THE RELATION OF CROSSING OVER TO CHROMOSOME ASSOCIATION IN ZEA-EUCHLAENA HYBRIDS

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THE PROBLEM

The problems to be considered in the present paper are concerned with the interrelations of various phases of the meiotic process. Conjugation is considered by most cytologists and geneticists to be necessary for the normal occurrence of crossing over, chiasma formation, post-diplotene chromosome association and chromosome disjunction. JANSSENS (1924), BELLING (1931), DARLINGTON (1931b), SAX (1930) and others hold the view that chiasmas are related to crossing over. DARLINGTON (1929, 1930, 1931b) has put forth the theory that chromosome association after diplotene is due to the presence of chiasmas. ANDERSON (1929) found that frequency of non-disjunction in Drosophila was inversely related to the frequency of crossing over. DOBZHANSKY (1931) has suggested that decreased frequency of crossing over in heterozygous translocations in Drosophila can be accounted for by assuming decreased frequency of conjugation in

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the regions affected. Certain of the hypotheses which have to do with the relations existing between various phases of the meiotic process have been tested experimentally. It is important that more such tests be made. The studies reported in the present paper were undertaken with such a purpose in mind.

It has been shown (EMERSON and BEADLE in press) that the maize chromosome which carries the *C* gene and others crosses over infrequently or not at all, in the $C-wx$ region, with its homolog from either the Durango or the Florida form of annual teosinte (*Euchlaena mexicana*). By making use of this chromosome of teosinte, it is possible to study chromosome behavior at meiosis under two conditions, namely, with and without crossing over. The studies here reported show a definite relation between crossing over and chromosome association after diplotene. This relation is considered in connection with various theories that have to do with the meiotic mechanism.

MATERIAL AND METHODS

Some fifteen genes in the *C* chromosome of maize have been worked with by various investigators. McCLINTOCK (1930) has demonstrated that the chromosome which carries the *C* group of linked genes is the ninth longest of the ten maize chromosomes. It will be referred to as chromosome 9 in the present paper. This chromosome has the spindle attachment so located that the ratio of the lengths of the two limbs of the chromosome is approximately 2:1 (McCLINTOCK 1930 and unpublished). The genes in chromosome 9 which have been used in the course of the studies here reported are listed as follows:

y_{g2}	Yellow green seedling and plant
C	Aleurone color
s_h	Shrunken endosperm
w_x	Waxy endosperm
v_1	Virescent seedling

A genetic map of this chromosome showing the position of the above genes is shown in figure 1. The teosinte homologs of this chromosome carry normal allelomorphs of these genes.

In addition to the above mentioned genes, a reciprocal translocation involving maize chromosomes 8 and 9 has been used in the course of the studies. This translocation, known as "semisterile-2," has been studied by BURNHAM (1930) and by McCLINTOCK (1930, 1931). Since this is the only translocation concerned in the present paper, it will be referred to merely as "translocation."

Some information as to the physical location of the genes in the ninth chromosome of maize is available. STADLER (1931) observed in a study of endosperm mosaics that the *C* and S_h genes were often lost (presumably by loss or injury of a part or all of the chromosome) without loss of W_x, but that loss of W_x was always accompanied by loss of *C* and S_h. This indicates that the spindle attachment is close to w_x. MCCLINTOCK (1930, 1931) has shown that the translocation concerned involves somewhat more than half the long limb of chromosome 9. She also demonstrated that the genes *C*, s_h and w_x lie in the same direction from the translocation point as does the short limb. BURNHAM has shown (unpublished data cited by MCCLINTOCK 1931) that the genes *C*, s_h and w_x lie in the order w_x, s_h, *C* from the translocation. The available evidence therefore indicates that the $C-w_x$ interval is included, at least for the most part, in the short limb of the chromosome.

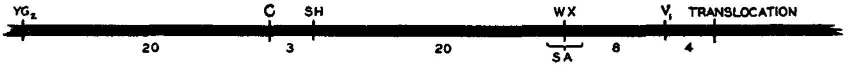

FIGURE 1.—Genetic map of the ninth chromosome of maize showing location of genes referred to in the present paper.

Homologs of the ninth chromosome of maize from the Durango and Florida forms of annual teosinte are involved in the studies presented in this paper. For convenience in technic and for other reasons which will be apparent later, these teosinte chromosomes were transferred to maize-like stocks by repeated backcrossing of the hybrids to maize. The plants used for study were from the third and fourth backcross generations. Since the teosinte chromosomes were marked only by the genes *c*, S_h and W_x, it is probable that any sections outside the marked region would, provided crossing over with the maize homolog occurs, be replaced by sections from maize chromosomes. Evidence to be presented suggests that the short limb of the teosinte chromosomes was retained. For convenience, the chromosomes which are made up of at least a certain section from teosinte will be referred to as teosinte chromosomes.

Genetic studies of crossing over were made using the derived teosinte chromosomes.

For cytological observations the maize translocation was introduced for the purpose of providing a physical marker for the chromosomes which it was desired to study.

Preparations for cytological observation were made by the aceto-carmine method. Material was killed in a mixture of 7 parts alcohol and 3 parts acetic acid. Preparations were best when material was left in this killer from one to several days. Sporocytes were stained and mounted either in aceto-carmine or a mixture of aceto-carmine and Ehrlich's haematoxylin as used by COOPER (COOPER and BRINK 1931). Sporocytes were flattened by gentle heating and by pressure of the cover glass as recommended by MCCLINTOCK (1929). All drawings were made with the aid of a camera lucida. Drawings of the chromosomes at diakinesis are semi-diagrammatic in that they are drawn with smooth outlines. Actually, by the methods used, diakinesis chromosomes in maize have irregular outlines.

