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MULTIPOLAR MEIOSIS IN DIPLOID CRESTED WHEATGRASS, AGROPYRON CRISTATUM[1]

WILLIAM TAI

Department of Botany and Plant Pathology, Michigan State University, East Lansing

ABSTRACT

A new model of spindle organizers is proposed: The spindle organizer in a higher plant is similar to the centriole of animal cells. It is a unit cell organelle which follows regular cell division cycles and is genome specific. Each genome carries its own spindle organizer. During fertilization, a male spindle organizer enters the egg cell. It may fuse with the female spindle organizer, or either one may degenerate. In a hybrid, both male and female spindle organizers may exist, and multipolar divisions separate different genomes into different groups. The same mechanism can be used to explain the formation of a polyhaploid from a polyploid. The chromosome behavior of an individual is believed to be an interaction of chromosome homology and the homology between chromosomes and their spindle organizers. This model is based on observations of multipolar meiosis which occurred in two cultures of diploid crested wheatgrass, *Agropyron cristatum* (L.) Gaertn. In these two cultures multipolar meiosis occurred at every stage after late diakinesis. The seven bivalents were separated into groups at late diakinesis. More than one equatorial plate was formed at metaphase I. Each micrometaphase plate behaved as an independent unit and had its own anaphase movement. Usually the chromosome complement separated into two groups with (4–3), (5–2), and (6–1) separations observed in about an equal number of cells. Cells with chromosomes divided into three or four groups were found less frequently. Multipolar meiosis may take place at either first or second division. Cell plates were formed across each spindle apparatus, cleaving each group of chromosomes into smaller micro-cells. At the "quartet" stage, 4- to 12-celled "quartets" were observed. Pollen stainability was measured at above 75% in both cultures. Stained pollen grains could be classified into two distinct size classes. Darkly stained, small pollen grains represented the result of multipolar meiosis and may have been viable. Multipolar cell divisions provided a mechanism which polyploids might reduce their ploidy level.

THE PHENOMENON by which the meiotic or mitotic chromosome complement is subdivided into two or more groups that function more or less independently within the cell has been described by various terms including "incompact spindle" (Darlington and Thomas, 1937), "double-plate metaphase" (Upcott, 1939; Vaarama, 1949), "somatic meiosis" (Huskins, 1948), "reductional groupings" (Wilson, 1950), "multipolar spindles" (Therman and Timonen, 1950; Knudson, 1958; Walters, 1958), "split spindles" (Upcott, 1939; Nielsen and Nath, 1961), and "complement fractionation" (Thompson, 1962). This phenomenon is characterized by the formation of two or more metaphase plates, appropriately called "microplates," within a single cell. The consequence of multiple plates and spindles is the production of daughter cells with variable chromosome numbers. The term "multipolar spindles" is preferred by this author, and the overall process shall be referred to as "multipolar" meiosis or division throughout this paper.

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Multipolar divisions have been reported in both animal and plant species. In animals, they have been observed in an inbred line of cattle (Knudsen, 1958), human cancer cells (Therman and Timonen, 1950; 1954), and an interracial *Drosophila* hybrid (Koller, 1934). More frequent reports of multipolar divisions occur among higher plants including haploid *Oryza sativa* (Morinaga and Fukushima, 1934), diploid *Zea mays* (Beadle and McClintock, 1928), polyploid *Ribes* (Vaarama, 1949), *Rubus* hybrids (Bammi, 1965), *Bromus* hybrids (Walters, 1958), and *Triticum-Aegilops* hybrids (Li and Tu, 1947).

Multipolar divisions can occur spontaneously, or they can be induced artificially by temperature shock (Huskins and Cheng, 1950), low concentrations of colchicine (Östergren, 1950), antibiotics (Wilson, 1950), irradiation (Puza and Srb, 1964), and other chemical agents (Kabarity, 1966). Plants with meiotic irregularities due to polyploidy and wide hybridization appear to be especially susceptible to multipolar divisions. Multipolar division is likely more common than is generally recognized. Huskins (1948) noted that, "It appears that many cytologists have observed somatic meiosis in plants. Few such

observations have been published because they are rarely 'well fixed' and are therefore usually dismissed as of doubtful validity." Recognition of multipolar divisions has increased in recent years, yet the data are scattered and fragmentary; and the significance of this phenomenon remains obscure.

At least part of the difficulties associated with a detailed description and analysis of multipolar meiosis arises from high chromosome numbers and meiotic irregularities found in most plants in which the phenomenon occurs. Diploid crested wheatgrass, *Agropyron cristatum* (L.) Gaertn. provides an excellent opportunity to study meiosis. It is a meiotically regular species with a low chromosome number, 2n = 14, and lends itself to cytological analysis. The recent discovery of two *A. cristatum* plants that exhibited multipolar meiosis provided the basis for this study, which describes the sequence of multipolar meiosis from late diakinesis through the quartet stage and pollen grain formation. The possible role of multipolar division in the evolution of chromosome number will be discussed, and a new hypothesis on the nature and action of "spindle organizers" will be presented.

