A PROOF THAT CROSSING OVER INVOLVES AN EXCHANGE OF SEGMENTS BETWEEN HOMOLOGOUS CHROMOSOMES[1]

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In his early work on Drosophila, MORGAN (1911) suggested that crossing over results from an exchange of parts between homologous chromosomes at some stage in the reduction divisions. This became at once a highly useful conception in genetics and, while definite proof for the view was lacking, a large amount of evidence accumulated which pointed strongly to the correctness of the assumption. Only recently has it been possible, however, to subject the idea to a decisive test. In a comprehensive study, STERN (1931) has demonstrated clearly that in Drosophila crossing over involves an interchange of chromosome segments. He examined genetically and cytologically the offspring of a race of flies which carried two translocations affecting the sex chromosomes. In one of the translocations, the X chromosome had been broken in two and one piece had become attached to the small fourth chromosome; in the other, the long arm of the Y chromosome was shifted to one end of the X chromosome. Two loci were marked by heterozygous genes in the segment of the X chromosome which had been rendered heteromorphic at both ends by the translocations. STERN examined 364 flies, of which 359 showed the correspondence in genetic composition and chromosome configuration expected on the Morgan hypothesis. Five exceptions were attributed to errors in classification. Using a stock of *Zea mays* carrying a reciprocal translocation between chromosomes 8 and 9 in which one arm of the ninth chromosome carrying the genes *c* and *wx* was differentiated from its mate by a terminal knob, CREIGHTON and MCCLINTOCK (1931) present evidence suggesting that crossing over between the two loci mentioned is accompanied by an exchange of chromosome segments in the arm marked by the knob. Only three individuals of the class which, if adequately sampled, affords a definitive test of the hypothesis, namely, plants which are crossovers in the region marked by genetic factors, were examined cytologically by these workers. These three plants conformed to expectation. Other results of a less decisive nature are also given.

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MATERIALS AND METHODS

In the present study use was made of a strain of maize homozygous for two reciprocal translocations involving opposite ends of chromosome 1[2] (*P–br* linkage group).[3] The origin of the stock has been described in an earlier publication (BRINK and COOPER, 1932). One end of the *P–br* chromosome in the doubly interchanged race is replaced by a terminal segment of chromosome 2 (*B–lg* linkage group). Earlier designated semi-sterile–1, the translocation is now named T1–2a. The other end of chromosome 1 has been exchanged for a distal segment of chromosome 6 (*Y–Pl* linkage group), the latter chromosome being distinguished from the other members of the set by its attachment to the nucleole. This translocation, formerly known as semisterile–5, is now designated T1–6a. Chromosomes 2 and 6 are correspondingly modified.

In the T1–2a reciprocal translocation, chromosome 1 was broken between a point near *ad* (adherent) and the locus of *gs* (green-striped) as shown by BRINK and COOPER (1931, 1932). In reciprocal translocation T1–6a, the break in chromosome 1 occurred between *P* (pericarp color) and *br* (brachytic). Evidence in addition to that submitted earlier by BRINK and COOPER (1932) for this conclusion is given in table 1, where

TABLE 1

Results of the backcross $\frac{(\mathrm{T1\text{–}6})\mathrm{a}\ br\,f}{+\ \ ++}\times\frac{+\ br\,f}{+\ br\,f}$.

PROGENY NO.	T1-6a/+				+/+			
	Br F	*br F*	*Br f*	*br f*	*Br F*	*br F*	*Br f*	*br f*
R855	82	0	1	4	6	2	0	60
R856	35	0	2	4	3	2	0	25
Totals	117	0	3	8	9	4	0	85

the results of backcrosses of two plants heterozygous for the translocation, *br* and *f* (fine-striped) to normal, *br f* are presented. The amount of crossing over between T1–6a and *br* is 7.5 percent; that between T1–6a and *f* is 10.6 percent; and that between *br* and *f* is 3.1 percent. The order is clearly T1–6a–*br–f*, although the crossover values are considerably lower than those obtained earlier. Two additional families containing 168 plants from the backcross $\frac{\mathrm{T1\text{–}6a}+}{+ts_2}\times\frac{+ts_2}{+ts_2}$ gave 11.8 percent crossing over between

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[2] In numbering the chromosomes the system adopted by EMERSON (Proc. Fourth Int. Genetics Congress, 1: 141–152) is followed.

[3] This statement is not intended to imply that the two translocations are necessarily in different arms of the chromosome.

