CYTOLOGICAL STUDIES IN ASYNAPTIC MAIZE[1]

O. L. MILLER, JR.[2]

Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, Minnesota

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ALTHOUGH the first study of recombination frequency within asynaptic (*as*) maize (BEADLE 1933) had indicated no significant crossover reduction in mutant plants, higher than normal single crossovers and much greater than normal double crossovers were subsequently reported for two genetic regions in both diploid and haploid eggs (RHOADES 1947; RHOADES and DEMPSEY 1949). A later study (DEMPSEY 1959), however, showed that on a basis of total ovules rather than viable seed set, single crossovers in the same two regions were reduced in haploid gametes to about 50 percent of normal values, but that double crossovers still were higher in *as* plants than in normal sibs. The apparent increase in crossing over based on seed set was interpreted as due to functional gametes being derived primarily from megasporocytes with more frequent chiasmata, and therefore more regular chromosome behavior, than occurred in those which produced abortive gametes.

Although diploid *as* eggs containing crossover chromatids were presumed in the earlier studies to come from meiocytes with few or no chiasmata, evidence indicating that such eggs may contain both sister and nonsister centromeres was later presented (DEMPSEY 1958). The suggestion was made that diploid gametes might arise from megasporocytes containing a mixture of bilalents and univalents by precocious separation of dyad centromeres and failure of division II following a division I in which both univalents and bivalents had divided.

In view of the anomalous genetic results in asynaptic maize, the objective of the present study has been to clarify various aspects of the cytological effects of the *as* gene, especially with respect to the mode of origin of diploid gametes containing crossover chromatids. This objective was approached through three primary avenues of investigation: first, to describe the different types and degrees of meiotic abnormalities encountered in various individual asynaptic maize plants; second, to demonstrate by using morphological chromosome markers the presence or absence of crossing over previous to the appearance of univalents in asynaptic plants; and, third, to determine the extent and location of early prophase pairing in plants with various degrees of asynapsis at diakinesis or metaphase I.

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[2] Present address: Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

MATERIALS AND METHODS

A stock carrying the recessive asynaptic gene (*as*) was obtained from the Maize Genetics Cooperative, Urbana, Illinois. Homozygous *as* plants segregating in these cultures were sampled for sporocytes and selfed, sibbed, or crossed with other maize types to produce additional material for study.

Detailed observations on the type and degree of abnormalities from diakinesis to pollen formation were made on 14 *as* plants from segregating stocks ranging from third generation selfs to double-cross hybrids. Chromosome pairing in early prophase was investigated in four *as* plants, two of which exhibited nearly complete asynapsis and two, medium to low asynapsis at diakinesis and metaphase I. Observations on the location and frequency of partial asynapsis in pachytene chromosomes were made on seven plants with low degrees of asynapsis at diakinesis and metaphase I.

To test the possibility that crossing over had occurred prior to univalent formation in *as* plants, it was necessary to produce an *as* stock heterozygous for chromosome markers identifiable at late diplotene or early diakinesis when univalents can be critically observed. Preliminary investigations indicated that the large heterochromatic knobs present on certain chromosomes in various maize stocks could be identified at these stages. A multi-knobbed, abnormal-chromosome 10 stock, containing most of the known knobs of maize plus a terminal heterochromatic segment on the long arm of chromosome 10, was crossed with the *as* stock, and appropriate plants containing identifiable knobs in heterozygous condition were obtained by selfing.

With the exception of a small number of root-tip squashes, cytological observations were limited to microsporogenesis and pollen formation in field-grown plants, and the techniques employed were those commonly used for maize cytology (SMITH 1947). Sporocytes were fixed in freshly mixed Carnoy's fluid (3:1) and the material stored in fixative at −10°C or in 70 percent alchohol at 0°C until examined with propiono-carmine staining. With few exceptions, visual observations and photography were restricted to temporary slides observed immediately after preparation. Pollen sterility was determined by empty-grain counts after staining with dilute iodine-potassium iodide solution.

OBSERVATIONS AND DISCUSSION

I. *Meiotic Abnormalities*

PRAKKEN (1943) classified asynaptic mutants from different species as having high, medium or low asynapsis at metaphase I and placed Zea in the high category. It is apparent, however, both from previous studies (BEADLE 1930, 1933) and from the present investigation (Table 1 and 2; Figures 2 and 4) that the degree of asynapsis in different maize plants may vary from complete to very low. In addition to highly variable asynapsis, a wide range in the expression of the *as* gene has been observed with regard to other cytological abnormalities. Since one of the primary purposes of this study was the clarification of the process

TABLE 1

Summaries of diakinesis and metaphase I bivalent counts in normal (As) *and in asynaptic plants* (as)

	Stage	Number of cells	Mean number pairs per cell	Range in number pairs per cell	Percent of pairs as ring bivalents*
As					
1	Diak	100	10.00	10 only	99.50
	M I	100	10.00	10 only	99.70
2	Diak	240	10.00	10 only	99.54
	M I	150	10.00	10 only	99.57
as					
1	Diak	500	0.00	0 only	
	M I	500	0.00	0 only	
2	Diak	250	0.00	0 only	
	M I	250	0.004	1 to 0	0.00
3	Diak	100	0.16	2 to 0	0.00
	M I	100	0.13	3 to 0	7.62
4	M I	50	2.00	7 to 0	53.00
5	Diak	100	5.12	10 to 0	35.16
	M I	100	3.03	10 to 0	17.16
6	Diak	100	7.61	10 to 1	51.77
	M I	100	6.46	10 to 0	66.10
7	M I	100	7.27	10 to 2	80.74
8	Diak	130	8.86	10 to 4	52.95
9	Diak	100	9.09	10 to 6	69.75
	M I	100	8.67	10 to 6	77.62
10	M I	50	9.00	10 to 3	84.89
11	Diak	100	9.44	10 to 6	76.06
	M I	100	8.91	10 to 4	67.34
12	Diak	100	9.79	10 to 8	86.41
	M I	100	9.73	10 to 8	90.03
13	Diak	350	9.96	10 to 8	89.64
	M I	100	9.93	10 to 9	93.55
14	Diak	100	9.95	10 to 9	88.04

* Based on the actual and not the potential number of pairs observed.

by which diploid gametes containing crossover chromatids may arise in *as* plants, this variability is discussed under several headings with emphasis on the possible and probable origin of such gametes.