TABLE 1

Backcross data on crossing over between maize chromosome 9 and its homolog from teosinte. The class carrying the dominant allelomorph of the first gene listed in column 1 is listed first, in all cases, under crossover class.

CONSTITUTION OF MAIZE CHROMOSOME	SOURCE OF TEOSINTE CHROMOSOME	CROSSOVERS IN REGION					TOTAL
		0	1	2	3	1, 3	
$y_{g2} w_x$	Durango	156 102	0 0 0 percent				258
$y_{g2}\ s_h\ w_x$	Durango	105 75	0 0 0 percent	0 0 0 percent			180 438
$y_{g2}\ s_h\ w_x$	Florida	550 475	3 0 0.3 percent	1 0 0.1 percent			1029
$C\ w_x$ translocation	Florida	37 37	0 0 0 percent	2 1 3.9 percent			77
C translocation	Florida	83 122	11 25 14.9 percent				241 318

Total crossing over in C-translocation (equivalent to w_x translocation) interval—12.3 percent.

$C\ s_h\ w_x\ v_1$	Durango	356 435	2 0 0.4 percent	0 0 0 percent	69 53 13.4 percent	2 0	917

Crossing over in region 3 based only on $c\ S_h\ W_x$ class—10.9 percent.

$C\ sh\ w_x\ v_1$	Florida	367 547	0 0 0 percent	0 0 0 percent	40 36 7.7 percent	0 0	990

Crossing over in region 3 based only on $c\ S_h\ W_x$ class—6.2 percent.

GENETIC STUDIES OF CROSSING OVER

Crosses of plants of the constitution $\frac{+ \; + \; + \text{ (teosinte)}}{y_{g2} \; C \; w_x \text{ (maize)}}$ or $\frac{+ \; + \; + \text{ (teosinte)}}{y_{g2} \; s_h \; w_x \text{ (maize)}}$ by maize, recessive for the genes concerned, are presented in table 1. It is evident from these data that there is little or no crossing over in the $y_{g2}-C$ interval. It has already been shown (EMERSON and BEADLE in press) that there is little or no crossing over in the $C-w_x$ interval in such crosses. The teosinte chromosomes from both Durango and Florida forms show similar behavior in the $y_{g2}-w_x$ interval in these crosses.

Data from the cross $\frac{+ + \quad + \text{ (teosinte)}}{C \; w_x \text{ translocation (maize)}}$ or $\frac{+ \quad + \text{ (teosinte)}}{C \text{ translocation (maize)}}$ by $c \; w_x$ maize are presented in table 1. Only the teosinte chromosome from the Florida form has been studied in this interval. As compared with crossing over in the w_x-translocation interval in pure maize (12 percent based on 1305 individuals—BURNHAM 1930 and unpublished), crossing over between the maize and teosinte chromosomes occurs with approximately normal frequency in this interval. Considering the history of the teosinte chromosome, it is probable that both chromosomes in part of this interval are made up of maize chromosome segments. There must have been at least some crossing over in this interval between the original teosinte chromosome and its maize homolog. It is not possible to say with what frequency it occurred.

Data from crosses of plants of the constitution $\frac{+ + + + \text{ (teosinte)}}{C \; s_h \; w_x \; v_1 \text{ (maize)}}$ by $c \; s_h \; w_x \; v_1$ maize are summarized in table 1. In these crosses, classification for v_1 was somewhat difficult in the $C \; s_h \; w_x$ class but was easily made in the $c \; S_h \; W_x$ class. The crossover value based on the $c \; S_h \; W_x$ class is therefore probably more reliable than that based on both classes. Both the Durango and Florida teosinte homologs of the ninth maize chromosome were studied in these crosses. The data show that crossing over occurs in the w_x-v_1 interval with about the same frequency as between two maize chromosomes (control value 8.3 percent based on 1104 individuals).

In the data summarized in table 1, a few apparent crossovers in the $y_{g2}-s_h$, $C-s_h$ and s_h-w_x regions are recorded. Such infrequently occurring crossover types have been found in previous experiments (EMERSON and BEADLE in press). All of these which have been tested have been shown not to be due to crossing over. The cases recorded in the present paper were not tested. They may have been the result of crossing over, contaminations or hetero-fertilization (SPRAGUE 1929).

The data indicate that crossing over between the ninth chromosome of maize and its teosinte homologs does not occur or occurs rarely in the short limb of the chromosome. Crossing over in the long limb probably occurs with about the same frequency as that observed in maize. The relation of frequency and distribution of crossing over in this chromosome to number and distribution of chiasmas and to association after diplotene will be considered later.