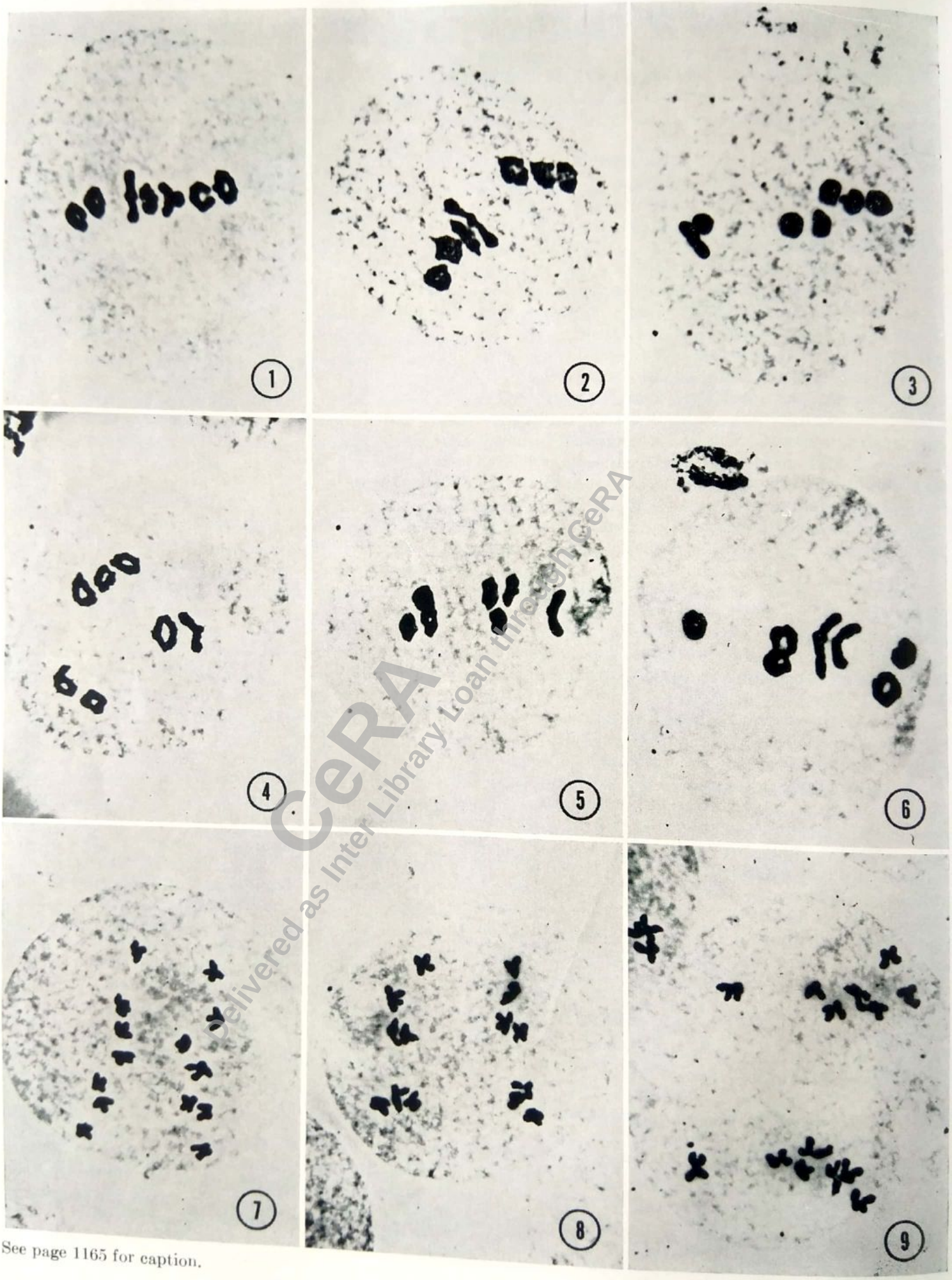
MATERIALS AND METHODS—One *A. cristatum* plant, CB-9-85, with multipolar meiosis was discovered during the course of an experiment involving induction of polyploidy (Tai and Dewey, 1966). This plant came from seed treated with a 0.1% colchicine solution for 12 hr. Polyploidy was not induced, and there is no evidence to indicate that the unusual meiosis in this plant resulted from the colchicine treatment. A second *A. cristatum* plant, CC-37-119, was found to exhibit the same type of irregular meiosis. However, this plant had received no treatment of any type. Meiosis was virtually identical in both plants, and the cytological data of the two are combined throughout this paper.

Spikes were fixed in Newcomer's (1953) solution and stored under refrigeration. All cytological observations were made on pollen-mother-cells stained with iron-acetocarmine. Pollen grains were stained in an aqueous I_2-KI solution. Photomicrographs of selected cells were taken through a phase contrast microscope equipped with a mounted camera.