Premeiotic syndiploidy: NYGREN (1946) used the term "synpolyploidy" to refer to the formation of polyploid syncytes by premeiotic fusion of cells. Although this phenomenon is extremely rare in normal maize, in the present study syncytes were observed in six of the 14 *as* plants on which extensive cytological examinations were made (Figure 1). The frequency of the polyploid cells varied from none to as high as one percent of the sporocytes, with 8n being the highest ploidy observed. The degree of asynapsis exhibited by any one plant had no relation to either the occurrence or frequency of syncytes. The diploid chromosome sets within the syncytes usually remained separated until diakinesis, but

TABLE 2

Frequency distribution in percent of cells with varying numbers of bivalents for 14 asynaptic plants

Plant	Number of bivalents per cell											Total cells	Average number bivalents per cell
	10	9	8	7	6	5	4	3	2	1	0		
1	..	..	..	..	..	..	..	..	..	..	100.0	1000	0.00
2	..	..	..	..	..	..	..	..	..	0.2	99.8	500	0.002
3	..	..	..	..	..	..	..	0.5	2.5	8.0	89.0	200	0.15
4	..	..	..	4.0	4.0	6.0	10.0	14.0	8.0	20.0	34.0	50*	2.00
5	4.5	7.5	6.5	6.5	6.5	10.5	11.0	12.5	9.0	6.5	19.0	200	4.75
6	17.0	21.0	15.5	13.5	9.0	6.5	4.0	4.5	3.0	4.0	2.0	200	7.03
7	15.0	18.0	20.0	16.0	13.0	4.0	5.0	7.0	2.0	..	..	100*	7.27
8	40.0	26.9	21.5	4.6	5.4	0.8	0.8	..	..	..	..	130†	8.86
9	34.5	33.5	21.5	6.5	4.0	..	..	..	..	..	..	200	8.88
10	43.0	30.0	12.0	4.0	..	4.0	..	2.0	..	..	..	50*	9.00
11	59.5	19.5	11.5	3.0	5.0	0.5	..	..	..	..	..	200	9.17
12	80.5	15.0	4.5	..	..	..	..	..	..	..	..	200	9.76
13	95.5	4.0	0.5	..	..	..	..	..	..	..	..	450	9.95
14	95.0	5.0	..	..	..	..	..	..	..	..	..	100†	9.95

Values are derived from combined diakinesis and metaphase I counts unless otherwise noted.
* = Metaphase I counts only.
† = Diakinesis counts only.

only one spindle was observed at metaphase I. No multivalent chromosome associations were seen, and the degree of asynapsis in the polyploid meiocytes was similar to that within diploid cells of the same plant.

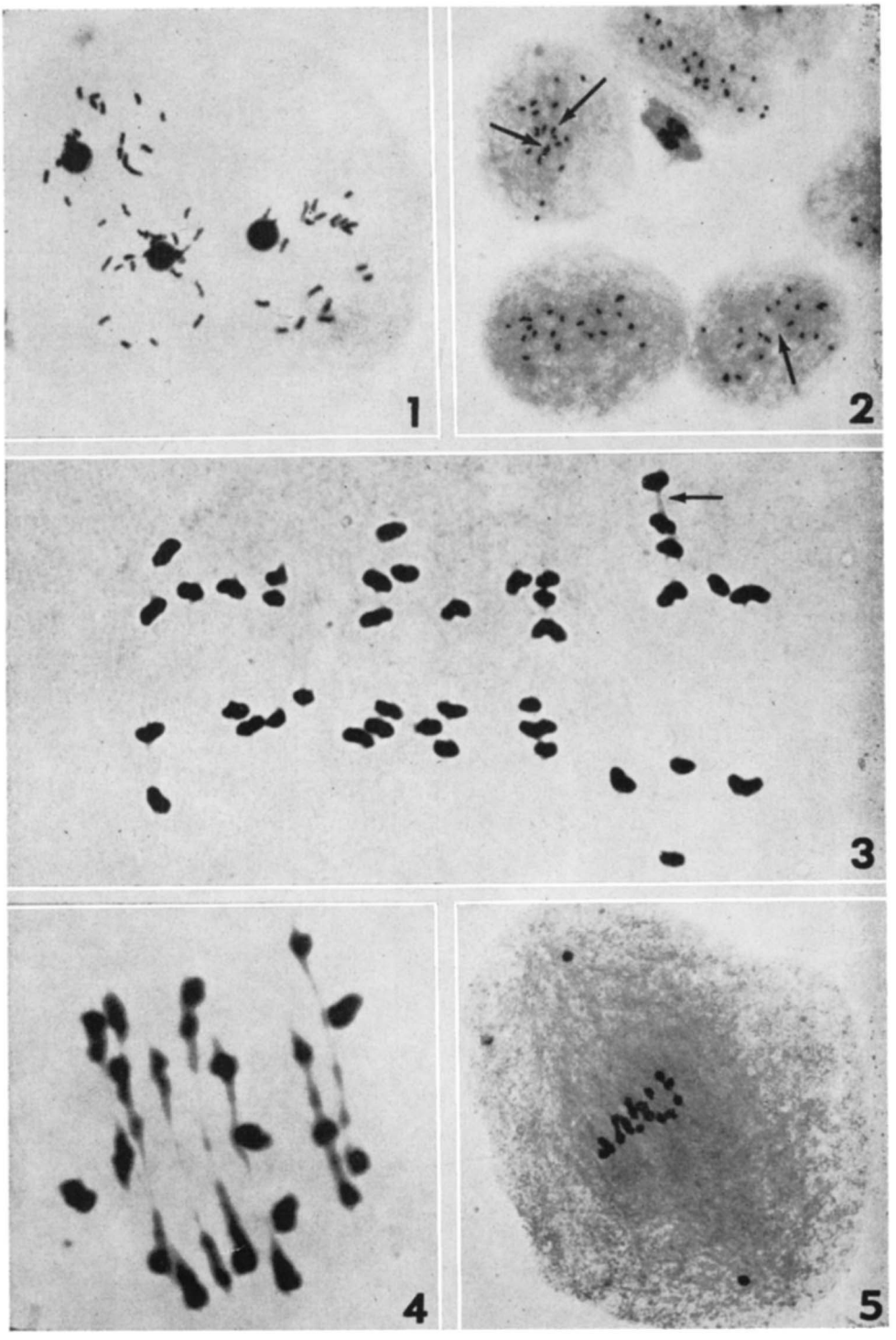
Although syndiploidy has been cited as the chief cause of polyploidy in plants and many animals (DARLINGTON 1937), it seems unlikely that the process is important as a source of diploid gametes in asynaptic maize plants. More than half of the plants in the study contained no syncytes. Furthermore, in those *as* plants which contain such cells, chromosome irregularities and consequent sterility occur in the polyploid as well as diploid meiocytes.

Asynapsis at diakinesis and metaphase I: MOFFETT (1932) found that in certain Anemone species terminal chiasmata present at diplotene had disappeared by metaphase I, and he suggested that lack of terminal affinity combined with strong terminalization of chiasmata would give univalents with crossovers at metaphase I. The possibility was considered that a similar resolution of chiasmata plus a subsequent failure of one of the meiotic divisions could produce diploid gametes containing crossover chromatids in *as* maize.

In the present study, comparisons of the number of pairs per cell at diakinesis and metaphase could be made in seven *as* plants (Table 1). All but one showed a slight drop in the mean number of pairs per cell at metaphase I, but five of the seven also showed an apparent increase in the percentage of ring bivalents. Certain errors in counting bivalents may account for these differences. The intimate association of the Number-6 chromosomes at the nucleolar organizer region at diakinesis was often counted as a chiasma, though none was present in that arm. This is indicated by the occurrence of this association at diakinesis in plants with complete asynapsis of all other chromosomes at diakinesis and of all chromosomes at metaphase I. Also, stickiness between chromosomes, often involving terminal regions, sometimes is present at diakinesis, and differentiation between true and pseudo terminal chiasmata in such cells often cannot be made. Both counting errors would cause apparent decreases in the average number of pairs and apparent increases in the percentage of ring bivalents from diakinesis to metaphase I.

