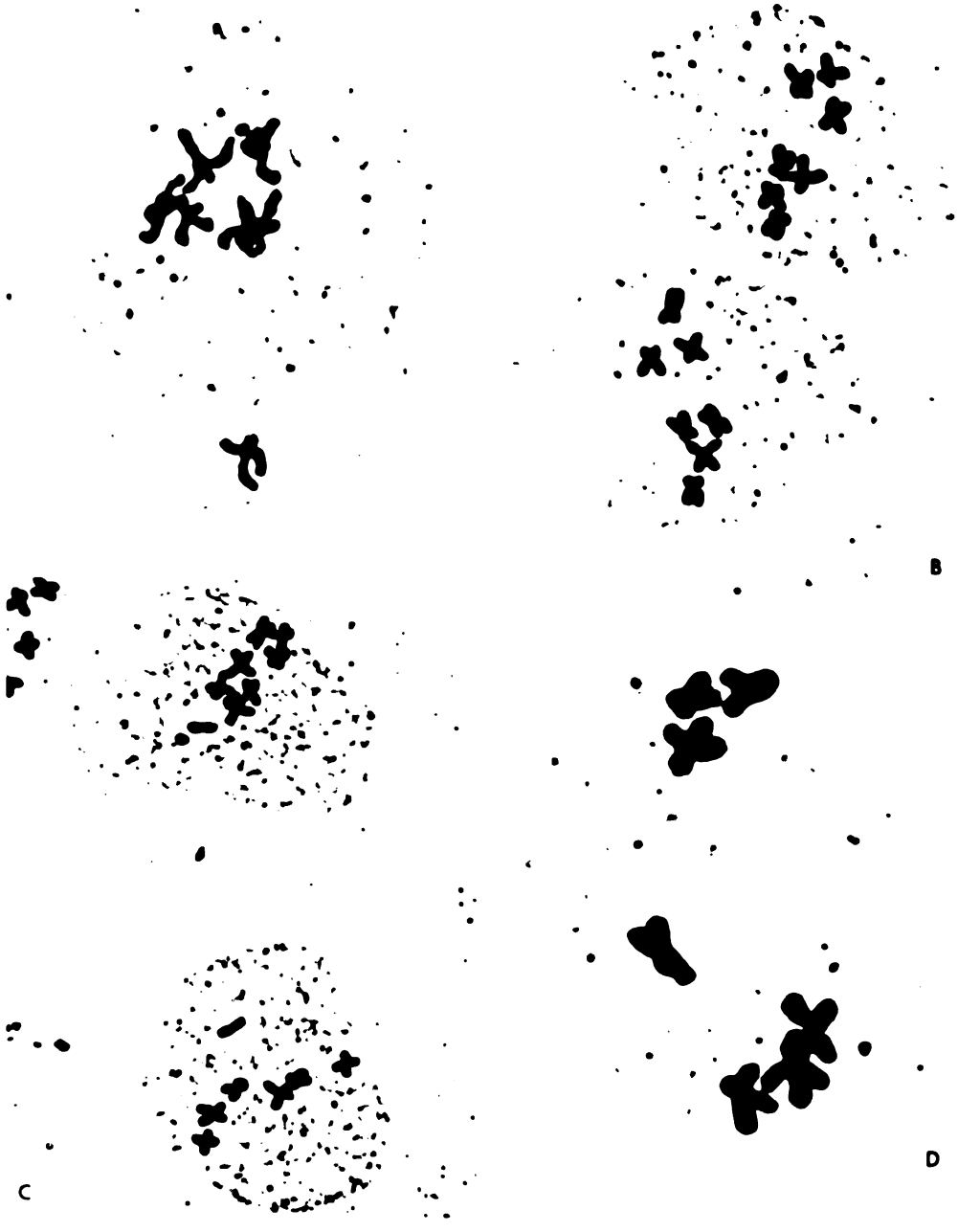

Figure 7

Table 9. Chromosome segregation at metaphase II in some F_1 progeny of CB-9-85 with seven bivalents.