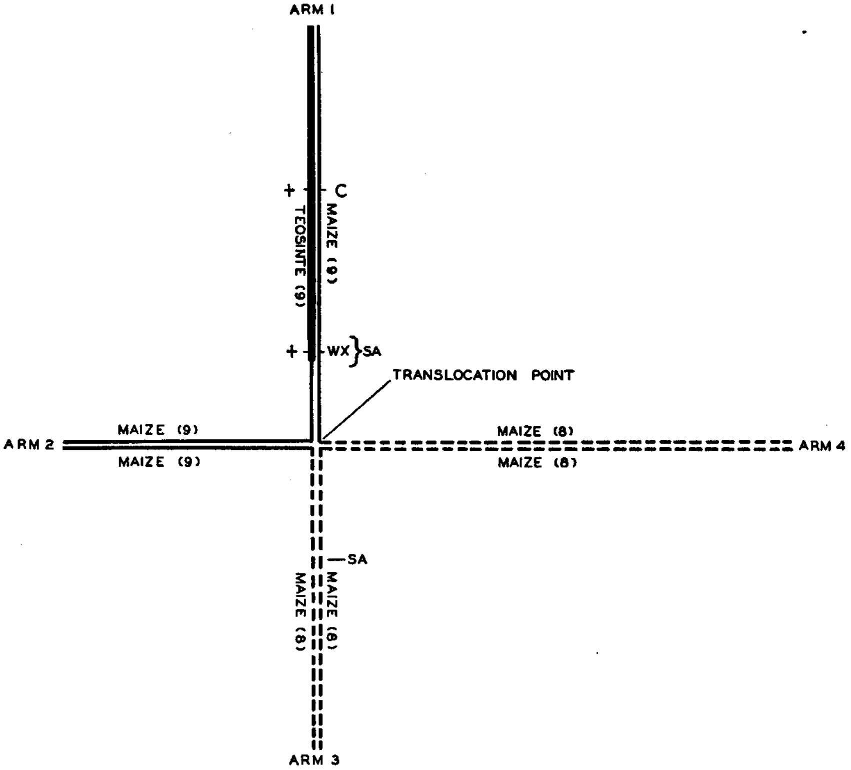

FIGURE 2.—Diagram of the set-up used for cytological observation. The portion of the non-translocated ninth chromosome known to have been derived from teosinte and known not to cross over with its corresponding maize section, is represented by a heavy line. Position of spindle attachments is indicated (SA). Lengths of the chromosomes are based on cytological determinations by MCCLINTOCK (1930).

CYTOLOGICAL OBSERVATIONS

Using ninth chromosome homologs from Durango and from Florida teosinte in combination with translocated maize chromosomes, plants of the constitution represented in figure 2 were obtained. Cytological observations on such plants were made for the purpose of studying pachytene pairing and post-diplotene association in arm 1 of the complex. It is this arm in which crossing over has just been considered. Pure maize plants heterozygous for the translocation were used as a control. In the control used for a study of the points of association at diakinesis in arm 1 of the complex, the longest translocated chromosome was marked with a deep-staining terminal knob (McClintock 1930). This knob provided a marker for this arm.

Pachytene chromosome association

Pachytene chromosome association in maize plants heterozygous for the translocation has been described by McClintock (1930). Parasynapsis of homologous parts takes place resulting in a cross-shaped configuration at pachytene such as is diagrammed in figure 2.

When the section of teosinte chromosome which does not cross over with its homologous maize parts is put into the complex, pachytene pairing in arm 1 of the complex is usually normal. Fourteen such cross-shaped figures were observed in complexes including a section of Durango teosinte chromosome. Two figures of such complexes in diplotene were observed. Of the complexes involving the Florida teosinte chromosome, fourteen pachytene cross-shaped figures were observed. Since with both teosinte chromosomes the results were the same, the two cases can be discussed together. Of the twenty-eight pachytene figures, 23 (11 Durango, 12 Florida) showed closely paired threads in all parts that could be followed. In these figures, no unpaired threads which might have been connected with the complex were observed. Numerous other pachytene figures were observed in which the translocation cross could not be definitely identified but in which threads throughout the nucleus could be traced and shown to be associated in pairs. Five pachytene translocation figures (3 Durango, 2 Florida) showed lack of pairing of threads in at least one arm of the cross. In one, part of one arm had apparently been torn off in the preparation of the slide. The remainder of the two threads of this arm were unpaired. Two figures showed a terminal portion (about one third) of one arm, which could not be identified, to be unpaired. One figure showed unpaired threads in approximately the median third of one arm. Another figure showed the segment beyond the spindle attachment of one arm to be unpaired (figure 3a). In this same figure, a portion of a second arm was unpaired.

From a consideration of relative lengths of the arms of the cross and from positions of the spindle attachments (McClintock 1930), the $C-s_h-w_x$

carrying arm could be identified in five figures and in all showed apparently normal pairing.

Of the two diplotene figures, one showed apparent association in all four arms of the cross. The other (figure 3b) showed the threads distal to the spindle attachment of what was probably the $C-s_h-w_x$ arm to be unpaired.

In none of the pachytene figures was reverse conjugation observed, such as McClintock (in press) has shown to be characteristic of at least two inversions in maize.