The possibility that differences in diakinesis and metaphase pairing may be due to environmental variations should also be considered. Although no studies have been made with asynaptic maize, differences in chromosome pairing due to environmental influences have been reported for comparable mutants in other species (SOOST 1951; GOODSPEED and AVERY 1939). In view of the probable counting errors and a possible environmental effect, it seems logical to regard the differences between diakinesis and metaphase I bivalent counts within the same *as* plant as being due to experimental error rather than resulting from complete resolution of chiasmata between the two stages. It seems doubtful, therefore, that such a mechanism plays any part in the origin of diploid gametes containing crossover chromatids in asynaptic maize.

Univalent behavior. (a.) *Equational division.*—Although no equational division of univalents during division I was observed in the earlier cytological studies of *as* maize, DEMPSEY (1958) reported that in cells containing a mixture of pairs and univalents, the univalents sometimes divided equationally after the dyads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had reached the poles. In the present study, equational division at metaphase I was highly variable both within and between plants. Division rarely occurred in completely asynaptic plants, although one plant with high asynapsis regularly showed univalent division (Figure 3). Either variable numbers of dividing univalents or lack of division was observed in plants with medium to low asynapsis. Nondividing univalents either remained near the metaphase group of bivalents (Figure 4), or had moved without apparent centromere activity to or near a spindle pole by that stage (Figure 5). In cells having bivalents, dividing univalents invariably congressed and divided after the disjunction of the pairs (Figure 6) although, at times, monads moved rapidly enough after division to reach the poles with the dyads.

(b.) *Misdivision.*—Although BEADLE (1930) observed fragmented chromosomes during meiosis in asynaptic maize, the origin of such fragments was not clear. In the present material, fragmentation of chromosomes was observed at both divisions, and the fragments clearly arose by misdivision of univalents or monads. Highly asynaptic plants generally exhibited a high rate of misdivision during division I (Figure 7), but although misdivision also was frequent in some plants with low asynapsis, it did not occur at all in others. In most of the cells showing misdivision during division I, anaphase movement of the misdividing univalents ceased before division was complete, and the chromosomes entered an interphase condition while still near the metaphase plate. Infrequently, misdivision was completed and the chromosome fragments included in the telophase I nuclei. A few instances of univalent misdivision were noted at anaphase II, but misdivision at that stage usually involved only monads (Figure 8), the frequency of the latter being dependent on the number of univalents that had divided equationally at division I.

Spindle abnormalities: BEADLE (1930, 1933) observed that first-division spindles often were much longer in *as* than in normal maize plants, and the abnormal elongation was presumed to be related in some manner to failure of metaphase pairing. Elongated spindles also have been observed in most of the *as* plants in this study. Such spindles usually lie to the side of the cell and curve around the periphery (Figure 9). In extreme cases, the spindle poles approach one another on the side of the cell opposite the metaphase I plate. A high frequency of curved spindles generally was correlated with high asynapsis. That lack of pairing itself

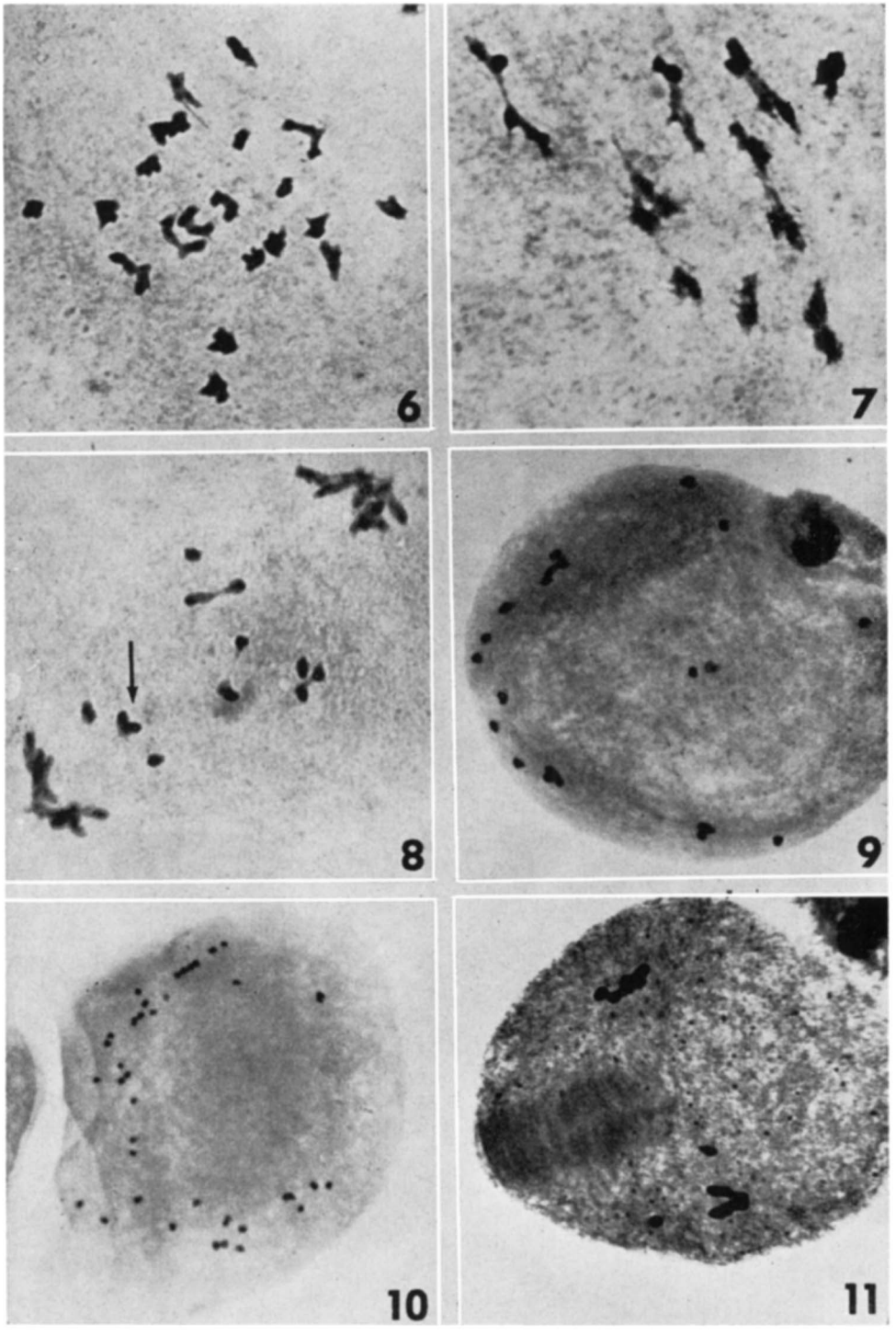
FIGURE 1.—Diakinesis (very high asynapsis). A hexaploid syncyte with the diploid sets well separated, indicating that premeiotic fusion of the meiocytes occurred at or after the last premeiotic mitosis.