Plant number	Type of grouping										
	7-7	2(6-1)	2(5-2)	2(4-3)	2(5-1-1)	2(4-2-1)	2(3-3-1)	2(3-2-2)	Others*	Total	%
A-1-3	72	8	3	2	-	1	1		2	89	19.1
A-1-4	35	2	1	5	-	1	-		1	45	22.2
A-1-13	19	4	6	3	-	1	1	4	8	46	58.7
A-1-16	10	10	22	14	-	4	7	3	14	84	85.9
A-1-24	57	5	1	-	-	1	3	-	8	75	24.0
A-1-28	29	1	1	2	-	-	-	-	1	34	14.8
A-1-33	25	5	1	7	3	1	2	2	12	58	56.9
A-1-34	36	4	-	2	1	-	3	-	5	51	29.5
A-1-37	26	2	-	1	-	6	-	4	14	53	50.9
A-1-44	11	10	9	16	1	1	2	6	10	66	83.3
A-1-45	33	19	26	30	1	30	10	19	4	172	80.8
Total	353	70	70	82	6	46	29	38	79	773	
%	46.5	9.0	9.0	10.6	0.1	5.9	3.7	4.9	10.2		

* denotes other types of chromosome grouping, such as 4-1-1-1, 3-2-1-1, 2-2-2-1, 3-1-1-1-1 and 2-2-1-1-1.

Table 10. Chromosome segregation at anaphase II in some F_1 progeny of CB-9-85 with seven bivalents.

	Type of grouping										
Plant number	7-7	2(6-1)	2(5-2)	2(4-3)	2(5-1-1)	2(4-2-1)	2(3-3-1)	2(3-2-2)	Others*	Total	%
A-1-3	35	1	4	1	2	6	2	1	4	56	37.5
A-1-4	21	-	-	3	-	2	1	4	1	32	34.4
A-1-13	23	4	7	1	2	8	2	1	12	60	61.7
A-1-16	17	1	-	3	-	-	-	3	3	27	37.0
A-1-24	35	1	2	5	3	-	2	2	-	50	30.0
A-1-28	20	1	2	1	4	-	1	1	-	30	33.3
A-1-33	16	2	1	1	-	2	1		5	28	42.9
A-1-34	27	-	5	-	1	-	-	1	2	36	25.0
A-1-37	19	3	1	-	-	4	1	1	3	32	41.0
A-1-44	8	2	4	1	-	3	1	2	1	22	63.6
A-1-45	12	4	3	2	-	1	1	1	-	24	50.0
Total	233	19	29	18	12	26	12	17	31	397	
%	58.7	4.8	7.3	4.5	3.0	6.6	3.0	4.3	7.8		

* denotes other types of chromosome grouping, such as 4-1-1-1, 3-2-1-1, 2-2-2-1, 3-1-1-1-1 and 2-2-1-1-1.

Figure 8. Cells, orginated from supernumerary cleavage of meiotic cell after metaphase II, contain different chromosomes.

A. Chromatid separation (1320x)
B. Without chromatid separation (1200x)
C. Single chromosome (1600x)
D. 5-5 and 2-2 separation at anaphase II (785x)

Figure 8

Figure 9. Supernumerary microcells and micronuclei occurring in quartet.

A. Supernumerary nuclei in 4-celled quartet (985x)
B. 6-celled quartet (985x)
C. 6-celled quartet containing 8 nuclei (1000x)
D. 5-celled quartet containing 9 nuclei (890x)

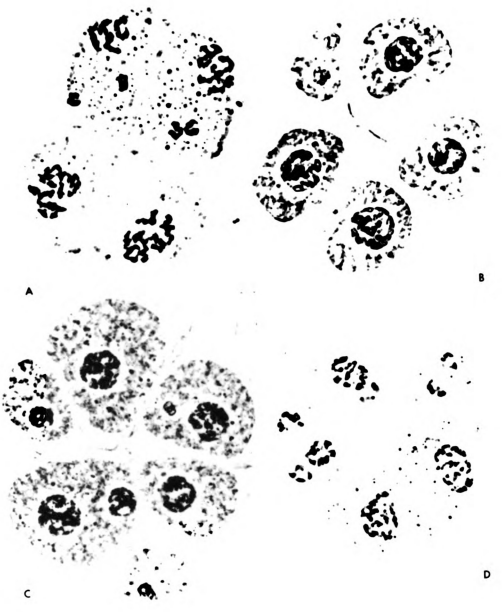

Figure 9

cells containing more than one nucleus are often observed (Table 11), and their average frequency is 60.9%. Each individual plant shows some variation, and in extreme cases, a quartet may contain 18 nuclei and a cell, 7 nuclei.