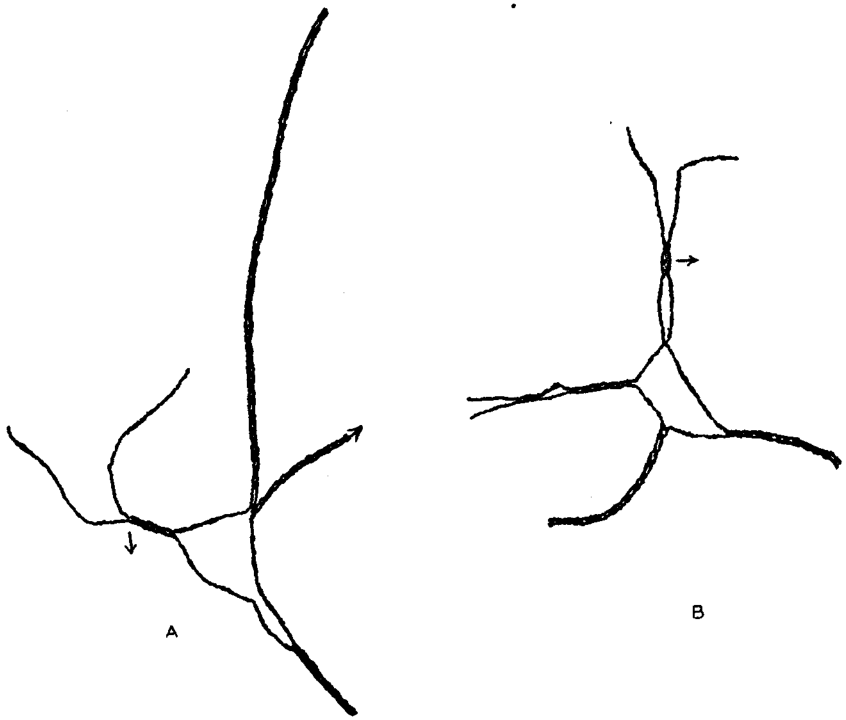

Figure 3.—A, Pachytene translocation complex including a Florida teosinte chromosome. Probable location of the spindle attachment of one chromosome is indicated by the lower arrow. B, Diplotene (early) translocation complex including a Durango teosinte chromosome. The spindle attachment in arm 1 of the complex is indicated by the arrow. Both figures A and B are semidiagrammatic. ×1565.

Diakinesis and metaphase chromosome association

As shown by the observations summarized in table 2, the heterozygous translocation in pure maize results in a ring configuration at diakinesis and metaphase in about 96 percent of the sporocytes. Diakinesis and metaphase observations agree as well as might be expected. Of 133 rings classi-

TABLE 2

Chromosome configurations of heterozygous translocation complexes in normal maize and in maize-teosinte hybrids.

ORIGIN OF NON-TRANSLOCATED CHROMOSOME 9	NUMBER OF PLANTS STUDIED	STAGE	OBSERVED FIGURES						PERCENT RINGS OF 4
			RING OF 4	CHAIN OF 4	TWO "PAIRS"	CHAIN OF 3 AND UNIVALENT	ONE "PAIR" AND 2 UNIVALENTS	TOTAL	
Maize	2	Diakinesis	235	7	0	0	0	242	97
		Metaphase	110	8	0	0	0	118	93
								360	96
Durango Teosinte	4	Diakinesis	46	183	4	4	0	237	19
		Metaphase	4	54	6	0	0	64	6
								301	17
Florida Teosinte	4	Diakinesis	7	37	156	6	2	208	3
		Metaphase	6	32	113	2	0	153	4
								361	4
Florida Teosinte	1	Diakinesis	9	25	2	0	0	36	25
		Metaphase	1	9	0	0	0	10	10
								46	22

fied at diakinesis with respect to the point of association in arm 1 (figure 2) of the translocation complex, 106 were associated only at the end of the arm (figure 4d, e). The remainder of the figures (27 or 20 percent) showed association at a point somewhere in the median region of non-translocated chromosome 9. The point of association was judged by its position to be in the region between the spindle attachment and the translocation point in arm 1 of the complex. In these figures, the chromosomes were also associated at the end of arm 1 of the complex. In most cases the chromosomes appeared to lie more or less parallel between these extreme points of association but in two figures they were opened out between the two points of association to form a figure 8 (figure 4f, g).

When the teosinte chromosome carrying the section which does not cross over with its maize homolog is put in the complex, the diakinesis and metaphase configurations are strikingly different from those observed in pure maize. Somewhat different results were obtained with the two forms of teosinte. The complex including a Durango teosinte chromosome gave ring configurations in 17 percent of the observed cells. The difference between diakinesis and metaphase counts is probably not significant. The diakinesis determinations are easier to make and therefore are probably more reliable. The observed configurations were predominantly chains of 4 at diakinesis and metaphase (table 2). Of 42 chains classified with respect to the position of the longest chromosome of the complex, 41 showed the longest chromosome in a terminal position (figure 4a, b). This shows that either arm 1 or 4 of the complex has opened out to form the chain. Since these plants represented the fourth backcross to maize, non-translocated chromosome 8 almost certainly is a maize chromosome in some of the plants from which counts are recorded. Arm 4 of the complex, being made up of maize chromosome segments, should show association at diakinesis and metaphase similar to that observed in the pure maize control. Arm 1 of the pachytene cross which includes the $y_{g2}-w_x$ region must be the one which opens out to form a chain. A careful study was made of the rings in the plants which included a Durango teosinte chromosome in the translocation complex. The chromosome segments in arm 1 of the complex were usually (38 out of 45 figures) associated only at a point in the median portion of non-translocated chromosome 9 (figure 4h, i, j). The points of association, as far as one could determine, were within the region of arm 1