FIGURE 2.—Metaphase I (very high asynapsis). Two cells contain rod bivalents (arrows) while the remaining cells show only univalents.

FIGURE 3.—Anaphase I (high asynapsis). Equational division of 20 univalents. At top right, one univalent is off the metaphase plate and is relatively late in dividing (arrow.)

FIGURE 4.—Metaphase I (medium asynapsis). Eight bivalents and four univalents are present. The bivalents show the attenuation characteristic of many asynaptic plants.

FIGURE 5.—Metaphase I (low asynapsis). Single univalents, perhaps homologous, have precociously moved to each pole.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is not the cause of the spindle abnormality, however, was indicated by the regular occurrence of elongated division I spindles in one plant with very low asynapsis, and by the presence in some *as* plants of curved spindles during division II where chromosome pairing would play no part.

Nuclear restitution due to failure of karyokinesis: DEMPSEY (1958) provided genetic evidence that individual diploid eggs probably contain both sister and nonsister centromeres, and suggested that such eggs may arise by precocious dyad centromere division and a failure of division II following a division I in which both univalents and bivalents had divided.

In the present material, chromosomes often were left near the plate following anaphase I (usually in cells with elongated spindles or misdividing chromosomes). In all instances, however, one or more chromosomes reached both poles, and no certain case of nuclear restitution due to failure of anaphase I was observed in any of the *as* plants examined.

One plant with very low asynapsis was found which exhibited cells with precocious division of dyad centromeres during anaphase I (Figure 10). No meiotic interphase or prophase II occurred, and all chromosomes remained contracted as at anaphase I. In no instance, however, was there a failure of division II spindle formation. The monads were distributed to the poles at random without orientation or centromere activity, and cytokinesis was normal following both divisions. In *as* plants with regular equational division of univalents during division I, prophase II cells with high numbers of monads were observed. At metaphase II, however, the few dyads present always oriented for division and no failure of anaphase II chromosome movement was observed.

In view of these observations and of evidence that cytological irregularities in *as* maize are similar in mega- and microsporogenesis (BEADLE 1930), it seems unlikely that diploid eggs in asynaptic plants arise either by restitution due to failure of chromosome movement at anaphase I or II, or to a complete lack of spindle formation.

Failure of cytokinesis and nuclear fusion: Although no failure of cytokinesis was reported in the earlier studies of the *as* gene in maize, complete or partial

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FIGURE 6.—Anaphase I (low asynapsis). Equational division of six univalents. Seven bivalents have separated normally.

FIGURE 7.—Anaphase I (complete asynapsis). Misdivision of univalents. The chromosomes appear to be entering an interphase state although division is not complete.

FIGURE 8.—Telophase II (low asynapsis). Misdivision of monads. Two have divided completely while three are in the process of division. A lagging monad appears at lower left (arrow).

FIGURE 9.—Anaphase I (very low asynapsis). The spindle extends almost the entire circumference of the cell. Dyads are staggered in the spindle due to different times of anaphase separation. A persistent nucleolus and a single univalent or dyad lie out of the spindle in center of cell.

FIGURE 10.—Anaphase I (very low asynapsis). Most of the dyads have separated precociously at the centromere before reaching the spindle poles.

FIGURE 11.—Telophase I (very low asynapsis). The phragmoplast has been formed only on one side of cell and will result in partial failure of wall formation.

failure of meiotic cell divisions was frequently observed in the present study (Figure 11). The abnormality was not correlated with degree of homozygosity, year of culture, or amount of asynapsis. In some *as* plants, abnormal spindles occurred but cell division was normal; in others, the spindle and chromosome movement appeared normal but cytokinesis failed. Cell division failed following karyokinesis at either division I or II singly, to give rise to diploid spores, or after both divisions in sequence, to produce tetraploid spores (Figure 12).

Chromosome counts at the first microspore mitosis in plants showing failure of cytokinesis at both meiotic divisions showed 10-, 20-, and 40-chromosome microspores undergoing apparently normal divisions. Staining of starch in mature pollen from these plants indicated that normal starch formation had occurred in many of the diploid- and tetraploid-sized grains (Figure 13). Use of similar pollen on a tetraploid female resulted in several tetraploid seedlings, showing that diploid spores derived by failure of cell division in *as* plants can be functional, and that *as* plants can be used as male as well as female parents for genetic studies if tetraploids are utilized as linkage testers.

In view of the fact that failure of cytokinesis during megasporogenesis has been demonstrated in maize (LEBEDEFF 1940), and since it is probable that the effect of the *as* gene is essentially the same in male and female meiocytes, the high incidence of failure of cytokinesis in the asynaptic plants in this study, and the absence of any other diploidization mechanism, strongly suggests that both male and female diploid gametes in *as* plants result from nuclear fusion following failure of cytokinesis at one or the other of the two meiotic divisions.

II. *Cytological Crossing Over*

The occurrence in *as* plants of crossover chromatids in diploid eggs, presumed to arise by nuclear restitution in megasporocytes with few or no chiasmata at metaphase I, might result from precocious resolution of chiasmata prior to metapase I or from the existence of a crossover mechanism unrelated to the postdiplotene association of chromosomes. In either case, crossover chromatids would be expected in asynapsed chromosomes at diakinesis, since asynapsed homologues show no association at or following this stage in *as* plants.

Since genetic recombination has cytologically observable consequences (STERN 1931; CREIGHTON and MCCLINTOCK 1931), it should be possible, by using cytologically visible markers, to determine whether asynapsed chromosomes at diakinesis in *as* plants contain crossover chromatids. Assuming that the majority of exchanges proximal to the markers would be single crossovers, recombination should result in univalent or rod pairs exhibiting equational disjunction of the markers. If no exchange had occurred previous to univalent or rod bivalent formation, marker separation would be reductional.

Two plants, suitable both in a low degree of asynapsis and knob constitution for the proposed crossing-over investigation, were derived from selfing hybrids between a multi-knobbed, abnormal-chromosome 10 stock and asynaptic plants. The first plant had a mean of 9.44 bivalents per sporocyte, was heterozygous for

the abnormal-10 segment, and contained three interstitial knobs. Because of difficulty in distinguishing between the interstitial knobs in many cells, observations were confined to the abnormal 10. The second plant had a mean of 9.20 bivalents and was heterozygous for two prominent knobs, a large interstitial knob on the long arm of chromosome 4 and a large knob terminal on the short arm of chromosome 9.

A summary of the abnormal-10 disjunction is given in Table 3, and an example is shown in Figure 14. Summaries for chromosome-4 and -9 knobs are presented in Table 4, while Figure 15 shows examples of these knobs. Reductional disjunction of the three markers was observed 102 times in univalent chromosomes (#4–47, #9–25, #10–30) and 185 times in rod bivalents (#4–74, #9–25, #10–86) while no case of equational disjunction was found. It is also evident that, at least in the three marked chromosomes, chiasmata occur more than twice as frequently in short as in long arms.

