CHROMOSOME LENGTH IN RELATION TO TRANSMISSION FREQUENCY OF MAIZE TRISOMES[1]

JOHN EINSET
N. Y. State Agricultural Experiment Station
Geneva, New York

Received December 3, 1942

TRISOMIC inheritance was first reported in maize by McCLINTOCK (1929) in a study of segregating progenies derived from a spontaneous triploid (RANDOLPH and McCLINTOCK 1926). Later McCLINTOCK and HILL (1931) identified the primary trisome of chromosome 10, the shortest member of the maize chromosome complement which carries the *r-g* linkage group, and certain general observations on the known primary trisomic types of maize were made by RHOADES and McCLINTOCK (1935). The present study was undertaken to compare the breeding behavior of the eight available primary trisomic types in maize.

MATERIAL AND METHODS

The trisomic stocks used in this study were procured from the MAIZE GENETICS COOPERATION, DEPARTMENT OF PLANT BREEDING, CORNELL UNIVERSITY, Ithaca, New York. The stocks were grown by DR. L. F. RANDOLPH in 1940 to isolate trisomic plants and establish cultures that did not possess B-type chromosomes. The trisomic plants so isolated were crossed by various stocks carrying known genetic markers and also by certain commercial inbred lines. The progenies of these crosses were grown in the summer of 1941. In the text and tables, these F_1 progenies are designated by a number which refers to the particular chromosome present in triplicate in the female parent, and the male parent, which was either recessive for a gene carried by a member of the trisome or an inbred line, is also specified.

Acetocarmine smear preparations were used to study the details of meiosis in the microsporocytes of these F_1 plants. The sporocyte samples were fixed in a mixture of one part of acetic acid to two parts of absolute alcohol. Somatic chromosome counts were made from root tips fixed in CRAF (RANDOLPH 1935), sectioned in paraffin and stained with crystal violet.

TRANSMISSION FREQUENCIES OF THE VARIOUS TRISOMES

If all sporocytes in a trisomic plant possessed a trivalent, and if two of the three members of the trivalent passed to one pole at the first meiotic anaphase

[1] These studies were made in the DEPARTMENTS OF BOTANY AND PLANT BREEDING, COLLEGE OF AGRICULTURE, CORNELL UNIVERSITY, Ithaca, New York. The writer wishes to express his sincere appreciation to DR. L. F. RANDOLPH of the DIVISION OF CEREAL CROPS AND DISEASES, BUREAU OF PLANT INDUSTRY, UNITED STATES DEPARTMENT OF AGRICULTURE and the NEW YORK STATE COLLEGE OF AGRICULTURE, Ithaca, New York, for advice and assistance throughout the course of this problem and for the many helpful suggestions in the interpretation and presentation of the material.

Approved by the Director of the N. Y. STATE AGRICULTURAL EXPERIMENT STATION for publication as Journal Paper No. 537. December 1, 1942.

with the third member passing to the opposite pole, 50 percent of the gametes would contain the n number of chromosomes and 50 percent would contain n+1 chromosomes. However, in the cross of 2n+1 × 2n, considerably less than 50 percent of the F_1 individuals were trisomic. The reduction from 50 percent is greater when the trisomic individual is the male parent. This has been established for maize, Datura, Matthiola, tomato and other plants. Since transmission of n+1 gametes through the male parent is limited by differential pollen tube growth, analysis of other factors also involved can best be made in connection with their transmission through the female gametophyte.

It has been found that the degree of transmission by the seed parent varies with the particular chromosome which is present in triplicate. In Datura (BLAKESLEE and AVERY 1938) the number of trisomic plants in the progeny of different trisomic parents varies from 3 percent in stocks of the 19·20 trisome to 32.7 percent transmission for the 23·24 chromosome. These transmission differences could not be correlated with differences in chromosome size. It has been shown that microspores carrying n and n+1 chromosomes apparently are formed in equal numbers in Datura (BLAKESLEE and AVERY 1938), and it was suggested that the same proportions also are present among the megaspores of these plants. It was also suggested that the deficiency of 2n+1 plants in the progeny was due to elimination of the extra chromosome or of cells containing the extra chromosome during some stage or stages of development of the gametophyte or zygote.

LESLEY (1928, 1932) found ovule transmission of the extra chromosome in tomato trisomics to vary from 18 percent to 32 percent. The loss of the extra chromosome at meiosis and zygotic lethality, suggested by the presence of many small inviable seeds in fruits of trisomic types, were thought to account for deficiency of ovule transmission of the extra chromosome.

In studies on *Nicotiana sylvestris*, GOODSPEED and AVERY (1939) found a variation in the transmission of the extra chromosome through the ovules of primary trisomic plants which ranged from 16.3 percent to 28.8 percent. This variation was not considered to be significant. The failure to approach the theoretical value of 50 percent transmission was attributed to the lagging of the extra chromosome in the meiotic divisions.

The deficiency of trisomics in the progeny of trisomic plants of Matthiola was attributed by FROST (1927) to disturbances of the reduction division and subsequent development of gametes and embryos caused by the presence of the extra chromosome, which created sufficient unbalance in the delicately adjusted developmental processes to produce lethal effects.