T1–6a and tassel seed–2. This value, likewise, is much lower than those obtained in two of the three progenies of like breeding reported in a former publication (BRINK and COOPER 1932). The cause of these variations in amount of crossing over is not known. For our present purposes, however, it is necessary only to know the order of the genes with reference to the point of break in the chromosome. This appears to be definitely established and is shown in figure 1. It has been found previously (BRINK and COOPER 1931) that the order of the *br* and *f* genes in relation to the other translocation involved is *br*–*f*–T1–2a, with *ad* very close to T1–2a.

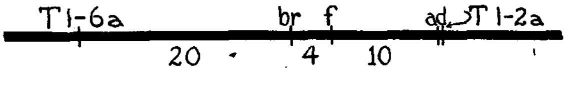

FIGURE 1.—A chromosome map of the interstitial region based on the crossover values obtained in the total population of which the individuals examined cytologically are a sample.

The loci of the *br* and *f* genes, therefore, lie between the two points of break in the chromosome. No undoubted case of crossing over between the T1–2a translocation and *ad* has been observed. Apparently the locus of this gene is near the point where chromosome 1 was broken. The factor is tentatively mapped as being left of the break; it might with equal justification be placed in a corresponding position to the right. The fact is that thus far (with two possible exceptions as noted later) T1–2a and *ad* have been found to assort together.

When the homozygous doubly interchanged line is crossed with normal maize the hybrids, $\frac{\text{T1–2a T1–6a}}{+ \quad +}$, are partially sterile and show at diakinesis a ring of 6 chromosomes attached to the nucleole and 7 bivalents. In prophase, a six-armed chromosome structure is found (BRINK and COOPER 1931). Only about 35 percent of the pollen appears to be functional, and seed production is correspondingly lowered. The high sterility is due presumably to the formation of many microspore and macrospore nuclei deficient in one or more chromosome segments involved in the translocations. When plants heterozygous for the two translocations in coupling series are used as pollen parents in crosses with normal maize four kinds of offspring, with reference to the structural relations of the chromosomes, are produced. These show the following respective configurations at diakinesis: (1) ring of 6 chromosomes attached to the nucleole plus 7 bivalents; (2) ring of 4 chromosomes attached to the nucleole plus 8 bivalents; (3) ring of 4 chromosomes free in the nucleus plus 8 bivalents; and (4) 10 bivalents (see figures 2 and 3). In the reciprocal cross, where the partially sterile plant serves as the pistillate parent, the same four classes of offspring result; and in addition a small proportion (about eight percent) of plants is produced with more, or sometimes possibly less, than the normal complement of chromatin. Many of these hyperploids or hypoploids may be detected on the ear as undersized seeds. The plants themselves are frequently atypical in form and irregular in fertility, consequent upon their abnormal chromosome make-up. As is commonly the case, it is largely macrospores of these exceptional types which function,