Pollen mitosis

Pollen mitosis was observed in cells from the first cytokinesis, which contained from 1 to 13 X-shaped chromosomes (Figure 10), and from the second cytokinesis, the normal process of pollen formation. Multipolar cell divisions were found in both kinds of cells during pollen mitosis. The micronuclei seen in the mature pollen may have originated from either meiosis or pollen mitosis.

3. Pollen fertility and Seed fertility

Pollen fertility, which is related to chromosome behavior and genetic balance, was examined by staining the pollen with I_2-KI solution. Individual plants had a frequency of darkly-stained pollen ranging from 47.2% to 87.9%, regardless of pollen size (Table 12). However, the overall staining frequency of the large cells (79.4%) was much higher than that of the small cells (39.1%).

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

Table 11. The number of nuclei at quartet stage in F_1 progeny of CB-9-85.

Nuclei	Plant number							
	A-1-13	A-1-37	A-1-44	A-1-45	A-1-9	A-1-10	A-1-27	Total
4	297	94	87	36	111	143	161	929
5	199	17	51	16	103	51	92	529
6	170	6	49	15	97	22	89	448
7	77	1	24	3	38	5	26	174
8	63	3	15	8	59	9	4	161
9	42		6		2	3	7	60
10	18		11	2	4	1	3	39
11	8		2					10
12	10		1					11
13	7							7
14	4							4
15	1							1
16								
17	1							1
18	1							1
Total	898	121	249	80	414	234	382	2378
Abnormality (%)	66.9	22.3	65.1	55.0	73.2	38.9	57.9	60.9

Figure 10. Pollen contain different chromosome number.