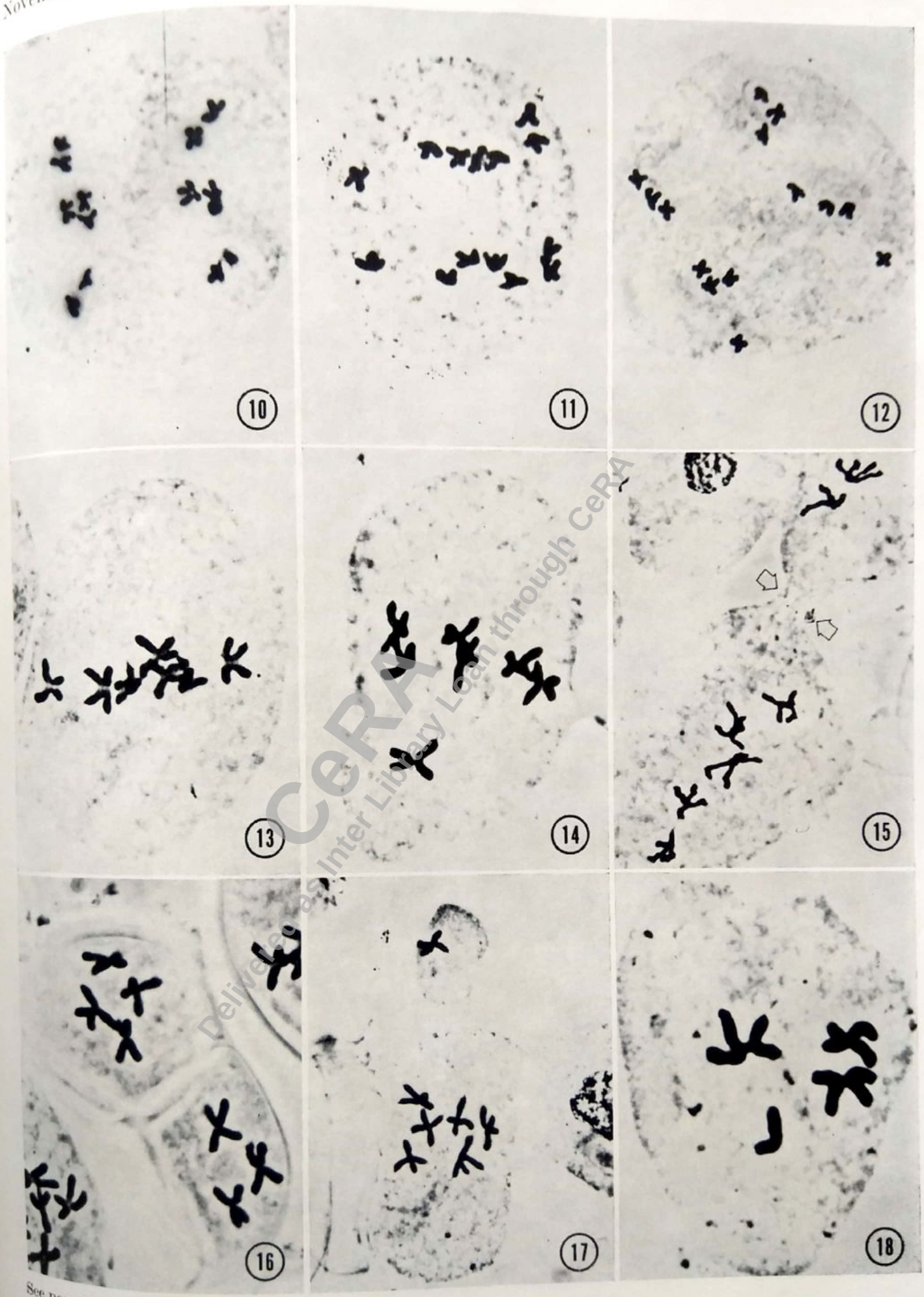
TABLE 1. *Chromosome groupings at different meiotic stages in cultures CB-9-85 and CC-37-119*

Stages	Groupings	Number of cells	Percent
Diakinesis	Normal	126	97.0
	(4–3)	2	1.5
	(6–1)	2	1.5
	Subtotal	130	
Metaphase I	Normal	377	65.6
	(4–3)	47	8.1
	(5–2)	81	14.1
	(6–1)	60	10.5
	3 groups[a]	6	1.0
	4 groups[b]	3	0.5
	Subtotal	574	
Anaphase I	Normal	93	60.4
	(4–3)–(4–3)	24	15.6
	(5–2)–(5–2)	18	11.7
	(6–1)–(6–1)	12	7.8
	3 groups[c]	6	3.9
	4 groups[d]	1	0.7
	Subtotal	154	
Metaphase II[e]	Normal	141	43.1
	(4–3)	53	16.2
	(5–2)	45	13.8
	(6–1)	40	12.2
	3 groups	34	10.3
	(2–2–2–1)	1	0.3
	Others[f]	13	3.9
	Subtotal	327	
Anaphase II	Normal	115	47.9
	(4–3)–(4–3)	35	14.6
	(5–2)–(5–2)	28	11.7
	(6–1)–(6–1)	30	12.5
	Others[g]	32	13.4
	Subtotal	240	
"Quartet" stage	Normal	128	40.1
	Micronucleated[h]	87	27.3
	Others[i]	104	32.6
	Subtotal	319	