MCCLINTOCK and HILL (1931) reported a transmission frequency of the extra chromosome of 33.06 percent in a cross of 2n+1 by 2n involving trisome 10 in maize. The deviation from the expected 50 percent was explained on the basis of irregularities at meiosis. Genetic evidence was also presented to show that 35 percent of the eggs carried n+1 chromosomes. RHOADES (1933) found a transmission frequency of 31 percent in a study involving trisome 5 in maize.

In the present study, random samples of 26 to 177 kernels were taken from

the ears derived from the trisomic stocks of the Maize Genetics Cooperation. In many of these stocks (trisomic for chromosomes 2, 3, 7, 8, or 10) samples from two or more ears were used. These ears in each case came from sister plants pollinated by a different male parent, either a known genetic stock or a commercial inbred line. The seeds were carefully germinated in trays. Chromosome numbers were determined from root tips taken from each seedling before it was transplanted to the field. Because the pollen parent was diploid and selection of seed was random, these determinations indicated the percentage transmission of the extra chromosome through the egg of the 2n+1 female parent.

The frequency of occurrence of 2n+1 individuals in the F_1 progeny of the 2n+1 × 2n crosses was not the same for all trisomics investigated. Also, the frequency of occurrence of 2n+1 individuals differed among the progeny of female parents possessing the same chromosome in triplicate (see trisomics 2, 3, and 10, table 1). There are several possible explanations for this variation. Inadequate sampling of certain stocks was a contributing factor. Likewise, certain genotypes among the genetically different stocks may have tended to promote a higher or lower rate of transmission. Variability of environmental conditions also might have influenced the results.

The various maize trisomes differed appreciably in the frequency with which they were transmitted to their progeny through the female gametophyte. The lowest transmission frequency was found to be 22 percent in a stock of trisome 9 and the highest transmission frequency was 52 percent in stocks of

TABLE 1

The viability of various trisomic stocks and the percentage of trisomics found in the progenies of 2n+1×2n *crosses.*

TRISOMIC STOCK	EAR FERTILITY %	NUMBER OF KERNELS	NUMBER OF KERNELS GERMINATED	NUMBER OF TRISOMICS	% TRISOMICS
2×LF inbred	95–100	148	134	65	49
2×inbred II	95–100	53	44	16	36
2×C11 inbred	95–100	100	93	48	52
2×*lg1*	90– 95	58	52	20	39
3×LF inbred	50+ poor ear	48	34	12	35
3×inbred II	75– 90	59	52	17	33
3×*lg2*	95–100	96	91	41	45
5×inbred II	95–100	101	89	46	52
6×*su2*	95–100	159	155	59	38
7×LF inbred	Tiny ear	26	22	10	45
7×inbred II	80– 90	81	58	23	40
8×LF inbred	90– 95	150	146	47	32
8×*j*	95–100	51	45	12	27
9×*wx*	100	123	113	25	22
10×LF inbred	95–100	177	149	38	26
10×*v18*	95–100	52	49	18	37

trisomes and 2 and 5. This unequal transmission of different trisomes through the egg is in agreement with data reported by other workers in Datura, Matthiola, Lycopersicum, Nicotiana, and other plants. The following explanations have been advanced by various workers to account for these differences:

Viability is decreased as a result of the unbalance caused by the extra chromosome. This unbalance may result in the abortion of some of the n+1 eggs or 2n+1 zygotes at different stages in ontogeny as suggested in Datura, Lycopersicum, and Matthiola.

The replacement of certain of the megaspores containing n+1 chromosomes in the chalazal position by megaspores containing the balanced number has been suggested by McCLINTOCK and HILL (1931).

The observed frequencies may be actual gametic frequencies, the extra chromosome being lost in the meiotic divisions.

With reference to viability, McCLINTOCK and HILL (1931) emphasized that the failure to find transmission frequencies approaching the theoretical 50 percent apparently was not due to lack of viability of the n+1 female gametes or 2n+1 plants, since trisomic plants were obtained with almost perfectly filled ears. In the mature ears of maize the regularity of the rows and kernel positions permits undeveloped kernels to be readily detected. In the present experiments an estimate was made of the fertility of all the ears of trisomic plants from which seed was taken. In table 1 these are noted as percent fertility. A rating of 95–100 percent indicates that the fertility is normal or indistinguishable from that of an average diploid ear. Since most of the ears used showed normal fertility, it may be concluded that any differential viability prior to seed germination plays an insignificant role in determining the proportion of trisomic individuals which appear in progenies.

The failure of 2n+1 seeds to germinate probably had no significant influence on the relative frequency of trisomics. Fourteen hundred and eighty-two seeds were started. Of these 1326 or 90 percent germinated. It is probably true that a greater percentage of the smaller seeds failed to germinate. Because the lighter seeds are more often trisomic than the heavier ones in all stocks used in the experiments (EINSET unpublished), the percentage of trisomics present in progenies may be slightly low. Of the 1326 seedlings that were transplanted to the field, 38 or 2.8 percent died before reaching maturity. Twenty of these were disomic, seven were trisomic, two were monosomic, one plant had 19 chromosomes plus a fragment chromosome, and in six the chromosome constitution was not determined. Most of these plants died soon after the transplanting, probably because of injuries sustained in handling. There was no indication of lessened viability caused by the presence of an extra chromosome.