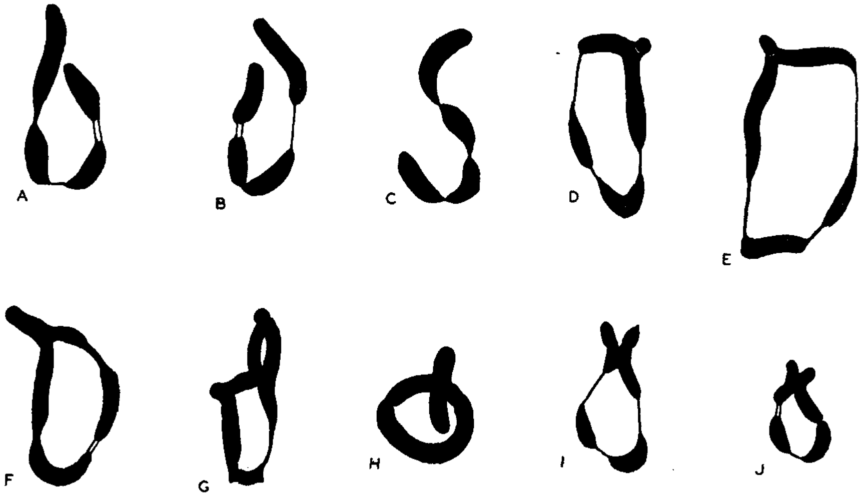

FIGURE 4.—Diakinesis figures of translocation complexes. ×1565. Semidiagrammatic. A, B, Chain of 4 including a Durango teosinte chromosome. C, Chain of 4 including a Florida teosinte chromosome. D, E, Ring of 4 in pure maize control showing end association in arm 1 of the complex which is marked by a terminal knob. F, G, Ring of 4 in maize control showing interstitial association in arm 1 of the complex. H, I, J, Ring complexes, including a Durango teosinte chromosome, showing interstitial association without end association in arm 1 of the complex.

which lies between the spindle attachment and the translocation point. Seven figures showed association of segments in arm 1 of the complex at the ends only. The significance of the region of association will be discussed later.

The translocation complex in four plants with a Florida teosinte chromosome gave ring configurations in 4 percent of the figures (table 2). In contrast to the situation where a Durango teosinte chromosome was put into the complex, the configurations were predominantly two "pairs" (table 2). The members of each "pair" were different in size and were attached only at one end at diakinesis (figure 6a). The smaller member of one "pair" was about equal in size to the larger member of the other "pair." During metaphase the members of these two "pairs" were separated to a greater extent than were the members of the other pairs in the same figure (figure 6b), presumably due to the fact that they were attached at only one end. One of 7 plants studied with a Florida teosinte chromosome in the complex gave predominantly chains of 4 (table 2) as did the Durango hybrids. The longest chromosome of the complex occupied a terminal position in the chain (figure 4c). Association in arm 1 of the complex, when it occurred, was mainly confined to the region about median to non-translocated chromosome 9. As in the Durango hybrids, opening out of the complex into a chain must have been the result of non-association in arm 1 of the complex.

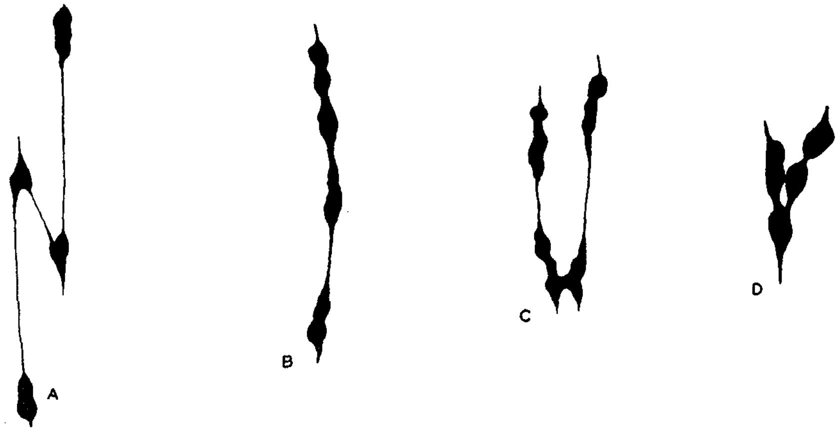

FIGURE 5.—Metaphase translocation chains of 4 including a Durango teosinte chromosome. ×1565. A, Adjacent chromosomes going to opposite poles. B, C, Two types of distribution in which adjacent chromosomes go to the same pole. D, Type of arrangement which may give 3-1 distribution of chromosomes.

In the complexes which gave two "pairs," arm 1 of the complex must have been one of those in which the segments separated. Arm 3 of the complex, then, must have been the other arm in which the segments separated. The reason for non-association of segments in arm 3 of the complex after diplotene is not clear. A section of teosinte chromosome may have been present in this arm though this is improbable if independent segregation of chromosomes in the original hybrid is assumed. A modified maize chromosome segment might have been introduced into arm 3 during the process of backcrossing to maize.

POLLEN STERILITY

Counts show that the heterozygous translocation in pure maize results in about 57 percent of visibly aborted pollen (BURNHAM 1930). Three plants, including in the translocation complex a Durango teosinte chromosome, showed 52 percent of aborted pollen (total number of pollen grains counted—1909). Three plants of similar constitution but including a Florida teosinte chromosome gave 57 percent of aborted pollen (total number of pollen grains counted—2084). The three plants including the Florida chromosome were of the group which gave mainly two "pairs" from the translocation complex. The relation of amount of pollen sterility to chromosome disjunction will be discussed later.