[a] (3–2–2) two cells, (3–3–1) two cells, (4–2–1) two cells.
[b] (2–2–2–1) one cell, (3–2–1–1) two cells.
[c] (3–3–1)–(3–3–1) three cells, (3–2–2)–(3–2–2) two cells, (4–2–1)–(4–2–1) one cell.
[d] (3–2–1–1)–(3–2–1–1) one cell.
[e] Since cytokinesis has taken place after meiosis I, each daughter cell is considered as a single unit.
[f] Cells having less than normal number (7) of dyads. Number of dyads per cell varies from 1–6. These cells represent the microcells which are possibly the result of supernumerary cytokineses taking place after multipolar meiosis I. (See text for details).
[g] Cells having less than normal number (7–7)–(7–7) of chromosomes. Number of chromosomes per cell varies from (1–1)–(6–6).
[h] The number of micronuclei per quartet varies from 1–6 with an average of 1.8 micronucleus per quartet.
[i] Having more than 4 spores per "quartet." The number of spores per "quartet" varies from 5–12. The most common types are 6-celled and 8-celled "quartets" with 36 and 44 "quartets," respectively.



See page 1165 for caption.



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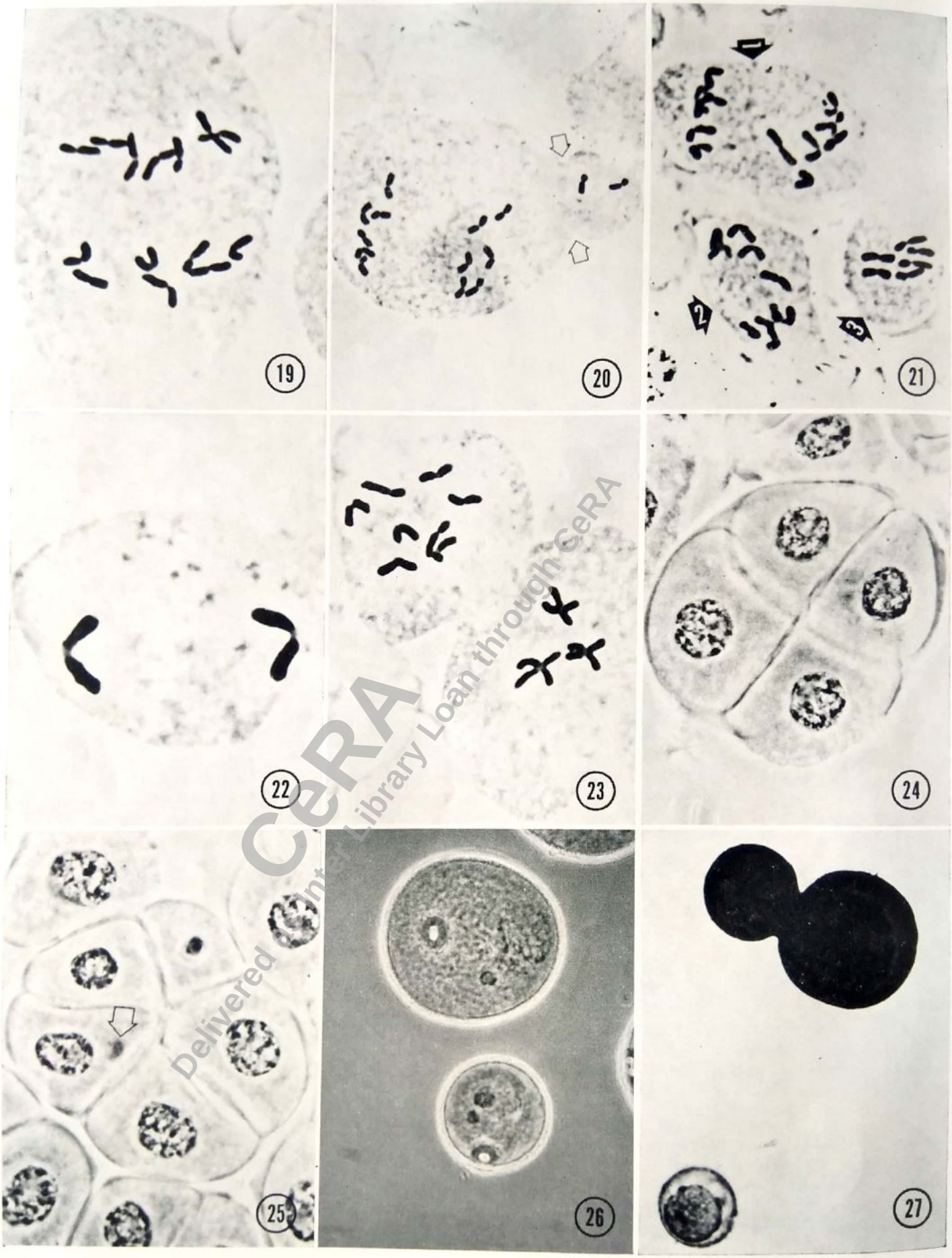