The possibility that the basal megaspore, if it contains the extra chromosome, does not function to produce the embryo sac but is replaced by one containing an n complement in a certain percentage of the cases was suggested by McCLINTOCK and HILL (1931). No indication of such substitution of megaspores was found by SINGLETON and MANGELSDORF (1940) in a study of gametic lethals on the fourth chromosome of maize. RHOADES (1942) followed the development of 200 embryo sacs in a study concerned with an abnormal type of

chromosome 10 and found that no replacement of chalazal megaspores occurred. To test the possibility of megaspore replacement in trisomic maize, a study of embryo sac development in a large number of ovules would be necessary. This study was not undertaken. However, chromosome number determinations of microspores are readily obtainable, and if the behavior of the extra chromosome during meiosis is comparable in mega- and microsporogenesis, a comparison of observed ratios of n: n+1 microspores in the parent trisomic plants with 2n and 2n+1 individuals in the progeny of these would indicate whether megaspore replacement was occurring. This comparison is shown in table 2. Although the chromosome counts of microspores were obtained from 2n+1 individuals in the F_1 progeny, the agreement in the ratio of n:n+1 microspores with 2n:2n+1 individuals is very close. If such a comparison is valid, one may conclude that the ratios of 2n:2n+1 individuals represent the true gametic ratios. It would also suggest that genic variability, known to be present among the different megaspores, female gametophytes and 2n+1 individuals, does not materially influence viabilities. The possibility that the observed ratio of n:n+1 microspores in a given sample may vary with the stage at which counts were made should be considered (McCLINTOCK 1938). If the rate of development of n and n+1 spores through prophase and metaphase is not comparable, then a sample in which only a small percentage of the microspore nuclei have entered division might give a ratio differing from that

TABLE 2

Progeny and microspore counts of trisomic plants and occurrence of univalents at metaphase I.

TRISOMIC STOCK	% TRISOMICS IN PROGENIES OF TRISOMIC PLANTS	N*	% MICROSPORES WITH n+1 CHROMOSOMES	N*	% MICROSPOROCYTES WITH UNIVALENTS AT METAPHASE I	N*
2×LF inbred	49	132	50	212	30	247
2×inbred II	37	43	47	149	17	202
2×C11 inbred	52	93	—	—	—	—
2×*lg1*	39	52	44	93	12	153
3×LF inbred	35	34	—	—	32	190
3×inbred II	33	52	—	—	—	—
3×*lg2*	45	91	41	167	24	152
5×inbred II	52	89	50	198	14	209
6×*su2*	38	155	34	109	28	109
7×LF inbred	45	22	50	193	30	136
7×inbred II	40	58	—	—	24	110
8×LF inbred	32	146	37	113	40	267
8×*j1*	27	45	36	132	—	—
9×*wx*	22	113	23	218	44	214
10×LF inbred	26	149	34	190	49	372
10×*v18*	37	49	33	109	37	300

* N equals the total number of observations.

obtained from a sample in which most of the spores have completed this division. Since the figures presented in table 2 represent composites from many anthers, in which different percentages of spores had completed the division, the possibility of such an effect presumably was eliminated.

The evidence presented above indicates that the observed differences in the frequencies of transmission of the extra chromosome through the egg, which characterized the various trisomic stocks in maize, were due to the difference in frequency with which n and n+1 megaspores were formed.

CHROMOSOME LENGTH AND TRANSMISSION FREQUENCY

The frequency of trisomics in progenies of trisomic plants and the percentage of microspores with n+1 chromosomes, presumably a measure of the frequencies of n and n+1 gametes, in stocks of different trisomics are summarized in table 3. The relative length at pachytene of each chromosome involved in this study, as determined by LONGLEY (1939), is indicated in the table. These eight chromosomes are arranged in three groups according to their relative lengths—namely, long (chromosomes 2, 3, and 5), medium (chromosomes 6, 7, and 8), and short (chromosomes 9 and 10).

It is evident that there is a positive correlation between the length of a

TABLE 3

Chromosome lengths and frequencies of trisomics in F_1 progenies, microspores with n+1 chromosomes, and microsporocytes with univalents at metaphase I.

RELATIVE CHROMOSOME LENGTHS*	TRISOMIC STOCK	% TRISOMICS IN PROGENIES OF TRISOMIC PLANTS	N†	% MICROSPORES WITH n+1	N†	% MICROSPOROCYTES WITH UNIVALENTS AT METAPHASE I	N†
80	2	47	320	48	454	20	602
74	3	45	91	41	167	28	342
73	5	52	89	50	198	14	209
Averages for long chrs.		48		46		21	
60	6	38	155	34	109	28	109
56	7	41	80	50	193	26	246
57	8	31	191	36	245	40	267
Averages for medium chrs.		37		40		31	
52	9	22	113	23	218	44	214
45	10	28	198	34	299	43	672
Averages for short chrs.		25		29		44	

* Relative lengths at pachytene, with chromosome 1 equal to 100, based on measurements on 33 Indian varieties of corn from the United States (LONGLEY, 1939).

† N equals the total number of observations.

chromosome and the frequency with which n+1 gametes involving different chromosomes are transmitted through the female gametophyte. Plants trisomic for the three longer chromosomes (chromosomes 2, 3, and 5) transmitted the extra chromosome through nearly 50 percent of its eggs; plants trisomic for the chromosomes of medium length (chromosomes 6, 7, and 8) transmitted the extra chromosome through 37 percent of the eggs, and plants trisomic for the short chromosomes (chromosomes 9 and 10) averaged only 25 percent transmission of the extra chromosome through the eggs. In some cases variation from the mean within each group was rather pronounced. Nevertheless a positive correlation is obvious.