For the crossing-over observations reported here to have significance, a rela-

TABLE 3

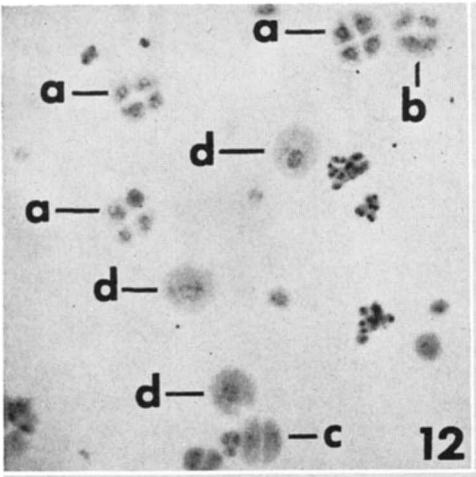
Observations on the location of chiasmata in chromosome 10 and the types of disjunction of the heterozygous abnormal-10 segment at diakinesis

	Slide						
	J	L	Q	P	N	Cell total	Percent
Chromosome 10 as univalents, and reductional for abnormal 10	1	14	11	3	1	30	17.14
Short-arm chiasma only, and reductional for abnormal 10	12	30	24	7	13	86	49.14
Long-arm chiasma only	6	3	6	0	1	16	9.14
Chromosome 10 as ring pair	4	16	10	2	11	43	24.58
	25	65	56	13	28	175	100.00

TABLE 4

Observations on the location of chiasmata in chromosomes 4 and 9 and the types of disjunction of the intercalary, long-arm knob on 4 and the terminal, short-arm knob on 9 at diakinesis

	Chromosome 9					
Chromosome 4	Univalents, reductional for knob	Long-arm chiasma, reductional for knob	Short-arm chiasma	Ring bivalent	Chromosome 4 totals	Percent
Univalents, reductional for knob	4	4	10	29	47	22.92
Long-arm chiasma	2	7	5	19	33	16.10
Short-arm chiasma, reductional for knob	6	5	9	54	74	36.10
Ring bivalent	13	9	29	..	51	24.88
Chromosome 9 totals	25	25	53	102	205	100.00
Percent	12.20	12.20	25.85	49.75	100.00	



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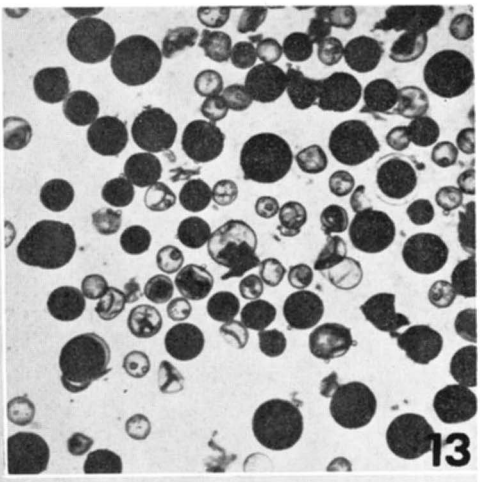

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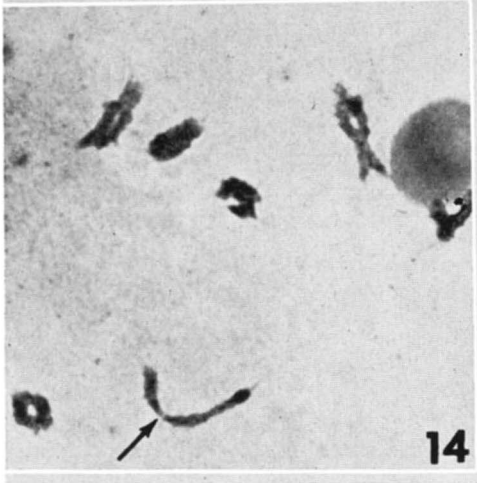

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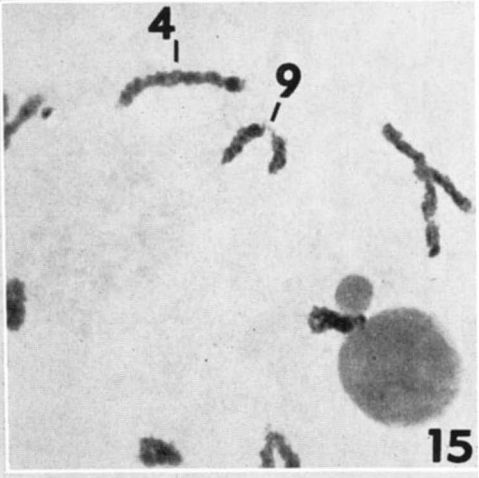

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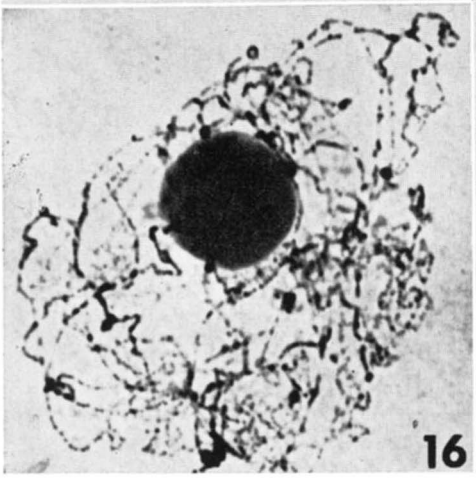

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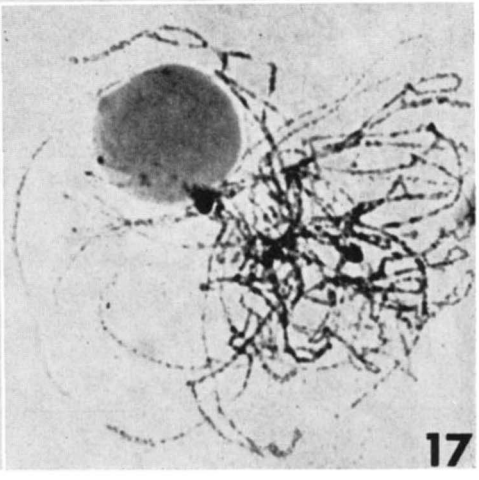

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tively high frequency of exchanges proximal to the markers must normally occur. Available genetic and cytogenetic data (HAYES, IMMER and SMITH 1955; MORGAN 1950; RHOADES 1942, 1950, 1956; KIKUDOME 1958; ANDERSON and KRAMER 1954) allow estimates of expected equational disjunction for the three markers, as follows: Chromosome-4 knob, equational disjunction expected in 95 percent of the sporocytes; chromosome-9 knob, in 94 percent of the sporocytes; and chromosome-10 knob, in 59 percent of the sporocytes. Thus, if crossing over had occurred in the asynapsed marker chromosomes at or near the frequency expected in normal plants, equational disjunction of the knob locus should have been evident for each of the knobs in a high percentage of sporocytes.

The complete absence of equational disjunction warrants the conclusion that exchanges have not occurred in asynapsed chromosomes present at diakinesis in asynaptic maize plants, and supports the conclusion that diploid gametes with crossover chromatids arise by nuclear fusion following failure of cytokinesis rather than by restitution following precocious resolution of chiasmata.