The two *A. cristatum* plants, CB-9-85 and CC-37-119, are maintained in field nurseries on the Evans Farm of the Utah Agricultural Experiment Station near Logan, Utah.

OBSERVATIONS—Normal meiosis in *A. cristatum* is comparable with that of other diploids. The 14 chromosomes typically form seven bivalents that align themselves on a single equatorial plate (Fig. 1) followed by a 7–7 bipolar anaphase I disjunction (Fig. 7) and a cytoplasmic cleavage that gives rise to two daughter cells each with seven dyads. Meiosis is synchronized so that sister cells proceed simultaneously through metaphase II (Fig. 13) and anaphase II (Fig. 19) and typical four-celled quartets are formed (Fig. 24).

The prophase stages of the two abnormal *A. cristatum* plants appeared to be fully regular, and all cells entered diakinesis with seven normal-appearing bivalents. By late diakinesis occasional cells, 4 of 130, showed a tendency for the chromosomes to accumulate into two groups. Casual examination of diakinesis in these plants revealed no unusual behavior.

The first conclusive evidence of multipolar meiosis was encountered at metaphase I. However, its effects could still be easily overlooked at this stage unless one was consciously searching for irregular behavior. Even with close examination, only one-third, 197 of 574, of the cells appeared to be abnormal. The abnormality consisted of clustering of the seven bivalents into two to four groups of one to six bivalents each. The most common type of grouping, accounting for 188 of the 197 abnormal cells, was separation into two groups of 4 and 3 (Fig. 2), 5 and 2 (Fig. 3), or 6 and 1 bivalents (Table 1). Bivalents formed into three groups in six cells (Fig. 4, 5), and four-group associations were observed in three cells (Fig. 6).

The true proportion of abnormal metaphase I cells may be higher than that recorded in Table 1. Two microplates might be forced together during squashing and appear as a single plate. On the other hand, a single plate would not likely be separated into segments unless undue pressure was applied to the cover slip during slide preparation. Considerable care was exercised to avoid breaking or disrupting the cell contents as the slides were prepared. The apparent underestimation of abnormal metaphase I cells is reflected in the slightly higher proportion of abnormal cells at anaphase I (Table 1).

The visible evidence of multipolar meiosis at anaphase I was similar to that at metaphase I and merely represented a continuation of the irregularities apparent at the earlier stage. Approximately 40% of the 154 anaphase I cells examined had abnormal groupings of dyads. The nature and relative frequency of the groups were more or less the same as those found at metaphase I (Table 1). Two-group dyad separations (Fig. 8, 9) were by far the most frequent, although three-group (Fig. 10, 11, 12) or four-group separations occurred in several cells.

Unlike many other plants, cytokinesis in *A.*

Fig. 1–9.—Fig. 1. Normal metaphase I showing five ring bivalents and two rod bivalents. × 840.—Fig. 2. Multipolar metaphase I with microplates of four and three (4–3) bivalents respectively. × 780.—Fig. 3. Multipolar metaphase I with (5–2) separation. × 840.—Fig. 4. Multipolar metaphase I showing (3–2–2) grouping in a polar view. × 780.—Fig. 5. Multipolar metaphase I with (3–3–1) separation. × 780.—Fig. 6. Metaphase I with seven bivalents separated into four groups with a (2–2–2–1) separation. × 840.—Fig. 7. Normal anaphase I with a (7–7) bipolar disjunction. × 800.—Fig. 8. Multipolar anaphase I showing (4–3) separation in each of the original two polar regions. × 780.—Fig. 9. Anaphase I with (6–1) separation in each of the original two polar regions. × 840.