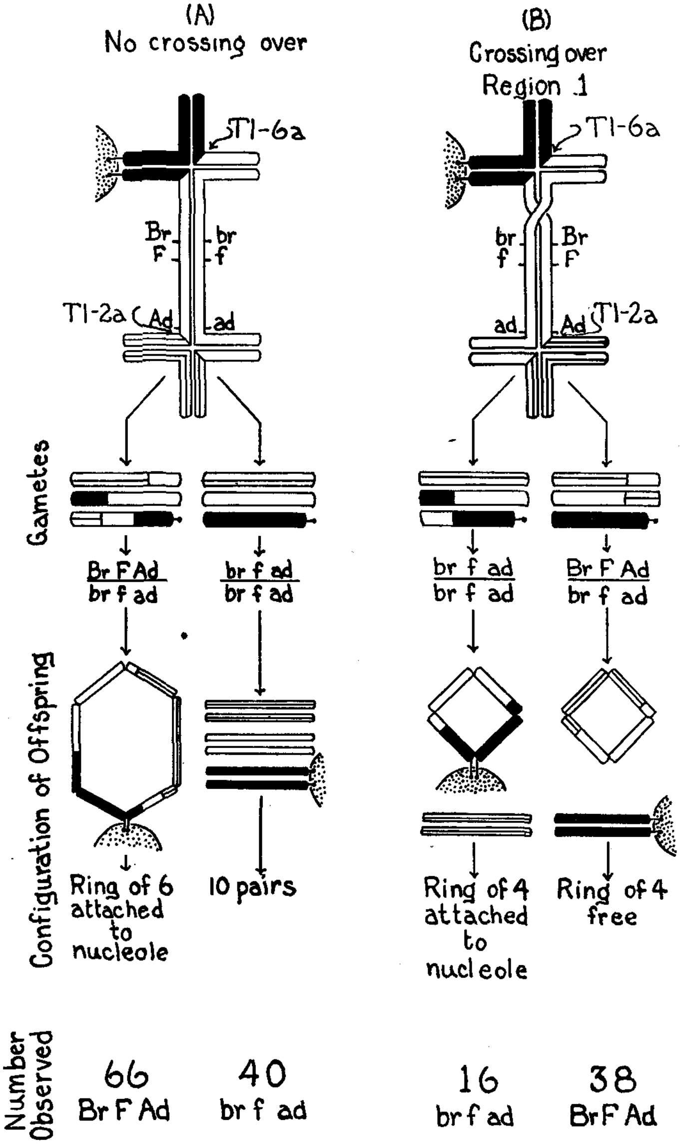

FIGURE 2.—Diagrammatic representation of the cytogenetic relations expected among the offspring of the backcross in which crossing over is not genetically detectable.

the corresponding microgametophytes frequently failing of complete development.

In the present consideration the exceptional aneuploid plants need not be discussed further. The origin of the other four types of plants differing in the structural relations of their chromosomes, however, is directly re-

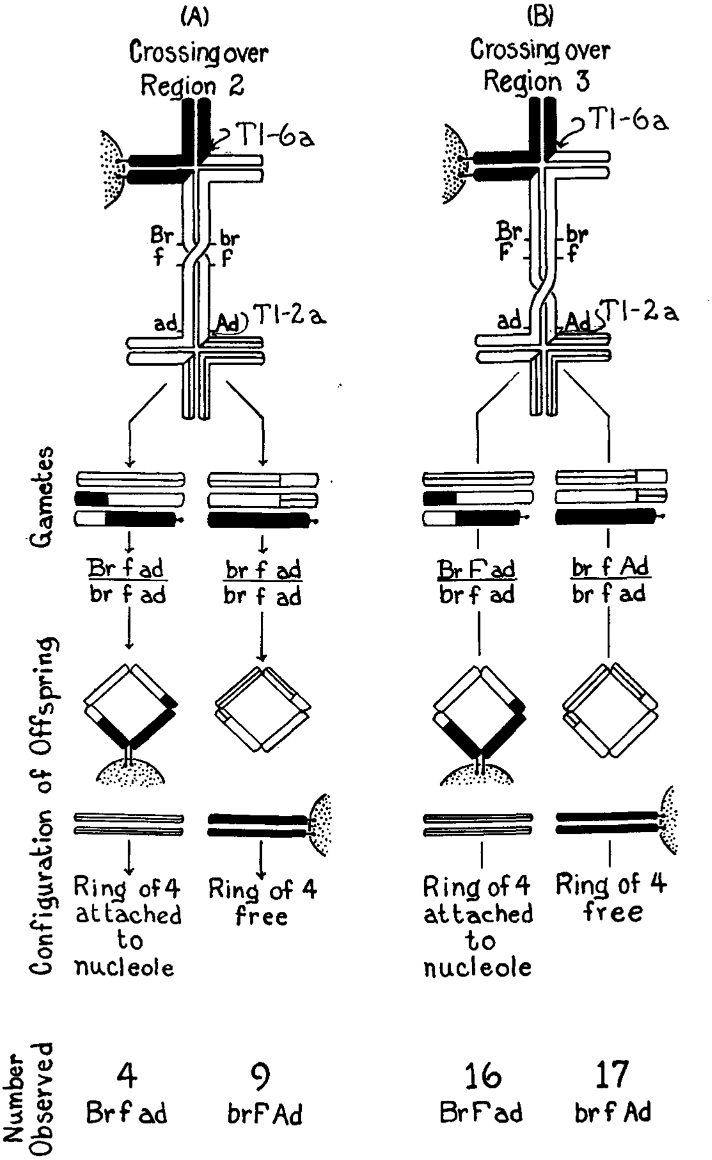

FIGURE 3.—Diagrammatic representation of the cytogenetic relations expected among the offspring of the backcross when genetic crossing over occurs (A) between the *br* and *f* loci and (B) between the *f* and *ad* loci.