III. *Prophase Pairing in Asynaptic Plants*

A. *Relation of early to late prophase pairing.*—In the previous cytological studies of the *as* gene (BEADLE 1930, 1933), the amount of mid- to late pachytene pairing was roughly correlated with the degree of metaphase I asynapsis. Complete synapsis at early pachytene followed by separation of homologous threads by mid- to late pachytene, however, was described for *as* plants with high asynapsis at metaphase I. If the latter were a regular phenomenon in *as* plants, the possibility of early prophase exchange and precocious resolution of chiasmata might be indicated.

FIGURE 12.—Quartet stage (very low asynapsis). Four types of sporad groups are present: a.—quartets resulting from normal cytokinesis after both divisions; b.—a triad of two haploid and one bi-nucleate diploid sporad following failure of cytokinesis in only one cell after division II; c.—a dyad of two mononucleate, diploid sporads resulting from failure of cytokinesis and nuclear fusion in both cells after division II; and d.—mononucleate, tetraploid sporads resulting from failure of cytokinesis after both divisions followed by nuclear fusion.

FIGURE 13.—Mature pollen (degree of asynapsis not determined). Sample shows haploid-, diploid-, and tetraploid-sized grains with normal starch deposition.

FIGURE 14.—Diakinesis (low asynapsis). Chromosome 10, heterozygous for abnormal-10 segment, is present as rod bivalent with short-arm chiasma (arrow). Disjunction of knob is reductional.

FIGURE 15.—Diakinesis (low asynapsis). Chromosome 9 with terminal knob on short arm and chromosome 4 with intercalary knob on long arm both occur as rod bivalents with short arm chiasmata. Disjunction of knobs appears reductional in each but it is possible that 9 has a chiasma proximal to knob which has not terminalized.

FIGURE 16.—Early prophase I (complete asynapsis). With exception of possible association of centromeres at upper right, no homologous pairing is evident in early prophase cell of plant which exhibited complete asynapsis at late prophase.

FIGURE 17.—Early prophase I (very high asynapsis). Homologous chromosomes above nucleolus show pairing at centromere and in distal segments. Other paired segments are present but no extensive pairing has occurred.

In the present study, the extent of early meiotic prophase pairing as compared with the degree of asynapsis at diakinesis and metaphase I was investigated in four *as* plants, one of which showed complete asynapsis, one very high asynapsis, and two medium asynapsis at late prophase. In each case, anthers from young tassel branches were systematically examined from early to late prophase stages (or, in the highly asynaptic plants, stages equivalent in chromatin condensation).

In the completely asynaptic plant, no pairs were observed in over 1000 cells at diakinesis and metaphase I. Early prophase observations also revealed essentially complete absence of pairing from leptotene to diplotene (Figure 16). In the highly asynaptic plant, only 29 bivalents were observed in over 200 cells at diakinesis and metaphase I. Although pairing of some segments at pachytene was clearly evident in a few cells (Figure 17), no extensive pairing was observed in any cell, and, in most of the cells examined no clearly homologous pairing could be found.

Each of the medium asynaptic plants showed 15 percent metaphase I sporocytes with ten bivalents, of which several usually were rod pairs. Cells with up to 20 univalents also were present. Stages from leptotene to pachytene in these plants showed that the amount of pairing ranged from cells with no paired threads, similar to those found in the highly asynaptic plants, to cells in which all the threads appeared to be paired.

Although limited numbers of plants were observed, the present study has shown that, in each case, the variability of zygotene to pachytene pairing, including the amount of initial synapsis of homologous threads, was similar to the variability of asynapsis exhibited at diakinesis or metaphase I within the same plant. The observations suggest that unpaired segments present in any one cell at late pachytene in *as* maize plants probably have not been paired at any time during previous stages of meiotic prophase within the same cell.

B. *Extent and location of partial asynapsis in pachytene chromosomes.*—The extent and location of nonpaired segments in pachytene chromosomes have been studied in seven plants in which the degree of asynapsis ranged from a mean of 5.62 pairs with 65 percent as rings to 9.95 pairs with 92 percent as rings at diakinesis or metaphase I. A comparison of partial asynapsis in the short arm versus the long arm at pachytene is shown in Table 5 for the five shorter chromosomes (Numbers 6–10). With the exception of chromosome 8, which often showed terminal asynapsis of the short arm (Figure 18), almost all of the asynapsis in the shorter chromosomes was intercalary, and was much more frequent in the long than in the short arms (Figure 19). Although no individual identifications of the longer chromosomes (Numbers 1–5) were made, general observations indicated that unpaired regions in these were more frequent than in the shorter chromosomes, but were usually intercalary in the long arms also (Figure 20). In both short and long chromosomes, centromere regions were always synapsed and the regions immediately adjacent to the centromeres usually were paired, although in some instances, the asynapsis appeared to extend to the centromere region (Figures 21, 22).

The observations on the location of asynapsis in pachytene chromosomes are of interest primarily in relation to the crossing-over studies with *as* maize. The gene

TABLE 5

Comparison of incidence of partial asynapsis in short and long arms of the five shorter maize chromosomes in plants showing low asynapsis

	Chromosomes showing partial asynapsis in:			
	Both arms	Short arm	Long arm	Total
Chromosome 6	1 (6%)	3 (19%)	12 (75%)	16
Chromosome 7	9 (17%)	3 (6%)	40 (77%)	52
Chromosome 8	5 (23%)	16 (73%)	1 (4%)	22
Chromosome 9	1 (33%)	0	2 (67%)	3
Chromosome 10	5 (25%)	3 (15%)	12 (60%)	20
Total	21	25	67	113

markers and the map distances for the three genetic regions used in the studies are given below (from summary in HAYES *et al.* 1955), and a summary of the genetic data is presented in Table 6.

Chromosome 2: white sheath-3 (ws_3)–11 units–liguleless leaf-1 (lg_1)–19 units–glossy seedling-2 (gl_2).

Chromosome 5: Anthocyanin-2 (A_2)–6 units–brown midrib-1 (bm_1)–2 units–brittle endosperm-1 (bt_1)–23 units–Purple aleurone (*Pr*).

Chromosome 9: Aleurone color (*C*)–3 units–shrunken endosperm-1 (sh_1)–30 units–waxy endosperm-1 (wx_1).

Information from inversion, translocation and deletion studies enable one to place these genes fairly well on the cytological map of maize chromosomes (ANDERSON 1941; CLARK 1956; MCCLINTOCK 1931, 1938, 1941; MORGAN 1950; PATTERSON 1952; RHOADES 1936). The *ws–lg–gl* region is located in the distal one fourth of the short arm of 2, with *lg* at or distal to 2S.83. The *C–sh–wx* region is located in the distal one half of the short arm of 9, with the *wx* locus near 9S.5. *C* is approximately at 9S.75, with *sh* proximal and very close to *C*. In chromosome 5, *bt* is very close to the centromere in the long arm while most of the *bt–pr* region probably lies in the proximal one half of the long arm. *bm* lies between 5S.0 and 5S.05, and, although no cytological placement of A_2 is known, it is distal to and shows only 6 percent recombination with *bm*. Thus, most of the A_2–*bm* region probably lies within the proximal half of the short arm of 5.