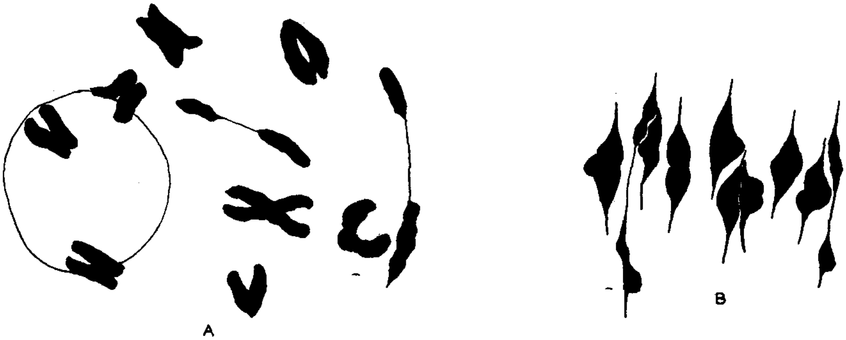

FIGURE 6.—A, Diakinesis showing translocation complex, including a Florida teosinte chromosome, broken up into two "pairs." B, Metaphase showing translocation complex, including a Florida teosinte chromosome, broken up into two "pairs."

DISCUSSION

Cause of reduction in crossing over

The genetic data show that crossing over does not occur or occurs rarely in the $y_{g2}-w_x$ interval, between the teosinte and maize chromosomes. It is possible that crossovers occur which lead to the formation of inviable spores. This would be expected if inversions were present. If such crossovers occur in the region under study, one should observe an amount of sterility equal to the frequency of crossing over. In the Durango hybrids there is a small amount of visible pollen abortion but probably no more than in normal maize stocks. The ears are of such a structure that it is difficult to estimate female sterility. In the Florida hybrids, a fair estimate of female sterility can be made. It is very low, probably not significantly higher than in pure maize. If any crossovers occur between the sections of teosinte and maize chromosomes under discussion, they must be rare.

The cytological observations do not show why the $y_{g2}-w_x$ section of the ninth chromosome of maize crosses over infrequently or not at all with its corresponding section of the teosinte chromosomes. The reduction cannot be due to lack of conjugation. This means that conjugation need not necessarily result in crossing over even where other chromosomes and other parts of the same chromosome show normal crossing over.

In Drosophila many crossover reducers are known to be inverted sections (STURTEVANT 1931a). Cytological observations on teosinte-maize hybrids give no evidence of inversions in the region under study. For two inversions in maize MCCLINTOCK (in press) has shown that reverse conjugation of the inverted section with its normal homologous section frequently occurs. However, other inversions, especially short ones, may not show reverse conjugation frequently enough to be detected cytologically. The inverted sections might well be mechanically pulled parallel and therefore appear conjugated.

Gene differences between the maize and teosinte chromosomes in the $y_{g2}-w_x$ section might perhaps account for the absence of crossing over. Such a gene difference, on SAX's theory of crossing over, might be expected to determine opening out a diplotene in the reductional plane only, which would result in no chiasmas and no crossovers.

Relation of crossing over to chromosome association and disjunction

For the purpose of the present discussion, five phases of the meiotic process are of interest. These are conjugation, chiasma formation, post-diplotene chromosome association, crossing over and chromosome disjunction.

Conjugation is the process of pairing of the chromosome threads as observed at zygotene. It is necessary for crossing over, chiasma formation, association of chromosomes after diplotene and normal disjunction of homologous chromosomes.

Theories of post-diplotene chromosome association

Two hypotheses to account for post-diplotene chromosome association will be considered. BELLING (1927) has postulated the existence of genes which have to do with association of chromosomes after diplotene in forms which show association of homologous chromosomes at the ends. This assumes special properties of chromosome ends which cause homologous ends to remain together after the non-terminal regions have separated at diplotene.

DARLINGTON (1929, 1930, 1931b) assumes that chiasmas (exchanges of partners among paired chromatids) are responsible for post-diplotene chromosome association. If chiasmas remain constant or nearly so in number and position through diakinesis and metaphase, then it is clear from cytological observations that these chiasmas might well be the only factors responsible for chromosome association at such stages. However, in forms in which the chromosomes are associated only or mainly at the ends at diakinesis and metaphase, as is the case in maize, the apparent difficulties of this hypothesis become greater. DARLINGTON assumes that the chiasmas have been reduced in number in such cases by a process of terminalization. The terminalized chiasmas are the agents responsible for the end association of homologous chromosomes. This hypothesis offers no satisfactory explanation of why the chiasmas stop at the ends of the chromosomes instead of slipping completely off and leaving the ends unassociated.

Assuming that something other than chiasmas holds chromosome ends together after terminalization is complete, chiasmas would still seem to be a prerequisite to such association. Cytological observations indicate that ends of homologous chromosomes are no more frequently associated at diplotene than are other points of a chromosome pair (see DARLINGTON 1931b for references to literature). In forms which show end associations at later stages, the simplest interpretation is that the ends are brought together after diplotene by terminalizing chiasmas. Doctor JACK SCHULTZ of this laboratory has suggested that the matrix which is known to surround the chromonemas at diakinesis in many forms (SANDS 1923, KAUFMANN 1926, KUWADA 1927, BABCOCK and CLAUSEN 1929 and others) may be concerned in end association of chromosomes after terminalization of chiasmas. If the matrix appears before terminalization is complete, then after terminalization the matrices which had been separate will become continuous. Final separation of the chromosomes would occur by attenuation and breakage of the matrix material between the two homologs. On this view, the chiasmas could completely terminalize and would not themselves hold the chromosome ends together but would be responsible for connecting homologous ends by making the matrix continuous from one homolog to the other. KUWADA (1927) shows a photograph (plate 2, figure 8) of an end association at metaphase in Tradescantia which is suggestive of the above view. The chromonemas are entirely separate but the matrix appears to be continuous between the homologous ends.