Fig. 10–18.—Fig. 10. Anaphase I with (3–2–2) separations. × 840.—Fig. 11. Anaphase I with (4–2–1) separations. × 780.—Fig. 12. Anaphase I with (3–3–1) separations. × 780.—Fig. 13. Normal metaphase II showing seven dyad chromosomes forming a single equatorial plate. × 1250.—Fig. 14. Multipolar metaphase II showing (2–2–2–1) separation. × 1250.—Fig. 15. Metaphase II with (5–2) separation. Open arrows point to the supernumerary cytokinesis. × 1200.—Fig. 16. Multipolar metaphase II. Supernumerary cytokinesis has taken place but chromosomes still show typical metaphase II morphology. A (4–3) separation is illustrated in this picture. × 1200.—Fig. 17. Metaphase II of (6–1) separation. The supernumerary cytokinesis has cleaved the cell into two microcells with one and six dyads respectively. × 740.—Fig. 18. A microcell with 3.5 dyad chromosomes at metaphase II, believed to be the results of multipolar meiosis and precocious division at an earlier stage. × 1500.

Fig. 19–27.—Fig. 19. Normal anaphase II showing (7–7) disjunction. × 1200.—Fig. 20. Anaphase II with (6–6) disjunction in one microcell and (1–1) disjunction in another microcell. Open arrows point to the supernumerary cytokinesis. × 900.—Fig. 21. Multipolar anaphase II. The intact cell shows a (7–6) disjunction (Arrow #1) and the two microcells show (4–4–1) (Arrow #2) and (3–3) (Arrow #3) respectively. The one lagging chromosome in the second microcell may have originated from the intact cell at first meiosis. × 720.—Fig. 22. Anaphase II. Microcell with (1–1) disjunction. × 2000.—Fig. 23. Asynchronization of multipolar meiosis. One cell has three dyads at metaphase II and another cell shows eight single-stranded chromosomes at anaphase II. × 1200.—Fig. 24. A normal quartet. × 880.—Fig. 25. A 6-celled "quartet" with the 6th nucleus out of the focal depth (open arrow). It is believed that the supernumerary sporads are results of multipolar divisions. × 880.—Fig. 26. Different sizes of pollen grains stained with acetocarmine. The smaller pollen is thought to have developed from a microcell. × 730.—Fig. 27. Pollen grains stained with I_2-KI. The large dark and small dark (arises from microcell) pollen grains are believed to be viable. × 660.

cristatum occurs after first meiosis and cleaves the primary microsporocyte into two secondary microsporocytes. Consequently, the whole of the second meiotic division takes place in two separate sister cells. In this report, each sister cell is considered as a single unit. No critical observations were made at prophase II because cells were seen only rarely at this stage, which apparently proceeds very rapidly.

Effects of the multipolar division at metaphase I became clearly apparent at metaphase II or shortly after. The chromosomes of more than half of the cells, 186 of 327, oriented themselves in two or more groups at the cell equator (Fig. 14, 15) in much the same fashion as at metaphase I. A second supernumerary cytoplasmic cleavage (Fig. 15, 16, 23) occurred in many of the abnormal cells at metaphase II and gave rise to microcells with one to six dyad chromosomes (Fig. 16, 17, 18). Other cells, both with grouped chromosomes and those without, proceeded into anaphase II normally with no supernumerary cytokinesis. Supernumerary cytokinesis could also take place together with regular cytokinesis after the first meiotic division, even though it was not observed in this investigation.

Sister microcells resulting from the supernumerary cytokinesis advanced to anaphase II more or less unsynchronized. One cell often remained at metaphase II while its sister cell proceeded to anaphase II (Fig. 23). Anaphase II in the other microcells was not particularly unusual except for the variable number of chromosomes per cell (Fig. 20, 21, 22).

The quartet stage reflected the abnormal behavior seen at the previous stages. Approximately 60% of the quartets contained micronuclei or consisted of more than four cells (Fig. 25). The number of cells per "quartet" is apparently dependent on the number of microplates formed at metaphase and the frequency of supernumerary cytokinesis. For example, a double microplate at metaphase I and supernumerary cytokinesis in both daughter cells at metaphase II would produce an 8-celled "quartet." A 12-celled "quartet" would result from a three-grouped metaphase I followed by two supernumerary cleavages at metaphase II. Supernumerary cytokinesis does not take place in every cell with multipolar spindles.

As might be expected, microspores and pollen grains from the abnormal *A. cristatum* plants were of variable sizes (Fig. 26, 27). More than 75% of the pollen stained darkly in I_2-KI (Table 2). Approximately one-third of the stained grains were small and likely contained fewer than seven chromosomes per gamete. Plant CB-9-85 averaged 1.18 seeds per spikelet under open pollination, whereas normal *A. cristatum* plants growing nearby averaged 2.55 seeds per spikelet. Seed set on plant CC-37-119 was not determined.

Discussion—Fragmentary observations of multipolar meiosis have been made in several plant species and hybrids; however, the overall sequence has never been followed closely in a low-chromosome number diploid such as *A. cristatum*. The advantages of studying the details of a complex meiotic irregularity in a plant with few chromosomes and no additional meiotic irregularities are obvious. Although the same mechanism may not be involved in multipolar divisions in all species or hybrids, the basic process is probably similar.