lated to the problem in hand. One of the chromosomes in the six-membered ring in $\frac{\text{T1–2a T1–6a}}{+ \quad +}$ plants consists of parts of what, in normal individuals, are three different chromosomes. One end of this tripartite member of the interchanged set is homologous with one end of chromosome 2; the other end is homologous with the distal portion of chromosome 6. The interstitial segment of the modified chromosome, on the other hand, corresponds with the middle part of chromosome 1. These relations are depicted in figure 2. It is the behavior of this interstitial segment in plants heterozygous for the two reciprocal translocations which, in matings with normal individuals, determines the mode of association of the chromosomes in the offspring. If an exchange of segments (cytological crossing over) does not occur in the region between the two points of break, and alternate chromosomes go to the same pole in the reduction divisions, two types of gametes may arise which, in conjunction with normal sperm or eggs, will produce individuals showing (1) a ring of 6 chromosomes attached to the nucleole plus 7 bivalents, and (2) 10 bivalents. In other words, gametes are formed corresponding to the two structural types which in association with each other give the configuration of the hybrid itself. If, on the other hand, a single exchange of chromosome segments occurs in the interstitial region, and the meiotic distribution of the chromosomes is such that the resulting macrospore and microspore nuclei receive full complements of chromatin, the two translocations are separated from each other and pass into different nuclei. The resulting chromosome complements in apposition to normal sets of chromosomes will produce individuals showing at diakinesis, (1) a ring of 4 chromosomes attached to the nucleole plus 8 bivalents, and (2) a ring of 4 chromosomes free in the nucleus plus 8 bivalents.

It is thus possible among the offspring of plants heterozygous for these two translocations to separate the individuals which have resulted from cytological crossing over in the interstitial segment from those which have not, and, furthermore, to identify the two complementary crossover classes when an exchange of chromosome parts occurs. The latter determination is rendered possible by the fact that the chromosome ring resulting from the T1–2a translocation lies free in the nucleus while that from the T1–6a translocation is attached to the nucleole. If a plant is made heterozygous for the two translocations and for two or more genes in the interstitial segment of the doubly modified chromosome and then backcrossed to the recessive type with normal chromosome structure one may determine the relation between exchange of chromosome parts in the region in question and genetical crossing over by cytological examination of the different classes of segregates.

Individuals homozygous for the two translocations T1–2a and T1–6a and carrying the dominant genes, *Br*, *F* and *Ad* were crossed with *br f ad* plants of normal chromosome composition. The partially sterile hybrids were backcrossed with a *br f ad* stock of normal chromosome structure. The results of a combined cytological and genetical study of 211 offspring from the backcross are presented in table 2. In obtaining family R900, the partially sterile hybrid was used as the pistillate parent in the backcross. The remaining families, R901 to R907, were derived from the reciprocal combination. The findings are essentially alike in the two cases and are combined in the totals.

TABLE 2

Cytogenetic classification of 208 offspring from the backcross $\frac{\mathrm{T(1\text{–}6)a}\ +++\ \mathrm{T(1\text{–}2)a}}{+\quad br\ f\ ad\quad +}\times\frac{+br\ f\ ad+}{+\,br\,f\ ad+}$.

PROGENY NUMBER	NON-CROSSOVERS: *br—ad* REGION				CROSSOVERS: *br—ad* REGION			
	Br F Ad		*br f ad*		*Br f ad*	*br F Ad*	*Br F ad*	*br f Ad*
	RING OF 6 ATTACHED TO NUCLEOLE AND 7 BIVALENTS	RING OF 4 FREE IN THE NUCLEUS AND 8 BIVALENTS	10 BIVALENTS	RING OF 4 ATTACHED TO NUCLEOLE AND 8 BIVALENTS	RING OF 4 ATTACHED TO NUCLEOLE AND 8 BIVALENTS	RING OF 4 FREE IN NUCLEUS AND 8 BIVALENTS	RING OF 4 ATTACHED TO NUCLEOLE AND 8 BIVALENTS	RING OF 4 FREE IN NUCLEUS AND 8 BIVALENTS
R900*	15	5	11	2	1	2	6	3
R901	12	5	8	3	0	0	1	3
R902	6	3	1	3	0	0	2	1
R903†	3	6	2	2	1	3	2	3
R904	8	5	7	0	2	2	3	4
R905	9	4	5	2	0	1	1	1
R906‡	5	2	4	1	0	0	0	1
R907	8	8	2	3	0	1	1	1
Totals	66	38	40	16	4	9	16	17
	160				46			

* This progeny also contained 2 *Br f Ad* plants which possessed a ring of 6 chromosomes attached to the nucleole and 7 bivalents.