With the exception of the *sh–wx* and *bt–pr* regions, which exhibited near normal recombination values, all of the regions tested in haploid gametes from *as* plants have shown considerably higher than normal crossing over. On a basis of total ovules, however, rather than number of seed set in *as* plants, single crossovers in chromosomes 2 and 9 are reduced well below normal, although double crossovers still remain higher. The increase in crossovers in haploid gametes when based on number of viable seed set was interpreted (DEMPSEY 1959) as due to

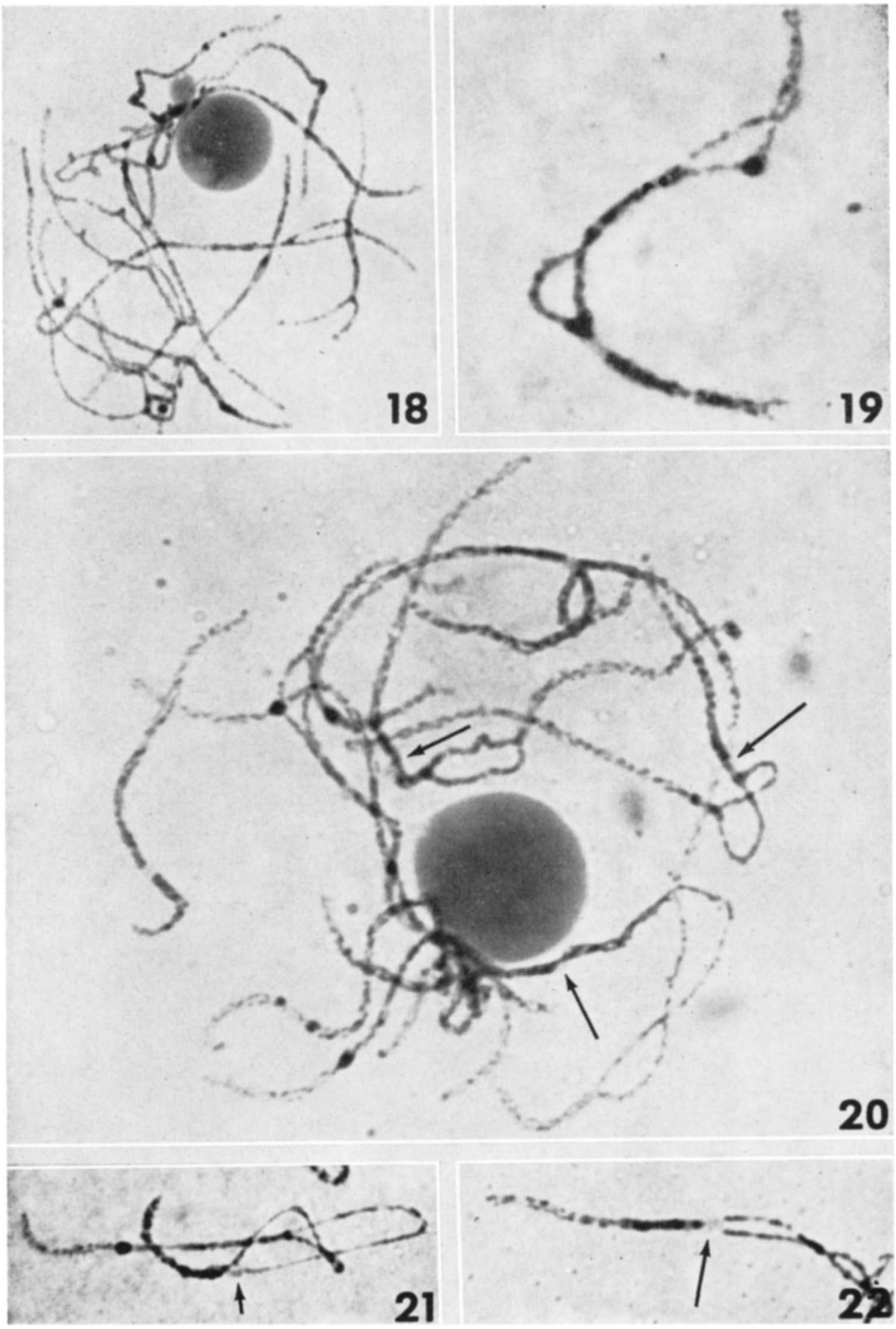
18
19
20
21
22

TABLE 6

Summary of genetic studies in asynaptic maize

	ws-lg	*lg-gl*	Percent double crossovers actual	expected	Coincidences	N	
Haploid gametes (RHOADES and DEMPSEY 1949)							
As	14.7	21.1	0.2	3.1	0.06	1000	
as	20.1	30.5	5.4	6.1	0.90	856	
Increase	37%	45%					
Haploid eggs (DEMPSEY 1959)							
As	8.4	18.9	0.2	1.6	0.13	1362	
as	14.5	29.0	2.1	4.2	0.50	1255	seeds
Increase	73%	53%					
(*as*)	4.8	9.6	0.7	0.5	1.52	3785	ovules
	C-sh	*sh-wx*					
As	3.7	21.2	0.2	0.8	0.25	2780	
as	6.3	21.2	0.7	1.3	0.52	2377	seeds
Increase	70%	0%					
(*as*)	2.3	8.7	0.3	0.2	1.50	5811	ovules

Haploid eggs (DEMPSEY unpublished)

	bm-bt		*A-bt*		*bt-pr*	
As	0.1	(4/2721)	4.3	(228/5322)	18.3	(618/3383)
as	0.8	(34/4416)	5.6	(312/5554)	19.6	(904/4614)
Increase	700%		30%		7%	

Diploid eggs (DEMPSEY 1958 and unpublished)

	A-bt	*bt-pr*	Chromatids tested	
Map	8.0	18.0	...	(Only *Bt* progeny used
as	2.5	14.8	318	in calculations)
Decrease	69%	23%		

	C-wx	Chromatids		*sh-wx*	Chromatids
Map	24.0	...	Map	21.0	...
as	11.2	596	*as*	21.4	126
Decrease	53%		Increase	2%	

Coincidences for the data are presented here for the first time and the repulsion and coupling backcross figures have been combined for the *C-wx* region, 1958 data. Increase and Decrease = percentage of increase or decrease in crossing over in *as* plants compared to normal sibs or map distances. (*as*) = *as* data calculated on basis of total ovules rather than number of seedlings.

FIGURE 18.—Pachytene (low asynapsis). The chromosome at right (probably No. 8) exhibits complete asynapsis of the short arm. Chromosome 6 with two knobs runs above the nucleolus and shows apparently normal pairing.

FIGURE 19.—Pachytene (low asynapsis). Chromosome 7 with two regions of nonpairing in long arm.

FIGURE 20.—Pachytene (low asynapsis). Three chromosomes (centromeres marked with arrows), unidentified but known not to be 6–10, show partial asynapsis in intercalary regions of long arms. Chromosome 8 at left center shows slight asynapsis in middle of long arm.