MEIOTIC BEHAVIOR OF THE TRISOMES

The behavior of the various trisomes was studied during microsporogenesis to determine whether the observed chromosome length-transmission frequency correlation could be related to some specific behavior of the chromosomes during meiosis. An explanation of the variability found in different stocks was likewise desired. Results obtained in such studies should be applicable to megasporogenesis, since the progeny and microspore counts showed such close agreement.

According to McCLINTOCK and HILL (1931) approximately two-thirds of the microsporocytes in plants trisomic for chromosome 10 contained nine bivalents and one trivalent at metaphase I; in the other sporocytes there were ten bivalents and a univalent. The univalent, when present, behaved very irregularly and often was not included in either telophase nucleus. Trivalents, on the other hand, seemed to disjoin regularly, two from one, in the first division of meiosis, and no irregularity was observed in division II. Consequently, it would be expected that 2n+1 plants having large numbers of microsporocytes with ten bivalents plus one univalent would produce fewer n+1 spores, and thus gametes, than plants having a lower number of such microsporocytes. The data presented in table 3 indicate that this was true.

The observed differences in univalent frequency at metaphase I were traced to differences in the frequency of disassociation of one member of the trivalent configurations during late prophase I. This was accomplished by examining progressive prophase stages between pachytene and metaphase I, the material being obtained from adjacent florets of the same tassel, fixed at the same time. The following observations were recorded on material from a plant trisomic for chromosome 3(3×LF inbred):

STAGE OF MEIOSIS	% OF CELLS SHOWING A UNIVALENT	TOTAL NUMBER OF CELLS OBSERVED
Pachytene	2- 4	112
Diplotene	7-11	104
Middle diakinesis	31	45
Late diakinesis	30	100
Metaphase I	32	190

The maximum values for cells showing a univalent at pachytene or diplotene were obtained by assuming that all doubtful cells, which were relatively few in number, contained the univalent. The pachytene observations indicated that in 96–98 percent of the microsporocytes synapsis was normal. Trivalents were formed regularly through the two-by-two synapsis of the three homologues at different levels. As the prophase progressed through diakinesis to metaphase I, a higher frequency of univalents was found.

From these data it may be concluded that most of the univalents observed at metaphase I result from the separation of one member from the trivalent complex either at diplotene or early diakinesis. This desynapsis could be attributed to the failure of chiasma formation following DARLINGTON'S (1933) theory of post-diplotene association by chiasmata.

At metaphase I, the three homologues were present either as a trivalent or as a bivalent and a univalent. Only rarely were three univalents observed. When a univalent is present at this stage, it may be found almost anywhere in the cell. Observations were made on 859 sporocytes possessing ten bivalents plus a univalent. In 132 or 15.4 percent of the cells the univalent chromosome was on the equatorial plate along with the bivalent chromosomes. In 136 cells (15.8 percent) the univalent was present in the cytoplasm outside the spindle In the remaining 591 cells (68.8 percent) the univalent was definitely off the equatorial plate but within the limits of the spindle.

The two sister chromatids of a univalent may show signs of separation as early as metaphase I or very early anaphase I. This is especially true if the univalent is located on the equatorial plate or near the plate within the spindle.

When a trivalent is present, disjunction at anaphase I is usually two from one. When a univalent is present, it may be included in one of the anaphase groups of disjoining bivalents and subsequently be included in a telophase nucleus, or it may fail to be included in either telophase nucleus. Data on the percentage of microsporocytes with univalents at metaphase I (table 3) and microspores with n+1 chromosomes indicate that in trisomic stocks involving the longer chromosomes most of the univalents were being transmitted to the microspores. This was substantiated by the low percentage of lagging at anaphase I (table 4). The medium and short chromosomes, when present as univalents, were lost to the telophase nuclei and not included in the microspores with a much higher relative frequency (table 3). The relative amount of lagging of univalents at anaphase I in stocks of trisomes 9 and 10 was also greater than in the stock of trisome 2 (table 4). When a univalent lags, in many instances the two-sister chromatids making up the univalent separate or show signs of separation. Rarely, however, do the separated chromatids (monads) reach the poles. Among 149 cells at late anaphase I none was found with ten dyads plus one monad at each pole. In 96 of these cells there were ten dyads at one pole and 11 dyads at the opposite pole. In 25 cells a univalent, either undivided or in the process of division, was present at the equatorial plate region. In the remaining 28 cases, the univalent occupied a position closer to one pole than the disjoining ten dyads, or was located outside the spindle

altogether. It seems probable, therefore, that although the two halves of a univalent often show signs of separation at metaphase I and anaphase I, this separation is ordinarily completed too late for the monads to be included in one or both of the telophase nuclei. However, RHOADES (1940) found that occasionally at least the daughter univalents of an unpaired telocentric chromosome did reach the poles in time to be included in the interphase nuclei. This was made evident by the observation of daughter univalents in prophase II. In the trisomic material a number of chromosome counts were made at pro-

TABLE 4

Frequency of univalents and micronuclei at meiosis I and II in various trisomic stocks, expressed in percentage.