Theories of crossing over

The three current theories of crossing over all agree in assuming a relation between chiasmas and crossing over. JANSSENS' (1924) chiasmatype theory as supported by DARLINGTON (1931b) assumes that for no apparent reason, two of four chromatids undergo crossing over sometime after conjugation and prior to diplotene. BELLING'S (1931) hypothesis provides a mechanism for the process of crossing over. Both of these hypotheses assume that every chiasma is a result of a crossover between two of four chromatids. SAX (1930) assumes, as have many earlier workers (see SAX for references), that chiasmas arise by opening out in different planes at diplotene. On SAX'S theory, crossovers occur by breaking of chiasmas subsequent to the time of their formation by opening out at diplotene. On the basis of the first two hypotheses, if post-diplotene association is the result of chiasma formation, it follows that crossing over is a necessary prerequisite to such association. SAX'S hypothesis is not necessarily inconsistent with DARLINGTON'S contention that chiasmas are responsible for association after diplotene. One must simply assume that not all chiasmas break. Those which do not break can be assumed to be responsible for the persistence of chromosome association. Chromosome association without crossing over, such as is known in the male Drosophila, is possible on such a view whereas it is difficult to reconcile with the view that chiasmas arise only by crossing over and that chiasmas occasion chromosome association after diplotene.

Crossing over and post-diplotene chromosome association

In discussing the data presented in this paper with respect to its bearing on the interrelations of conjugation, chiasma formation, post-diplotene chromosome association, crossing over and chromosome disjunction, it is only necessary to consider arm 1 (figure 2) of the translocation complex. Conjugation takes place in this arm. Let us consider, first, the relation between crossing over and association of chromosomes after diplotene. The data show a definite relation between the two. When crossing over is reduced, association at diakinesis and metaphase is likewise reduced. Crossing over occurs only in the w_x-translocation interval (except possibly at the distal end of the arm beyond y_{g2}). Chromosome association is limited to the region which, so far as can be determined, coincides with the interval in which crossing over is known to occur. Both crossing over and association of chromosomes in this region are approximately equal in frequency to corresponding determinations in the control. The agreement between crossing over and chromosome association constitutes definite evidence that the two are related.

Chiasmas and post-diplotene association of chromosomes

There is no direct cytological evidence that the associations observed in the present study between homologous chromosome segments were chiasmas. However, there is cytological evidence in many forms which indicates that interstitial points of association between homologous chromosomes during and after diplotene are chiasmas (see BELLING 1931 and DARLINGTON 1930, 1931b for discussion and references to literature). Wherever the methods have been adequate, such interstitial points of association have been shown to be real chiasmas. The interstitial nodes or points of association in maize are therefore assumed to be chiasmas. If so, it follows that chiasmas are related to both crossing over and post-diplotene association of chromosomes.

DARLINGTON (1930) has studied frequency of association of whole chromosomes and of fragments in *Fritillaria imperialis*. He found that frequency of association during and after diplotene was approximately proportional to length of chromosome. This relation is offered as evidence in favor of his hypothesis that association after diplotene is occasioned by chiasmas. The case by no means constitutes a critical test of the theory. The observed result could equally well be predicted on the assumption that association is due to something other than chiasmas and is reduced in frequency in short fragments because of reduced frequency of conjugation. Pachytene pairing was not studied in the case of the fragments.

Chiasmas and end association of chromosomes

The relation between interstitial chiasmas and chromosome association has been discussed. The bearing of the observations presented above on the relation of chiasmas and end associations of homologous chromosomes will now be considered. The ends of chromosome segments in arm 1 of the translocation complex including a teosinte chromosome were not often associated during diakinesis and metaphase. There was some crossing over and chiasma formation in this arm but both were, for the most part, limited to the region between the spindle attachment and the translocation point. The spindle attachment presumably prevents movement of chiasmas through it (argument by analogy with Drosophila where the first meiotic division is almost certainly reductional for the spindle attachment [BRIDGES and ANDERSON 1925, ANDERSON 1925, MORGAN 1925, ANDERSON 1929, REDFIELD 1930, RHOADES 1931 and STURTEVANT 1931b]). Cytological observations on the method of conjugation and opening out of translocation complexes in maize (McCLINTOCK 1930, COOPER and BRINK 1931) show that opening out occurs in the reductional plane at the translocation point. Chiasmas would therefore be unable to terminalize past such a point (see also DARLINGTON 1931a). In the translocation complex discussed in this paper, the chiasmas in arm 1 between the spindle attachment and the translocation point are unable to terminalize. Since there is little or no crossing over between the spindle attachment and the end of the short limb of the chromosome, few or no chiasmas would be expected in this region. If chiasmas are necessary for end association, few or no such associations would be observed. The data are consistent with this expectation. This does not necessarily mean that end associations are chiasmas. It was suggested above that terminalizing chiasmas may result in a continuous matrix between homologous ends and thereby be only indirectly responsible for end associations.