† This progeny also contained 1 *br f Ad* plant with a ring of 4 chromosomes attached to the nucleole plus 8 bivalents, and 1 *br f Ad* plant with 10 pairs of chromosomes. These two individuals are apparent exceptions.

‡ This progeny also contained 1 *Br F ad* plant which possessed 10 bivalent chromosomes.

ACCURACY OF CLASSIFICATION

Classification in the field for the *Br*, *br* pair of characters seldom presents any difficulty, and it is considered very unlikely that there are any errors in the table in regard to them. Fine-striped, likewise, is usually distinct from the allelic condition. Occasionally, however, the striping in a plant of this class is so reduced as to be seen only as a very narrow chlorophyll-deficient line at the base of a single leaf blade. But there are very few individuals which do not give at least this much evidence of their composition with respect to the *F*, *f* pair. To check the accuracy of the field classification for fine-striped, pollen from nine plants which were classified as *br F*, that is, as apparent crossovers between these two loci, in the families on which table 2 is based, was applied to homozygous fine-striped individuals. The offspring were tested in the greenhouse. Under the reduced light conditions of winter particularly, fine-striped occasions a marked reduction in chlorophyll development in the seedling stage. All nine plants were found to have been correctly classified as *F*. Another individual similarly tested which had been marked in the field as "doubtfully *f*" was shown to be *F*. The degree of accuracy attained in the classification of the *F*, *f* pairs, therefore, would appear to be satisfactory.

The adherent character is manifested more or less conspicuously at two stages in the development of the plant. In adherent seedlings, the laminae of two or more leaves are usually, although not always, stuck together. If considerable elimination of plants of this type is to be avoided in the early stages of growth the leaves must be parted by hand. At the flowering stage the branches of the tassel in typical adherent individuals are closely appressed in the direction of the main axis and more or less firmly held together by a mucilaginous substance. By marking the plants in the seedling stage and checking them again at flowering time most individuals are readily enough classified for adherent. Neither criterion, however, is infallible, and occasional individuals are found in which it is not certain whether either the seedling leaves are actually attached or the tassel branches stuck together. Attempts were made in these cases to secure outcrosses to homozygous adherent. The only stock available to the writers for that purpose at the time was also carrying brachytic and fine-striped and was weak and comparatively unfruitful. Consequently most of the outcrosses failed. No check is available, therefore, on the classification of the two plants in progeny R903 listed in the table as possible exceptions, the doubtful point being whether they were actually non-adherent. It is believed that the other entries in table 2 are correct in this regard.

CYTOLOGICAL PROCEDURE

As in previous studies (Brink and Cooper 1931) aceto-carmine smears of pollen mother cells were used in determining the chromosome configurations. Attention was directed particularly to diakinesis figures. The requirement for a study of this kind that indisputable morphological differences in the chromosome complements of the four expected classes of plants be detectable with the microscope is amply met. Each of the groups is distinguished by an easily recognizable cytological feature which is diagnostic. As pointed out above, one non-crossover class (with reference to the interstitial segment) is unique in having a ring of 6 chromosomes attached to the nucleole, plus 7 bivalents; the other has a normal complement of 10 bivalent chromosomes. The two crossover classes have rings of 4 chromosomes, which are not only smaller than the ring occurring in the first mentioned non-crossover class but are accompanied by 8 rather than 7 bivalents. The crossover classes are no less sharply distinguishable from each other by virtue of the fact that in one the ring of 4 chromosomes is always attached to the nucleole whereas in the other it lies free in the nucleus.

EXPECTED CYTOGENETIC RELATIONSHIPS

The expected relationships among the offspring of the backcross $\frac{\mathrm{T1-6a}\ +\ +\ +\ \mathrm{T1-2a}}{+\ br\ f\ ad\ +} \times \frac{+\ br\ f\ ad\ +}{+\ br\ f\ ad\ +}$ are illustrated in figures 2, 3, and 4. There are three regions in the interstitial segment in which crossing over occurs with measurable frequency. The amounts of crossing over in these as computed on unpublished data from the total population of which the individuals studied cytologically are a sample are shown in figure 1 and are as follows: (1) T1–6a–*br*, 20 percent; (2) *br–f*, 4 percent; (3)*f–ad* (T1–2a)10 percent. As mentioned earlier, crossing over between *ad* and T1–2a has not been proved. These points are considered together, therefore, as marking one end of the interstitial region.