UNIVALENT AND MICRONUCLEI FREQUENCY, IN %	TRISOMIC STOCK									
	2×LF	N*	3×LF	N	9×*wx*	N	10×LF	N	10×*v*18	N
Total number of univalents present										
Diakinesis	28	275	30	145	32	195	—		33	100
Metaphase I	30	247	32	190	44	214	49	372	37	300
Detached univalents or micronuclei										
Anaphase I	3	60	—		41	39	29	45	19	32
Telophase	4	53	31	71	15	126	24	151	13	80
Interphase	—		—		—		19	136	—	
Metaphase II	—		11	93	—		—		38	101
Anaphase II	—		15	13	—		—		12	33
Telophase II	—		13	85	—		—		15	300
Spore quartets with micronuclei	—		—		—		6	100	7	57
Spores with n+1 chromosomes	50	212	—		23	218	34	190	33	109

* N equals number of sporocytes, number of pairs of cells in division II or number of quartets of spores.

phase II, but in no case were ten dyads plus a monad found in each of the daughter cells. Univalents that were included in the telophase I nuclei passed undivided to one of the two poles of the spindle.

At telophase I the univalent or its two separated halves that were not included in the daughter nuclei may be found anywhere within the cell, either at the plate region, near one of the telophase nuclei or elsewhere in the cytoplasm. This excluded chromatin may be present as a compact chromatin mass or becomes organized as a distinct micronucleus. Often there are two such compact

chromatin masses or micronuclei, probably representing the two sister halves of a former univalent. Occasionally a fragment or a chromatin bridge was noted.

In general, the second meiotic division was freer of irregularities than the first. Relatively little chromosome lagging was observed. Observations were made on paired or daughter cells at telophase II. Occasionally one of these two cells contained a compact chromatin mass or a micronucleus. In other cases, each of the two cells contained one such body, presumably representing the sister halves of a former univalent. Certain paired cells were observed in which there were two micronuclei in one daughter cell and none in the other.

BEHAVIOR OF MICRONUCLEI IN TRISOMIC PLANTS

During the meiotic divisions univalent chromosomes which remain in the cytoplasm may be transformed into micronuclei, or they may degenerate and subsequently disappear. It is also possible that such excluded chromosomes or micronuclei are not permanently "lost" but may be reincorporated in the macronuclei. If this happens, the frequency of apparent loss of the univalent during division I in trisomic plants cannot be utilized to estimate the frequency of occurrence of n+1 chromosome complements in spores and gametes. WATKINS (1924) reported on univalent behavior at microsporogenesis in F_3 plants from a cross of *Triticum turgidum* × *T. vulgare*. The univalents arrived at the equatorial plate after the bivalents and split, but the halves were often not included in the daughter nuclei. Many of these lagging chromatids were included in the nuclei after the second meiotic division. Micronuclei decreased in frequency as the microspore developed, and the assumption was made that odd chromosomes degenerated during pollen development. CHANDLER, PORTERFIELD, and STOUT (1937) in a study of triploid types of *Lilium tigrinum* found that "the lagging chromatin elements in the cytoplasm undergo noticeable and rapid disintegration." "Microcysts" or compact chromatin masses were observed to form directly from lagging chromosomes or by degeneration of micronuclei. Later, these chromatin masses disappeared. They also noted that fragments or lagging elements near the equatorial plate region were swept to the periphery of the cell as the phragmoplast expanded at the telophase I. These lagging elements remained at the periphery of the cell and either degenerated or formed micronuclei.

MYERS (1941a) studied the behavior of univalents in certain plants of *Lolium perenne* showing abnormal meiotic behavior. Lagging univalents divided equationally in all observed cases, and it was concluded that a majority of the daughter half-chromosomes from anaphase I laggards were included in the daughter nuclei prior to the interphase stage. Similar behavior was observed in a hybrid of *Phleum pratense* × *Phleum subulatum* (MYERS 1941b). However, in the latter case the univalents were more regularly oriented on the equatorial plane along with the bivalents than in the plants of *Lolium perenne*, and there was also a more regular equational division of the univalents at anaphase I.

Table 4 summarizes the data on the occurrence of univalents and micronuclei at different stages of meiosis in several stocks of maize. There was a decrease in the percentage of cells showing lagging chromosomes or micronuclei from telophase I to the spore quartet stage. The question arises as to whether this decrease was due to incorporation of micronuclei into macronuclei, to disintegration of micronuclei, or to the incorporation of the micronuclei in separate microcytes. The answer may be found in table 4. The trisomic culture 10 × LF inbred may be used as an example. Chromosome counts at the first microspore division showed that 34 percent of these spores were n+1. If the extra chromosome was lost in 30 percent of the microsporocytes at division I, 35 percent of the microspores would be expected to have an extra chromosome. It is, of course, realized that there may be some division of the univalent in the first followed by chromatid lagging in the second division, but this and other anomalous types of behavior are relatively rare as previously shown. Anaphase I showed 29 percent of the cells with a lagging univalent or a univalent in the cytoplasm. Thus the percentage of anaphases with detached univalents corresponded very closely to the expected 30 percent loss of univalents calculated from the known fact that 35 percent of the spores had n+1 chromosomes.