Bearing of the data on theories of crossing over

The data on relation of crossing over and chromosome association are not inconsistent with any of the three theories of crossing over outlined above. Either JANSSENS' or BELLING'S theory, assuming chiasmas to be responsible for post-diplotene association of chromosomes, leads to a definite relation between crossing over and frequency of association of chromosomes. Since each chiasma is the result of a crossover between two of four chromatids, short segments of chromosomes should be associated after diplotene with a frequency equal to twice the frequency of crossing over. The genetic data indicate that the crossovers in the w_x-translocation interval in the complexes including a teosinte chromosome are about as frequent as in pure maize. The control value is about 12 percent (BURNHAM 1930 and unpublished). The frequency of association of homologous segments at diakinesis (the most reliable determination) in the same arm of the complex was about 20 percent. The frequency of association expected from the crossover data is 24 percent. The agreement is fairly good.

As pointed out above, SAX'S theory can be reconciled with the view that chiasmas are responsible for chromosome association after diplotene. If put on this basis, this theory does not require a definite relation between frequency of crossing over and frequency of association. The fact that a fair agreement was found between observed association and the expectation on JANSSENS' or BELLING'S hypotheses does not disprove SAX'S theory. On SAX'S hypothesis, if chiasmas are formed between two points where opening out occurs in the reductional plane, at least two must be formed. Both need not break, in which case an agreement with the observations discussed above, though improbable, is possible.

Association and disjunction of chromosomes

The reciprocal 8–9 translocation in maize which was made use of in the present study results in about 57 percent of aborted pollen (BURNHAM 1930). MCCLINTOCK (1930) has shown that there are formed the six possible types of spores expected from random distribution of the four chromosomes in the ring. The amount of sterility indicates, however, that they are not formed in the frequencies expected on random distribution. MCCLINTOCK assumes that adjacent chromosomes go to the same pole in approximately half the cases. This accounts for the observed sterility.

The translocation complexes which were broken up into a chain of 4 by the introduction of a teosinte chromosome gave 52 percent aborted pollen. Metaphase figures were observed (figure 5) which showed that the six possible types of spores were formed. As in the ring of 4, adjacent (or end) chromosomes in the chain must go to the same pole in approximately half the cases. The slightly lower sterility may be due to there being more 3–1 distributions in the chain than in the ring (figure 5d). In the translocation complex broken up into two "pairs" the sterility was approximately the same as in the case of the ring of 4 (57.0 and 56.9 percent respectively). The possible types of spores are here reduced from six to four. The expected sterility is 50 percent since the four types of spores should be formed in equal numbers, two fertile and two sterile. The observed increase over the expected 50 percent may or may not be significant.

If, as has been argued, the breaking up of the translocation complex into two "pairs" is the result of non-association of segments in arms 1 and 3 (figure 2) then homologous spindle attachments and segments of both chromosomes 8 and 9 must undergo non-disjunction in about 50 percent of the meiotic divisions. Homologous spindle attachments are distributed approximately at random with respect to each other. This shows clearly that spindle attachment homology, in itself, need have nothing to do with chromosome disjunction. A similar conclusion was arrived at by DOBZHANSKY and STURTEVANT (1931) in a study of translocations in Drosophila. The relation of crossing over to association and disjunction of chromosomes is clear in this translocation complex which breaks up into two "pairs." ANDERSON (1929), DOBZHANSKY (1932), and REDFIELD (unpublished) have shown that chromosomes which cross over with high (normal) frequency usually go to opposite poles at the first meiotic division whereas chromosomes which undergo little or no crossing over frequently go to the same pole (non-disjunction). GOWEN (1928, 1931) has reported a case in Drosophila in which absence of crossing over in the female is accompanied by a high frequency of non-disjunction of all chromosomes. The facts presented in the present paper make it clear that reduced crossing over and high non-disjunction are not necessarily due to reduced frequency of conjugation of the chromosome segments concerned. The Drosophila data are entirely consistent with the view that normal disjunction is dependent on post-diplotene association of chromosomes which is dependent on chiasma formation which, in turn, is related (either as cause or effect) to crossing over.

SUMMARY

Crossing over in the $y_{g2}-w_x$ interval (about 45 units) between chromosome 9 of maize and its homologs from Durango and Florida forms of annual teosinte occurs infrequently or not at all. Crossing over occurs in the w_x-v_1 interval (about 8 units long) in the hybrids. Since the spindle attachment is probably close to w_x, it is assumed that crossing over between maize and teosinte chromosome 9 is confined to the long limb.

Lack of crossing over in the shorter limb between maize and teosinte chromosome 9 cannot be accounted for by lack of conjugation. It is concluded from this that conjugation or apparent conjugation does not necessarily lead to crossing over even in a form which shows normal crossing over in other chromosomes and other parts of the same chromosome. The reduction in crossing over may be due to a segment or segments of the short limb of chromosome 9 being inverted in one species with respect to the arrangment in the other. There is no direct cytological evidence in favor of this assumption. The reduction in crossing over may be due to general gene differences in this region which are not accompanied by differences in sequence. Such differences might inhibit crossing over without observably affecting conjugation.

In arm 1 (figure 2) of the translocation complex including a chromosome from teosinte, crossing over has been shown genetically to be mainly confined to the region near the translocation point. Association of chromosomes after diplotene in this arm of the complex is mainly confined to the region in which there is crossing over, presumably because chiasmas in this region cannot terminalize. Furthermore, this association is about equal to twice the frequency of crossing over. This relation supports DARLINGTON'S

hypothesis that chiasmas are responsible for post-diplotene chromosome association.

None of the three theories of crossing over considered are necessarily inconsistent with the data reported.

A case of independent assortment of homologous spindle attachments and chromosome segments at meiosis is reported. It is concluded that spindle attachment homology, in itself, has nothing to do with chromosome disjunction.

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