It should be noted, perhaps, that the entries in table 2 do not represent random samples of the eight progenies used. In collecting material for cytological study an effort was made to get as large a representation as possible of plants which were genetically crossovers since the results on these individuals were expected to be definitive. Likewise, the genetically non-crossovers, *Br F Ad*, and *br f ad*, are weighted in the T1–2a and T1–6 classes.

EXPERIMENTAL RESULTS

If the chromosomes cross over in region 1, that is, between T1–6a and *br*, the original relations between the *Br br*, *F f* and *Ad ad* genes are maintained (figure 3). While the event is not detectable genetically, therefore, it is reflected in a change in the amount of pollen abortion in the crossover plants. In contrast with about 35 percent functional pollen and all functional pollen in the two respective non-crossover types of offspring, the crossovers show 50 percent empty grains. The part of the interstitial region not marked with genes is continuous and at one end because of the near coincidence of the *ad* locus with the point of break in the T1–2a translocation. In view of this fact, a definite relation is expected on the Morgan theory of crossing over between chromosome configuration and genetic composition of these two classes of plants. As illustrated in figure 2, all *Br F Ad* individuals by hypothesis should show either a ring of 6 chromosomes plus 7 bivalents or a ring of 4 chromosomes free in the nucleus and 8 bivalents. Referring to table 2 it may be seen that of the 104 *Br F Ad* plants examined cytologically 66 were of the first kind and 39 of the second. Similarly, all *br f ad* segregates should possess either 10 bivalent chromosomes or a ring of 4, in this case attached to the nucleole, and 8 bivalents (figure 2). Of the 56 *br f ad* plants studied 40 were of the first type and 16 of the second. Expectation in these two groups of plants, therefore, is fully met.

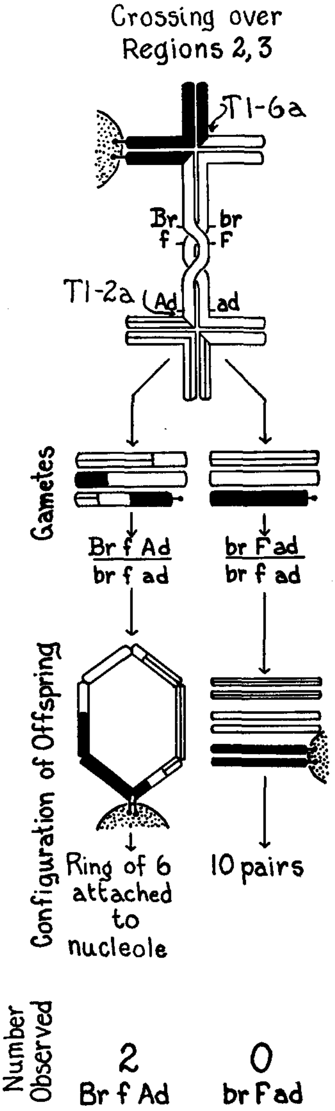

FIGURE 4.—Diagrammatic representation of the cytogenetic relations expected among the offspring of the backcross when genetically detectable double crossing over occurs in the interstitial region.

Thirteen plants which were genetically crossovers between the *br* and *f* loci were examined cytologically (table 2). Reference to figure 3 (A) will show that the *Br f ad* class is expected to have at diakinesis a ring of 4 chromosomes attached to the nucleole and 8 bivalents. The 4 *Br f ad* plants studied showed this configuration. The complementary crossover class, *br F Ad* on the other hand, should possess a ring of 4 chromosomes free in the nucleus plus 8 bivalents. The 9 plants showing the *br F Ad* characters which were tested all showed this configuration.

The structural character of the chromosome complement was determined for 33 plants which were genetically crossovers between the *f* and *ad* loci. The results are given in table 2. As figure 3 (B) illustrates, *Br F ad* plants are expected to possess a ring of 4 chromosomes attached to the nucleole plus 8 bivalents. Of the 17 *Br F ad* plants examined, 16 fulfilled this expectation; one plant did not conform, and is considered further below. The complementary crossover class, *br f Ad*, should show a ring of 4 chromosomes free in the nucleus and 8 bivalents. Of the 19 *br f Ad* plants studied, 17 were of this configuration. There were two apparent exceptions which are discussed below.