A. 6-6-3-1 (750x)
B. 13-7-4-2 (750x)
C. 5 (750x)
D. 7 (1045x)
E. 8-4 (750x)
F. 7 (750x)
G. 8 (750x)
H. 8-7-6 (750x)

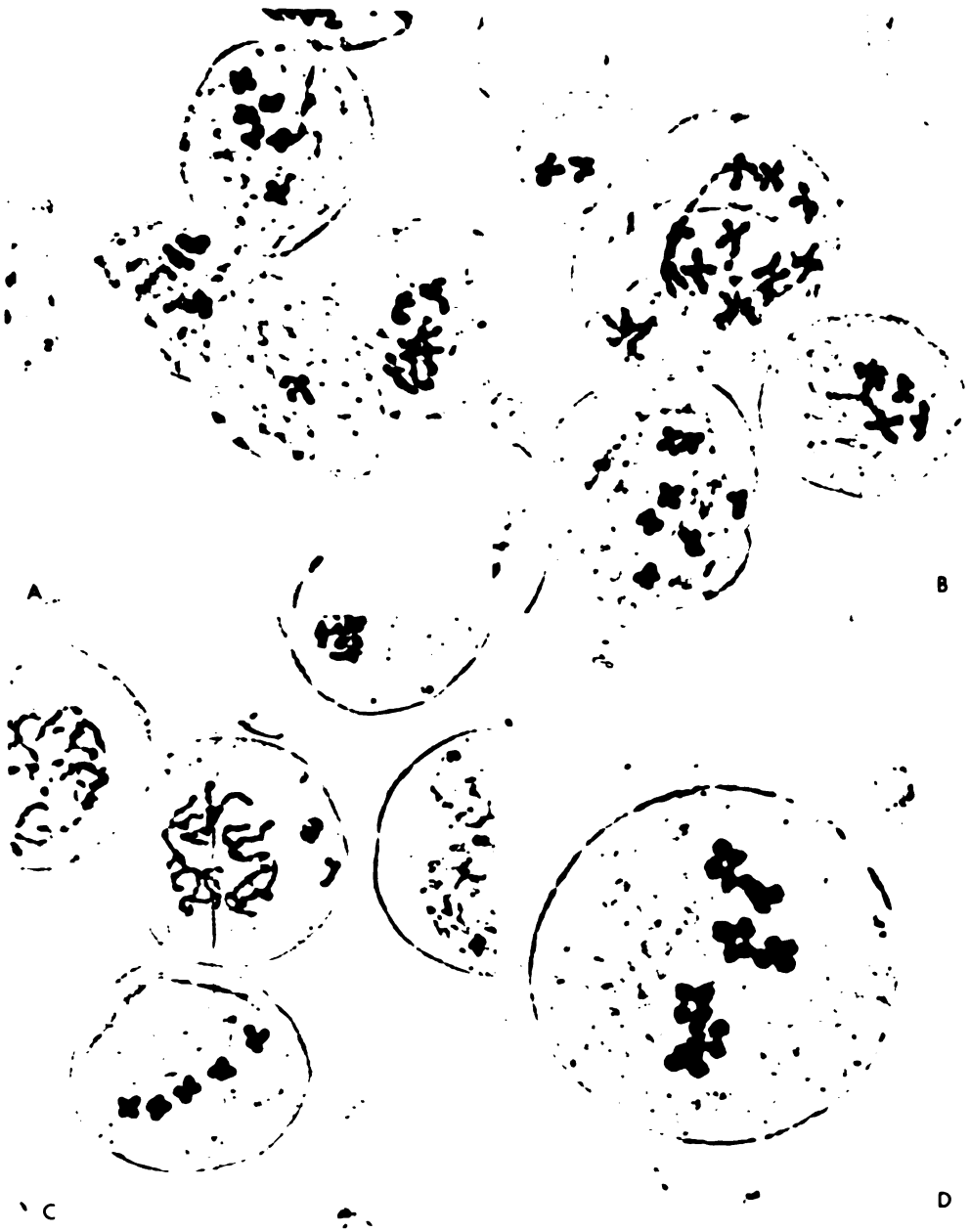

Figure 10

Figure 10

Table 12. Pollen fertility in F_1 progeny of CB-9-85

Plant Number	Large grains		Small grains		Total	%
	Stained	Yellowish	Stained	Yellowish		
A-1-3	215	24	28	71	338	71.9
A-1-4	268	47	0	52	366	73.2
A-1-6	197	63	17	96	373	57.3
A-1-8	284	77	0	120	481	59.0
A-1-9	213	131	8	116	468	47.2
A-1-10	341	165	21	324	851	42.5
A-1-11	266	79	0	98	443	60.0
A-1-13	138	20	149	56	363	79.0
A-1-24	235	2	130	48	415	87.9
A-1-27	358	124	73	165	720	59.9
A-1-31	262	86	45	147	540	56.9
A-1-34	249	27	112	73	461	78.3
A-1-37	172	15	143	34	364	86.5
A-1-44	365	78	188	114	745	74.2
A-1-45	215	41	106	72	434	73.9
Total	3778	979	1020	1586	7363	65.1
%	79.4		39.1			

DISCUSSION

1. The meaning of multipolar cell division in chromosome evolution

As previously reported, multipolar cell divison is found in various hybrids of plants and animals and may arise spontaneously or artifically. 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 division, univalents, chromosome bridges, unequal disjunction and micronuclei in the hybrids of common onion, Allium cepa X 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 Kozlovskaya, 1974), endomitosis (Jensen, 1974), abortive mitosis (Ohno, 1966), endoreduplication (Schmid, 1965; Heneen, 1970) and nuclear fusion (Pera, 1970), also have great influence on the phenomenon of multipolar cell division. In cultured rat-kangaroo cell, Heneen (1970) indicated that the frequency of multipolar cell division and number of poles per cell increases with the ploidy. Moreover, he showed multipolar configurations may have either mononucleate or multinucleate origin. Colchicine treatment provides an excellent tool to study the correlation between progressive polyploidization and multipolar cell division both in frequency and multipolarity (Palitti and

Rizzoni, 1972). In addition to arresting cell division, colchicine also increases the frequency of chromosome changes (Sakharov, et al, 1969). Tai (1970) observed that multipolar cell division and supernumerary secondary cleavages occurred in the colchicine-treated diploid A. cristatum CB-9-85. The present study shows that multipolar cell division occurred in the F_1 progeny of CB-9-85 and pollen chromosome number varied from 1 to 13. From the above information, a scheme (Figure 11) 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, 1957). In his analysis of rat liver chromosomes by length, position of kinetochore, and differentially stained segments, he found that metaphase in triploids resulted in distinct genome groupings of the types (2n-1n and 1n-1n-1n). Similiar 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 anaphase I, the parental genomes segregate in a very regular manner. In Daphnia, the complete set of paternal chromosomes becomes heterchromatic during early embryogeny, and the maternal set remains euchromatic and functioning in the nuclei (Brown and Nur, 1964). Quantitative