From anaphase I to the spore quartet stage a steady decrease in number of micronuclei was observed. In culture 10 × LF, only 6 percent of the quartets possessed micronuclei. In other cultures this same tendency was observed. Microspores with micronuclei were considerably less frequent than was to be expected from the observed irregular meiotic behavior of the univalents. The incorporation of the micronuclei into macronuclei will not explain this reduction. If this occurred, the number of microspores with n+1 chromosomes would be considerably greater than was observed. Since there was no evidence of microcyte formation or other related phenomena that might account for their disappearance, it was concluded that the micronuclei disintegrated in the cytoplasm during the interim from anaphase I to spore formation.

CHROMOSOME VARIANTS OTHER THAN PRIMARY TRISOMICS

Several chromosome types other than primary trisomics appeared in the crosses of 2n+1 × 2n plants. Among the 1916 plants in which chromosome counts were made for the primary purpose of identifying trisomic individuals, there were, in addition to 658 trisomics, five monosomics (19 chromosome plants), one plant with 19 chromosomes plus a fragment chromosome, one with 20 plus a fragment, three with 21 plus a fragment, two haploids and three triploids. The chromosome fragments present in five of these plants were not supernumerary B chromosomes but presumably represented deletions or additions involving members of the normal complement. The frequency of 19 chromosome plants or monosomics, six among 1916, was higher in the trisomic stocks than was expected, since they previously have been observed infrequently in maize. Unfortunately, five of these six plants were extremely weak and died soon after transplanting to the field, and it was not possible to identify the

individual monosomics. In order to determine whether or not the observed frequency of monosomics in these trisomic cultures differed significantly from their spontaneous occurrence in diploid maize, it would be necessary to make chromosome determinations or critical genetical tests of large numbers of plants produced from very carefully germinated seeds of normal diploid ears. In ordinary field plantings, most monosomics probably would be eliminated early in ontogeny due to a lack of vigor; and this probably accounts in part at least for the very low frequency of monosomics ordinarily observed. Of the five plants with fragments which were found, only one, a plant with 21 chromosomes plus a fragment, grew past the seedling stage. It was extremely weak and produced no tassel or ear. Fragment types have also been derived from progenies of trisomics in Zea, Datura, Lycopersicum, and *Nicotiana sylvestris*, but apparently have not resulted in the unexpected lethality which in these experiments accompanied the presence of chromosome fragments. The frequency of haploidy in these stocks, two among 1916 plants, does not differ significantly from the normal spontaneous frequency of approximately one in 2000 (RANDOLPH unpublished). The four triploid plants which occurred in the trisomic progenies cannot definitely be said to have originated spontaneously, since the trisomic stocks were grown in proximity to tetraploid maize with which they might have been contaminated.

DISCUSSION

Among the various plant species in which trisomic inheritance has been studied, specificity of effect of different trisomes may be apparent not only in the phenotypic appearance of the individual, but also in its breeding behavior. The extra chromosome, theoretically expected to be present in 50 percent of the gametes, has been found in most of the forms studied to be transmitted rarely through the pollen and transmitted with varying frequencies through the egg. The specific effects of different chromosomes in Datura are manifested in differential elimination of the extra chromosomes and differential reduction of viability of spores, gametes, zygotes, embryos, and seeds containing the extra chromosome. Zygotic lethality and reduced seed viability are interpreted as being due to influences of the trisomes similar to those which are partially responsible for the differences in the transmission of different chromosomes through the egg, that have been noted in Matthiola, Lycopersicum, and *Nicotiana sylvestris*.

In Zea no such explanation can be advanced. Any unbalance causing lethality of spores, eggs, or zygotes would be detected in the lowered fertility of the ear. The ears of trisomic plants, however, are wholly fertile. Therefore, the observed differences in transmission frequency of specific maize trisomes must mean that spores with n and n+1 chromosomes are not formed in equal numbers as in Datura microspores, but in whatever ratio the progenies indicate. That this is true of the megaspores as well as the microspores is indicated by the strong positive correlation between microspore counts and transmission frequencies of the various trisomes through the seed parent. This same expla-

nation has been suggested as a partial cause of the differences observed in Matthiola, Lycopersicum, and *Nicotiana sylvestris*. Meiotic irregularities due to the presence of a univalent resulted in the frequent loss of the extra chromosome. That the presence of a trivalent, on the other hand, imposes greater regularity is supported by various investigations.

The data presented in this paper indicate that in maize the extra chromosome is present in the male and female gametes from 22 percent to 52 percent of the time, depending on the particular trisome that is involved. There is a definite positive correlation between the length of the chromosome and the frequency with which it is present in spores or transmitted through the egg. Differences in the frequency of loss of the extra chromosome at meiosis is a function of the length of the extra chromosome (varying from relative values of 80 to 45 in the trisomic stocks of maize studied). BELLING (1924) studied the chromosome numbers of 52 microspores of triploid hyacinths which contain long, medium, and short chromosomes. He concluded that the chromosomes of different length were distributed according to chance. His data, however, suggested that there was some slight tendency for the shorter chromosomes to be lost more often, or to be less frequently present in the microspores than were the medium and long chromosomes.

Data which are here presented on the frequency of trivalents at metaphase I indicate that there are more trivalents present when the trisome is made up of the longer chromosomes of the set. Similar observations were made by BELLING (1925) in certain triploid hyacinths, by NEWTON and DARLINGTON (1929), and DARLINGTON and MATHER (1932) in triploid Tulipa, and by others. In *Nicotiana sylvestris*, GOODSPEED and AVERY (1939) found no differences in trivalent frequencies among the four different trisomics which they studied. Neither did they find length differences in the extra chromosomes, nor significant differences in transmission frequencies of these four chromosomes when present in triplicate.