It is thus seen that 46 plants which were genetically detectable single crossovers in the interstitial region afford direct evidence, in the structural relations of their nuclear complements, of an exchange of chromosome segments.

The four types of chromosome complements found in the backcross population are illustrated in figure 5.

In progeny R900, two *Br f Ad* plants occurred. These are genetically double crossovers and, by hypothesis, as illustrated in figure 4, should show a ring of 6 chromosomes attached to the nucleole and 7 bivalents. Both individuals exhibited this configuration.

The one *Br F ad* plant in progeny R906 which possessed 10 bivalent chromosomes rather than the ring of 4 attached to the nucleole plus 8 bivalents as in other plants of this class may, likewise, be a double crossover. Its genetic make-up shows that crossing over occurred in the *f–ad* region. If a second crossover occurred between the T1–6a translocation and the *br* locus, an event which might be expected occasionally, its chromosome complement is that expected by hypothesis.

POSSIBLE EXCEPTIONS

In progeny R903 two plants occur which are possible exceptions. To account for a *br f Ad* plant showing a ring of 4 chromosomes attached to the nucleole plus 8 bivalents, it is necessary to assume three simultaneous crossovers, one of which is between the *ad* locus and the point of break in the T1–2a translocation. As pointed out earlier, no crossovers of the latter kind have been found previously, although they have been looked for in a few families comprising several hundreds of plants. The other apparently exceptional plant was classified as *br f Ad* and showed 10 bivalent chromosomes. Such an individual could arise from simultaneous crossing over between *f* and *ad* and between *ad* and the T1–2a translocation. The same doubt consequently arises here, although possibly the case is regular. A more likely explanation, however, is that both these plants were misclassified as non-adherent. If they were genetically adherent they would fall into two of the expected categories. Unfortunately outcrosses to test the correctness of their classification failed.

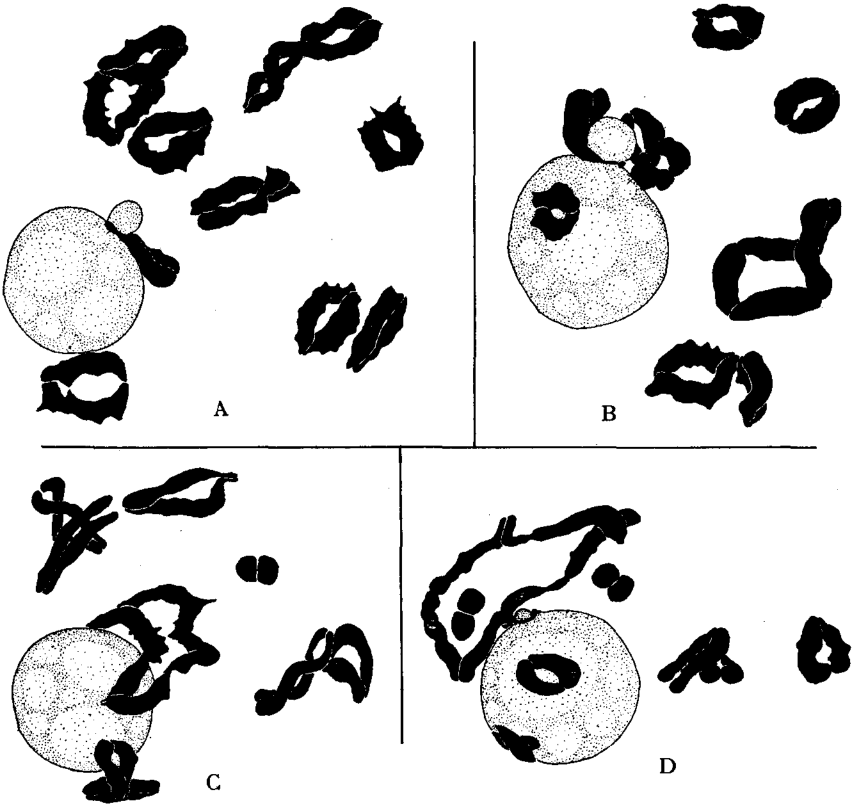

FIGURE 5.—Diakinesis figures of (A) normal plants, showing ten bivalent chromosomes, (B) plants carrying the T1–2a translocation, showing a ring of 4 chromosomes free in the nucleus and 8 bivalents, (C) plants carrying the T1–6a translocation, showing a ring of 4 chromosomes attached to the nucleole and 8 bivalents, and (D) plants carrying both the T1–2a and T1–6a translocations, and showing a ring of 6 chromosomes attached to the nucleole and 7 bivalents.