FIGURE 21.—Pachytene (low asynapsis). Partial asynapsis in the long arm which extends to the centromere (arrow). Note that the short arm is more condensed than the long.

FIGURE 22.—Pachytene (low asynapsis). Partial asynapsis in the long arm. The nonpairing extends to the centromere (arrow).

functional gametes being derived primarily from megasporocytes with more frequent chiasmata and, therefore, more regular chromosome behavior than occurred in those giving rise to abortive gametes. If similar increases on a basis of seed set could be demonstrated for all genetic regions of maize chromosomes, however, meiocytes giving rise to functional haploid gametes in *as* plants would have had a higher chiasma frequency per sporocyte than occurs in normal maize. In view of the chiasma frequencies demonstrated in *as* plants, such an event would be highly unlikely, even if counts are limited to cells with ten bivalents present at metaphase I. Selective recovery of crossover chromatids in functional gametes also is probably not a factor in the increased recombination rates, since DEMPSEY (1959) has shown that no evident association of crossover strands occurs in single gametes when two genetic regions are tested simultaneously.

The observations on pairing at pachytene in *as* plants suggest that some mechanism in addition to selection of gametes from meiocytes with relatively high chiasma frequencies must account for the increased recombination in the genetic regions so far tested in haploid gametes. Unpaired segments in plants with low asynapsis usually were intercalary and were more extensive and more frequent in the long arms. In plants with high asynapsis, the limited amount of pairing present always involved terminal or centromere regions of the chromosomes. In addition, the cytological crossing-over studies show that, at least in three chromosomes, chiasmata in rod bivalents occur in the short arms more than twice as often as in the long arms. Since the genetic regions so far used to test crossing over are mainly located in distal short-arm and centromeric regions, it appears that the increased recombination in haploid gametes may represent a true increase in chiasma frequency owing to an abnormal localization of chiasmata in the terminal and centromeric portions of the chromosomes.

Since these regions sometimes will not have been paired in asynaptic material but are always paired in normal plants, the increased crossing over must also represent an actual increase in chiasma frequency per number of pairings within those regions. In others words, the pairing in *as* plants must be more effective in formation of chiasmata within the paired regions than that within the same regions in normal plants. On this basis, it can be predicted that crossing over will be reduced below normal rates in most intercalary regions, and that crossing over in the terminal regions of the long arms will be less than that in terminal regions of the short arms, with the possible exception of chromosome 8 which shows frequent terminal asynapsis of the short arm. The *sh–wx* and *bt–pr* exceptions in haploid gametes can thus be explained by the fact that portions of these regions are in intercalary areas where pairing is infrequent.

Because of the variability in degree of asynapsis in *as* plants, valid comparisons of rates of crossing over in different regions or in haploid and diploid gametes can be made only when the regions are tested in the same plants. It also will be expected that haploid gametes will show higher rates of recombination than diploid gametes of the same plants, since, based on the cytological observations, viable haploid gametes, on the average, should have arisen from sporocytes having a higher frequency of bivalents per cell. For example, random segregation of

monads during division II would have no effect on the viability of diploid gametes resulting from nuclear fusion following failure of cytokinesis as long as the monads were included in the two telophase II nuclei. On the other hand, random segregation of monads at division II following equational division of univalents at division I would result in a high percentage of inviable haploid gametes.

The increased crossing over observed in haploid gametes of *as* plants may be comparable to the compensatory recombination found in certain other genetic studies. In maize (RHOADES and DEMPSEY 1953), decreases in crossing over within heterozygous inversion regions was paralleled by increases immediately proximal to the inversion in the same chromosome. Intrachromosomal decrease and compensation also have been described for heterozygous X chromosome inversions in Drosophila (GRELL 1963) and interspecific Gossypium crosses (GILES 1961; RHYNE 1958; STEPHENS 1961). Interchromosomal compensation in Drosophila has been reported for heterozygous inversions (review by SCHULTZ and REDFIELD 1951; SUZUKI 1962), XXY females (Morgan *et al.* 1932, 1933, and compound and ring X chromosomes (SUZUKI 1962a).

Although there is no evidence to indicate similar or different causal mechanisms for inter- and intrachromosomal compensation, it seems likely that some type of abnormal pairing may be involved, either a lack of pairing such as occurs in *as* maize and probably certain of the Drosophila aberrations, or some degree of nonhomologous pairing within the heterozygous aberrations.

STEPHENS (1961) has discussed the possibility that total recombination frequency per bivalent or genome may be genetically limited. One might further speculate that the amount of crossing over is dependent on a substance which normally is limited in quantity in meiocytes and is competed for with differential success by all homologously paired regions of the chromosomes. When the amount of the substance is increased for normally paired regions, either by asynapsis or nonhomologous pairing within individuals heterozygous for aberrations or by partial asynapsis in *as* plants, compensatory recombination may be produced in other chromosome regions.

SUMMARY

Fourteen asynaptic (*as*) maize plants were examined from diakinesis through the quartet or first microspore mitosis stage. The mean number of bivalents at metaphase I ranged from 0 to 9.95 in separate plants. Cytological irregularities other than asynapsis included polyploid meiocytes, elongated and curved spindles, misdivision of univalent and monad centromeres, and partial or complete failure of cytokinesis after the meiotic divisions. Nuclear restitution due to spindle failure was not observed at either division. At the first microspore mitosis, microspores with 10, 20, and 40 chromosomes were observed. It was concluded that polyploid gametes in *as* plants result from nuclear fusion following failure of cytokinesis after one or both of the meiotic divisions.

Three different heterochromatic knobs were incorporated in heterozygous condition into plants with low asynapsis. Although genetic data from normal stocks

indicate that chiasmata frequently occur proximal to the knob locations, no equational disjunction of the knobs was observed in 287 chromosome pairs present at diakinesis as univalents or as rod bivalents with chiasmata in the knobless arm. It was concluded that chromosomes showing asynapsis at diakinesis or metaphase I have not undergone cytological crossing over.

Plants with varying degrees of asynapsis at metaphase I were examined during successive stages from leptotene to metaphase I. The amount of pairing in the early prophase stages was correlated with the degree of asynapsis at late prophase, and it was concluded that chromosome segments unpaired at late pachytene had not been previously paired.

In plants with low asynapsis, unpaired segments in pachytene chromosomes usually were intercalary in location, and more often in long than in short arms. Centromere regions always were paired and terminal regions usually paired. At diakinesis in plants with low asynapsis, chiasmata in rod bivalents of chromosomes marked with knobs occurred more than twice as often in the short arms as in the long arms.

It is suggested that the higher than normal rates of crossing over observed in previous genetic studies of haploid gametes from *as* plants are due to the fact that the genetic regions previously tested are in distal segments of the short arm or in segments near the centromere where pairing occurs more frequently than in intercalary regions. Both the demonstration of localization of chiasmata in short arms and the observed pairing of terminal segments in partially asynapsed chromosomes agree with this conclusion. Data on crossing over in intercalary regions are needed. These values should be lower than normal in *as* plants.

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