It has been shown that these differences in trivalent frequencies at diakinesis and metaphase I in the maize material here studied are due to desynapsis of one of the three homologous members of the trivalent. On the basis of DARLINGTON'S theory of post-diplotene association by chiasmata, this early separation of previously synapsed homologues in the late prophase would be due to the failure of one of the three homologous chromosomes to form a chiasma with either of the other two. The frequency with which chiasmata, that would prevent desynapsis, might be formed would then be conditioned by the variation in length of the pairing chromosomes and in the number of chiasmata formed in any given length (DARLINGTON and MATHER 1932).

DARLINGTON, MATHER, and others have published extensively on chiasma behavior and find, in general, a correlation between chromosome length and the number of chiasmata formed. In diploid maize as well as in trisomic maize (DARLINGTON 1933; MATHER 1939) the Stenobothrus type (DARLINGTON and DARK 1932) of relationship exists, the correlation being roughly linear, but the shortest chromosomes have a proportionately higher chiasma frequency

than the longer ones. DARLINGTON (1933) found relatively little reduction in chiasma frequency through terminalization in maize. BEADLE (1933), however, found an average of 3.7 chiasmata per bivalent at diplotene but only 1.8 at diakinesis in maize.

In the trisomic stocks of maize involving the long chromosomes the opportunity for any two chromosomes to be synapsed over relatively long regions is greater than it is among the shorter chromosomes, and for this reason it follows that the opportunity for chiasma formation is greater; there is less desynapsis and consequently more trivalents at metaphase I. If there is little terminalization in maize, or the same relative amount for long and short chromosomes, it is improbable that this phenomenon would account for the greater prevalence of univalents in plants trisomic for the shorter chromosomes.

Briefly, when a short chromosome is present in triplicate, fewer trivalents and more univalents are present at metaphase I. Irregular assortment of the univalents in anaphase I results in their frequent elimination, and this elimination of the univalents reduces the number of spores and gametes containing the extra chromosome. Since n and n+1 gametes and the zygotes which they form are equally functional, there are fewer trisomic individuals in the progeny of plants trisomic for the shorter chromosomes than in the progeny of those trisomic for the longer chromosomes.

There was a relatively great amount of variability in transmission frequency among various stocks of the same trisome as well as a discrepancy between the value for trisome 5 (31 percent) reported by RHOADES (1933) and the value here reported (52 percent). This variability cannot be correlated with chromosome length, but may possibly be explained on the basis of variability in the number of univalents at metaphase. RHOADES (1933) found a low value (10–15 percent) for the frequency of univalents at metaphase, although transmission was only 31 percent. Different samples from the same trisomic stocks sometimes showed rather extreme fluctuations in univalent frequency. One sample of 8×LF had 30 percent of the sporocytes with univalents at metaphase I, another had 48 percent of the sporocytes with univalents. In the case of 2×LF inbred the counts indicated that the frequency of univalents at metaphase I was 30 percent. Chromosome counts in microspores and progenies of this trisomic indicated that 50 percent of the gametes were of the n+1 type. If a large percentage of the univalents is lost, how is it possible to find such high frequencies? LESLEY (1928), working with 3n tomatoes, observed considerable variation in meiotic chromosome behavior in samples examined the same day, even within the same anther. The literature dealing with abnormalities in meiosis caused by heat, cold, and other environmental effects is extensive. STOW (1927) found very marked decrease in conjugation at a temperature of 25–30°. MATSUURA (1937) noted more univalents in metaphase I under high temperature conditions. OEHLKERS (1935) also made extensive studies of the varying effects of temperature on crossing over and chiasma formation. It seems, therefore, that the observed variability within

stocks may have been caused by environmental effects, or possibly by genic effects which might have altered either the usual synaptic relations of the trisome or more likely chiasma formation and the occurrence of univalents at meiosis. More extensive studies in a carefully controlled environment should throw additional light on this question.

SUMMARY

A study of the breeding behavior of eight of the ten primary trisomes of maize (trisomes 1 and 4 were not studied) revealed marked differences in the frequency with which the extra chromosome was transmitted to the progeny through the egg. Transmission frequencies determined by somatic chromosome counts of 1916 plants ranged from approximately 50 percent for the longer chromosomes to approximately 25 percent for the shorter chromosomes, and the chromosomes of intermediate lengths exhibited intermediate transmission frequencies.

Failure of the extra chromosome to be transmitted to 50 percent of the progeny in $2n+1 \times 2n$ crosses apparently was not due to replacement of $n+1$ megaspores by spores containing the n number, since there was very close agreement in specific crosses between the percentage of microspores containing the extra chromosome and the percentage of trisomic plants in the progeny. Neither was it due to differential viability of zygotes, since the ears of trisomic plants were as well filled as those of their disomic sibs, and their seeds were equally viable.

The failure of the extra chromosome to be transmitted to 50 percent of the progeny through the egg apparently was due to the elimination of the extra chromosome as a univalent in the meiotic divisions.

Desynapsis of one member of the trivalent to form a univalent occurred more frequently in trisomic plants involving the shorter chromosomes than in those involving the longer chromosomes. This desynapsis took place in late prophase; there was regular two-by-two synapsis of the three homologues at pachytene.