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SUMMARY AND CONCLUSION

1. The requirements for a test of the relationship between exchange of corresponding chromosome segments and genetic crossing over were found in a strain of *Zea mays* carrying two reciprocal translocations involving opposite ends of chromosome 1. One of the translocations, T1–6a, represents an interchange with a part of the distal arm of chromosome 6 which is attached proximally to the nucleole; the other, T1–2a, involves chromosome 2, which is not attached to the nucleole. A plant carrying translocation T1–6a only, shows at diakinesis of the pollen mother cell a ring of 4 chromosomes attached to the nucleole and 8 bivalents. Individuals possessing the T1–2a translocation alone, on the other hand, form a ring of 4 chromosomes free in the nucleus and 8 bivalent chromosomes. When both translocations are present simultaneously a ring of 6 chromosomes attached to the nucleole and 7 bivalents occurs.

2. A stock carrying both reciprocal translocations, T1–2a and T1–6a, was crossed with a normal race possessing 10 bivalent chromosomes and carrying the three recessive genes *br* (brachytic), *f* (fine-striped) and *ad* (adherent) in the portion of chromosome 1 corresponding to the interstitial segment in the doubly interchanged line. The order of the marked points was known from previous studies to be T1–6a–*br*–*f*–*ad* T1–2a, no crossing over having been found between the latter two. The partially sterile hybrids, $\frac{\text{T1}-6\text{a} + + + \text{T1}-2\text{a}}{+\ br\ f\ ad\ +}$, were backcrossed to the normal, *br f ad* line, and the genetic composition and chromosome configuration of 211 offspring determined.

3. On the hypothesis that genetic crossing over involves the exchange of chromosome segments, the *Br F Ad* segregates from the backcross should show cytologically either a ring of 6 chromosomes attached to the nucleole plus 7 bivalents or a ring of 4 free in the nucleus plus 8 bivalents. Of the 104 *Br F Ad* plants examined 66 were of the former type and 38 of the latter. Similarly, the *br f ad* segregates should possess either 10 bivalents or a ring of 4 chromosomes attached to the nucleole plus 8 bivalents. Fifty-six *br f ad* individuals were studied cytologically, and 40 were found to be in the first category and 16 in the second.

4. Single crossing over in the portion of the interstitial segment between the *br* and *f* loci is detectable genetically and should give (1) *Br f* plants

possessing a ring of 4 chromosomes attached to the nucleole plus 8 bivalents, and (2) *br F* individuals showing a ring of 4 free in the nucleus and 8 bivalents. Four plants of the first class and 9 of the latter met these requirements. There were no exceptions.

5. Crossing over in the *f–ad* region is likewise reflected in the offspring of the backcross and, on hypothesis, should result in (1) *F ad* plants showing a ring of 4 attached to the nucleole plus 8 bivalents and (2) *f Ad* individuals having a ring of 4 chromosomes free in the nucleus plus 8 bivalents. Of the 17 *F ad* plants studied cytologically 16 agreed with this expectation and one possessed 10 bivalent chromosomes. The latter may have been a crossover in the T1–6a–*br* region also and, if so, it is consistent with theory. Chromosome configurations were determined on 19 *f Ad* segregates. Seventeen showed rings of 4 chromosomes free in the nucleus plus 8 bivalents as expected, and two appeared to be exceptions. It is considered not unlikely that these apparent exceptions were incorrectly classified for the *Ad, ad* pair of characters (see text).

6. Two double crossover plants, *Br f Ad,* were found to have rings of 6 chromosomes attached to the nucleole and 7 bivalents as expected.

7. In summary, 46 plants which showed genetic crossing over between the *br* and *ad* loci possessed the particular chromosome configurations called for by theory. Two possible exceptions may have been due to errors in classification. The 160 individuals of the two complementary noncrossover classes, *Br F Ad* and *br f ad,* likewise, fell into one or the other of the two respective cytological classes expected for each of them.

8. With doubt attaching to but two plants in 211, the analysis affords convincing proof of the correctness of MORGAN'S view that genetic crossing over involves the exchange of segments between corresponding chromosomes.

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