Thompson (1962) emphasized that after "complement fractionation," each group of chromosomes is an independent unit. This point is also demonstrated in the present study. The percentage of multipolar meiotic cells increases from one meiotic stage to the next, except anaphase II at which the percentage of normal cells is slightly higher than the normal cell percentage found at metaphase II (Table 1). This indicates that multipolar meiosis can occur at any stage, and as the meiotic process proceeds, the number of multipolar cells accumulates. It also demonstrates that once microplates are formed, they are not regrouped even though they may be positioned very close to each other at telophase I or II. Multipolar meiosis as an independent event is also evident in those cells where asynchrony was observed. Figure 21 shows an intact cell and two microcells. The total number and the distribution of chromosomes indicate that all three cells

Table 2. *Pollen stainability with I_2-KI solution in cultures CB-9-85 and CC-37-119*

	Stained					Yellowish			
	Large		Small			Large		Small	
	Number	Percent		Number	Percent	Number	Percent	Number	Percent
CB-9-85	780	52.0%	77.5%	383	25.5%	217	14.5%	120	8.0%
CC-37-119	810	54.0%	79.1%	361	24.1%	197	13.1%	132	8.8%
Total	1590	53.0%	77.8%	744	24.8%	414	13.8%	252	8.4%
							22.5%		
							21.9%		
							22.2%		

come from the same primary microsporocyte, yet multipolar division occurs in one secondary microsporocyte with no influence on the other.

The separation of chromosomes into groups is another independent operation. If the numbers of cells at different stages are added together, the total number of cells with chromosomes separated into two groups is 473. The cells of (4–3), (5–2), and (6–1) separations are represented by approximately equal numbers of 33.6%, 36.4%, and 30.0%, respectively. The number of microcells at metaphsae and anaphase II are not included in this calculation because a microcell with three chromosomes may result either from (4–3) or (3–3–1) separation.

The independent operation of each microplate provides evidence for the existence of a cell organelle, in the two *A. cristatum* cultures studied, similar to the centriole in animal cells. From evidence reported in the literature and her own experiment, Thompson (1962) suggested two origins of multipolar cell divisions. One explanation is that the chromosomes are carried by a split spindle. The alternative is that the chromosomes are first grouped and then form their own split spindles. Neither hypothesis can be proven cytologically. No matter whether first the spindles split or the chromosomes group, without centers at the polar regions for the chromosomes and spindles to orient themselves at telophase, the microplates will be regrouped to form a single nucleus. Although no centriole or similar structure has been seen in any angiosperm cell (Swanson, 1952), its physical existence is probable.

Based on their observations of "incompact spindles," Darlington and Thomas (1937) proposed that the spindles are developed through the cooperation between centromere and "pole-determinants." They also indicated that "pole determinants" exist as diffuse particles which coalesce or congregate at the moment when spindle poles are normally formed and exist separately at other times. The polar determinants have the function of centrosomes and possess their continuity as single and visible coherent bodies. Swanson and Nelson (1942) attributed the multipolar spindles in *Mentha* to extra-pole determinants, probably arising de novo. Supernumerary centrioles were also reported to be associated with multipolar divisions in animal cells (Therman and Timonen, 1954; Puza and Srb, 1964).

The "spindle organizer" described by Walters (1958) is essentially the same as a pole determinant. She suggested that the spindle organizer may be a compound structure, usually single and following a regular division cycle. It is, however, capable of supernumerary division under extraordinary condition into substructures that may also be effective in spindle organization. In *Bromus* hybrids she noted that "one or both organizers may divide again. Some separation of the new organizer occurs, and a number of chromosomes somewhat proportional to the size (or quantity of components) of each spindle organizer is attracted to it."

The above models of "polar determinants" or "spindle organizers" can explain normal chromosome behavior and some multipolar cell divisions satisfactorily. However, they all fail to explain the following facts: (1) multipolar division occurs very rarely in a natural diploid population; (2) it occurs in many hybrids and polyploids; (3) the grouping of chromosomes is often based on the genome origin of the chromosomes. The genome separation of chromosomes has been observed in species hybrids of *Rubus* (Bammi, 1965), in an amphidiploid of *Triticum* and *Aegilops* (Li and Tu, 1947), tetraploid *Rhoeo* (Huskins and Chouinard, 1950), triploid *Lycopersicum* (Gottschalk, in Thompson, 1962), triploid *Rubus* (Thompson, 1962), and in other species.

A new model is presented here to describe this cell organelle. The fact that multipolar meiosis has never taken place at a stage earlier than prometaphase I indicates that the grouping of chromosomes is somewhat linked with the formation of spindle fibers. The name "spindle organizer" is therefore adopted.