It is suggested that this desynapsis was due to the failure of chiasma formation between the chromosome that desynapsed and either of the other two, chiasmata presumably preventing complete separation of synapsed homologues during late prophase. On the basis of the chromosome length-chiasma frequency correlation, this failure would be more frequent in trivalents composed of short chromosomes than in trivalents made up of long chromosomes.

Univalents frequently lagged in the meiotic divisions and failed to be incorporated in the daughter nuclei. Thereafter they sometimes formed micronuclei, but more often they disappeared, probably as a result of disintegration in the cytoplasm.

In a population of 1916 plants derived from the cross $2n+1 \times 2n$, 658 plants (34.34 percent) were trisomic, two were haploids, six were monosomics, four were triploids, and five carried fragment chromosomes in addition to the complement of normal chromosomes.

LITERATURE CITED

BEADLE, G. W., 1933 Further studies of asynaptic maize. Cytologia **4**: 269–287.

BELLING, J., 1924 The distribution of chromosomes in the pollen-grains of a triploid hyacinth. Amer. Nat. **58**: 440–446.

1925 Homologous and similar chromosomes in diploid and triploid hyacinths. Genetics **10**: 59–71.

BLAKESLEE, A. F., and A. G. AVERY, 1938 Fifteen-year breeding records of 2n+1 types in *Datura Stramonium*. Cooperation in Research, Carnegie Inst. Washington Publ. **501**, 315–351.

CHANDLER, C., W. M. PORTERFIELD, and A. B. STOUT, 1937 Microsporogenesis in diploid and triploid types of *Lilium tigrinum* with special reference to abortions. Cytologia, Fujii Jubilee Volume: 756–784.

DARLINGTON, C. D., 1933 The origin and behavior of chiasmata. VII. *Zea Mays*. Z.I.A.V. **67**: 96–114.

DARLINGTON, C. D., and S. O. S. DARK, 1932 The origin and behavior of chiasmata. II. *Stenobothrus parallelus*. Cytologia **3**: 169–185.

DARLINGTON, C. D., and K. MATHER, 1932 The origin and behavior of chiasmata. III. Triploid Tulipa. Cytologia **4**: 1–15.

FROST, H. B., 1927 Chromosome-mutant types in Stocks (*Matthiola incana* R. Br.). I. Characters due to extra chromosomes. J. Hered. **18**: 474–486.

GOODSPEED, T. H., and P. AVERY, 1939 Trisomic and other types in *Nicotiana sylvestris*. J. Genet. **38**: 381–458.

LESLEY, J. W., 1928 A cytological and genetical study of progenies of triploid tomatoes. Genetics **13**: 1–43.

1932 Trisomic types of the tomato and their relation to the genes. Genetics **17**: 545–559.

LONGLEY, A. E., 1939 Knob positions on corn chromosomes. J. Agric. Res. **59**: 475–490.

MCCLINTOCK, B., 1929 A cytological and genetical study of triploid maize. Genetics **14**: 180–222.

1938 The fusion of broken ends of sister half-chromatids following chromatid breakage at meiotic anaphases. Missouri Agric. Expt. Sta. Research Bull. **290**: 1–48.

MCCLINTOCK, B., and H. E. HILL, 1931 The cytological identification of the chromosome associated with the r-g linkage group in *Zea Mays*. Genetics **16**: 175–190.

MATHER, K., 1939 Chiasma frequencies in trisomic maize. (Abstract) Genetics **24**: 104.

MATSUURA, H., 1937 Chromosome studies on *Trillium kamtschaticum Pall.* V. Abnormal meiotic divisions due to high temperatures. Cytologia, Fujii Jubilee Volume: 20–34.

MYERS, W. M., 1941a Variations in chromosomal behavior during meiosis among plants of *Lolium perenne*. Cytologia **11**: 388–406.

1941b Meiotic behavior of *Phleum pratense*, *Phleum subulatum*, and their F_1 hybrid. J. Agric. Res. **63**: 649–659.

NEWTON, W. C. F., and C. D. DARLINGTON, 1929 Meiosis in polyploids. I. J. Genet. **21**: 1–16.

OEHLKERS, F., 1935 Untersuchungen zur Physiologie der Meiosis. I. Z. Bot. **29**: 1–53.

RANDOLPH, L. F., 1935 A new fixing fluid and a revised schedule for the paraffin method in plant cytology. Stain Tech. **10**: 95–96.

RANDOLPH, L. F., and B. MCCLINTOCK, 1926 Polyploidy in *Zea Mays* L. Amer. Nat. **60**: 99–102.

RHOADES, M. M., 1933 An experimental and theoretical study of chromatid crossing over. Genetics **18**: 535–555.

1940 Studies of a telocentric chromosome in maize with reference to the stability of its centromere. Genetics **25**: 483–520.

1942 Preferential segregation in maize. Genetics **27**: 395–407.

RHOADES, M. M., and B. MCCLINTOCK, 1935 The cytogenetics of maize. Bot. Rev. **1**: 292–325.

SINGLETON, W. R., and P. C. MANGELSDORF, 1940 Gametic lethals on the fourth chromosome of maize. Genetics **25**: 366–390.

STOW, I., 1927 A cytological study on pollen sterility in *Solanum tuberosum* L. Japan. J. Bot. **3**: 217–238.

WATKINS, A. E., 1924 Genetic and cytological studies in wheat. I. J. Genet. **14**: 129–171.