Figure 11. The role of multipolar cell division in the change of chromosome number

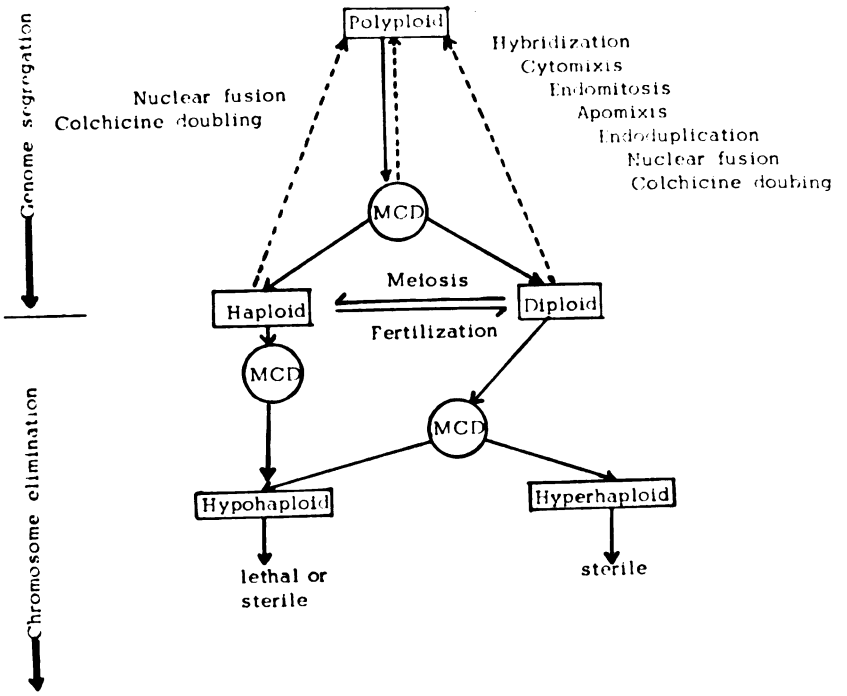
Polyploid
Hybridization
Cytomixis
Endomitosis
Apomixis
Endoduplication
Nuclear fusion
Colchicine doubling
Nuclear fusion
Colchicine doubling
Genome segregation
MCD
Meiosis
Haploid
Diploid
Fertilization
MCD
MCD
Hypohaploid
Hyperhaploid
lethal or
sterile
sterile
Chromosome elimination

Figure 11

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 (Tepliz, et al, 1968), although aneuploid cells were of exceptionally low frequency. The aneuploids were not all oncogeneous, but most of the neoplasiae 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 (Levis, 1962; Fenter and Potter, 1965; Heneen, et al, 1970; Tai, 1970 and Dvorak, et al, 1973), has been described previously. Both physical and chemical methods for multipolar cell division induction, generally used in agriculture and medicine, seem rather haphazard 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 by Sakharov, et al (1969) in rootlet cells of Crepis capillaris. Their work indicated that prolonged treatment with colchicine caused change in ploidy, aneuploidy, 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 (Table 1), and some of them died before reaching maturity. In microsporogenesis of F_1 plants, multipolar cell division occurred in association with other abnormalities. This 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 to the poles (Bajer and Mole'-Bajer, 1972; and Nicklas, 1974), but they all conclude that the spindle fibers of microtubules which are oriented between 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 comes from: 1) the centrioles seem to be involved in the formation of protozoan flagella; 2) centrioles migrate prior to spindle formation; and 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 might 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 or rejection of the latter point must be based on knowledge of the chemical action of mercaptoethanol which breaks the disulfide (-S-S-) 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 forms and operates 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-irradiation (Levis and Martin, 1963); 5) the kinetochore equivalent is responsible for spindle formation in the fungus, Polysticus versicolor (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 amphipolypooids and autopolyploids. The term, microtubule-organzier-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 has 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. Furrowing

Furrowing, cytokinesis without the formation of a phragmoplast or cell plate, occurs in pollen and has been described by Esau (1965). Detailed information about the furrowing process in microsporogenesis, is still very limited (Farr, 1916). Experimental results coming from the study of cleavage in animals and lower plants, algae and fungi, may lead to a better understanding of the process.