I propose that the spindle organizer is a single organelle. Its function is similar to that of a centriole in animal cells. In a somatic cell of a diploid species there are two organizers functioning as two poles during mitosis. In a male gametic cell there is only one spindle organizer. It enters the egg during fertilization together with a genome of chromosomes. This action is similar to the entrance of a male centriole into egg cells in *Ascaris* and sea urchin species. After the male spindle organizer enters the egg, it may fuse with the female organizer to form a single unit. Or after the entrance of male organizer, either female or male organizer disintegrates. The disintegration of male organizer may be more probable because parallel phenomena have been observed in animal species. Subsequently, the remaining spindle organizer may divide again before or during early mitosis as centrosomes do in animal cells. The spindle organizer apparently applies its influence on chromosome behavior through the fibers connecting the centromere and the spindle organizer.

I propose that the spindle organizer is genome specific. This means that each genome carries its own spindle organizer, and the movement of this genome of chromosomes is controlled by its own spindle organizer. In a species hybrid where different genomes are combined, heterozygous spindle organizers exist within the same protoplast. If either male or female spindle organizer disintegrates, the affinity between spindle organizers of one species and chromosomes of another species may be little or none. Therefore, chromo-

somes without their own spindle organizer will move rather randomly, and many of them will be lagging. Another possibility is that in a hybrid neither spindle organizer degenerates; rather, they serve as opposite poles, and the lack of interaction between the poles causes the irregular chromosome behavior. In an amphidiploid each chromosome finds its own homologous partner, and each genome finds its own spindle organizer. If the spindle organizers of different genomes fuse, normal chromosome behavior is recovered. If the spindle organizers are not fused and remain as separate units, multipolar division should result, and the chromosomes will be grouped according to their genomic origins. Apparently, chromosome behavior in a species is an interaction of homology between chromosomes and the homology between chromosomes and their spindle organizers.

The same reasoning can be used to explain genome separation in polyploids and colchiploids. If each genome carries its own spindle organizer and the spindle organizers do not disintegrate or fuse, multipolar division will occur. Apparently, genome separation in a multipolar division is gene-controlled. Even in an autopolyploid a slight difference in genetic composition may enable the spindle organizer to recognize its own genome.

Usually a spindle organizer is a single-unit cell organelle. It can be broken by chemical treatment (Wilson, 1950; Kabarity, 1966), irradiation (Puza and Srb, 1964), and low temperature (Huskins and Cheng, 1950). It may also be broken spontaneously as observed in culture CC-37–119 in the present study. When a spindle organizer is broken, multipolar division arises.

A unit spindle organizer usually is broken randomly. This can be demonstrated by the random grouping of chromosomes in induction experiments. It is clear that the spindle organizers of CB-9–85 and CC-37–119 are single units broken randomly because the groupings of chromosomes are completely random. I believe that the number of chromosomes attracted to it is proportional to the size of the broken piece.

Cytokinesis seems to occur as an interaction between any two spindle organizers. No genome specificity was observed in this regard. Therefore, wherever there is a spindle organizer, cytokinesis takes place, cleaving the cytoplasm and keeping the constant ratio of one spindle organizer per each daughter cell. As the number of spindle organizers increases, the number of cytokineses increases with it, and there will be more microcells. If a double plate first metaphase occurs, an "octet" or 8-celled "quartet" should be produced. The close relationship between the spindle poles and the "division furrow" is discussed in detail by DuPraw (1968) based on a few observations with animal materials.

Avers (1957) observed 17 bivalents in a hybrid between *Aster turbinellus* (n = 50) and *A. cordifolius* (n = 9). If multipolar cell division is taken into consideration, this result is not hard to explain cytologically. Similar reports can be found in many articles dealing with plant cytogenetics. In most cases "unknown" has been marked as the cause of these mysterious hybrid chromosome numbers.

The significance of multipolar divisions has not been recognized previously. The existence of multipolar divisions is not mentioned in recent cytology or cytogenetics textbooks. In a review of haploid angiosperms, Kimber and Riley (1963) stated that there was no known technique which could induce haploidy.

In Fig. 26 and 27 stainable pollen grains of different sizes indicate that some of the microcells may be functional. More significant data may be obtained when the few seeds collected from cultures CB-9–85 and CC-37–119 are planted and studied. Fertile offspring after "complement fractionation" in *Rubus* were also reported by Thompson (1962). She suggested that multipolar division provided a method to decrease the level of ploidy. Thus polyploidy is a reversible process, and "ancient polyploids are an untapped reservoir of variability that can be drawn upon." Raven and Thompson (1964) stated that "there appears to be no theoretical reason or experimental basis for excluding polyhaploidy as an evolutionary mechanism of some importance in polyploid groups or organisms." It is apparent that in the study of genome relationships and evolutionary pathways of a group of plants, the study of multipolar divisions deserves at least as much attention as the induction of polyploids by colchicine treatment. In addition, the significance of multipolar division to plant genetics and plant breeding should also be recognized. It could provide a simple method for obtaining an individual which is homozygous for every gene locus. It would require only two steps, induction of haploidy by multipolar cell divisions and redoubling of the chromosome complement in the haploids.

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