Karyokinesis, nuclear division, and cytokinesis appear to follow each other so closely that mitosis and cytokinesis appears to be one phenomenon; however, the two processes may be separate. In certain animal cells, which have entered metaphase, cleavage can occur even if the spindle is removed or destroyed (Mazia, 1961). In cultured human amnion cells in which cytokinesis has begun, application of p-DL-fluro-phenyl alanine will not stop cleavage, but chromosome movement does slow down in anaphase (Sisken, 1973). In plant cells, cytokinesis can be inhibited by certain concentrations of caffeine (Pickett-Heaps, 1969) deoxyguanine (Brulfert, et al, 1974) without interrupting nuclear division. In potato meiocytes, the cytoplasm can undergo cleavage and form a quartet, triad or dyad, irrespective of the division or the restitution of the nuclei (Ramama, 1974). In the colchicine-treated, diploid A. cristatum, secondary, supernumerary cleavage occurs following metaphase II (Tai, 1970). In the present study unsynchronized cell division was observed in one meiotic cell, and cytokinesis occurred in any meiotic stage after metaphase I.

Cytoplasmic cleavage in animal cell always seem to be coordinated by the microtubules (Tamma et al, 1969; Williams, 1975), Margulis (1973) indicated that mibrotubules clearly underlie the development of asymmetric cell shapes, and that slow morphogenetic movement, which involves microtubular polymerization, tends to be colchicine sensitive. Moreover, this study states that conformation changes in microtubular protein may account for the chemosensitivity of the nervous system of higher animals. This concept of microtubules as cellular skeletons has been supported by data from plant materials. In Chlamydomonas (Johnson and Porter, 1968) and in Nassula (Tucker, 1971), cytoplasmic cleavage is associated with a contractile ring of microfilaments formed perpendicular to the plane of the furrow. After telophase the microtubules are oriented in approximately in the same plane and within the ring of microfilaments. In zoosporogenesis of Thraustochytrium (Kazama, 1975), subplasmalemma microtubules crossed the furrow, at oblique angles. A similar furrowing process was observed in the present study, using a light microscope. It is suggested that furrowing cleaves the cytoplasm by the means of a shearing force, both twisting and pulling the daughter cells apart, rather than by the formation of a contractile ring.

There are many similarities between plant and animal miocytes, both of which undergo cytoplasmic cleavage without the formation of a cell plate. Therefore, the furrowing process, as seen in microsporogenesis, is viewed as intermediate between the conventional types of cytokinesis cited to separate plants and animals. Perhaps furrowing, represents an efficient mechanism conserved through the otherwise divergent evolution of the two kingdoms.

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 as early as 1916, Farr noted that presence of the furrowing process in numerous dicot genera including Nicotiana, Primula, Tropaelum, Ambrosia, Chrysanthemum, Helianthus and others. 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 quartets. The quartet may provide for metabolite exchange between the microgametes 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 exchange genetically unbalanced pollen may be capable of successful pollination and fertilization. Quartet metabolite exchange provides a suitable explanation for the survival of gametes which can assure the creation, evolution and survival of hybrid and polyploid.

SUMMARY

Microsporogenesis and multipolar cell division were studied in the F_1 progeny of the colchicine-treated, diploid Agropyron cristatum, CB-9-85. Since multipolar cell division was observed in all of the F_1 progeny, it was proposed that colchicine-induced mutation is inheritable.

The occurrence of multipolar cell division was associated with the occurrence of univalents, unequal disjunction, precocious division, chromosome fragments, chromosome bridges, and micronuclei. Therefore, multipolar cell division was interpreted to be the main source of multinucleate and aneuploid pollen grains.

Normally the furrowing process was observed to occur immediately after meiosis I and meiosis II. However, supernumerary cytoplasmic cleavage, which is related to chromosome elimination, was observed at all stages after metaphase I.

Pollen fertility was related to the size of pollen grains, with small grains having the lowest fertility. Seed fertility was correlated with the formation of pollen quartets. In plants which failed to form quartets, seed fertility was zero.

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