CYTOGENETIC STUDIES OF AN INTERCHANGE BETWEEN CHROMOSOMES 8 AND 9 IN MAIZE

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The partially sterile line of maize considered in this paper originated as one of three partially sterile plants in a culture of sixty plants from an ear of red aleurone (*pr*) waxy (*wx*) maize which was being used as a standard normal stock. The stock had not been subjected to X-ray treatment. This partially sterile line of maize has been described in a preliminary note (BURNHAM 1930) as semisterile-2. It was reported then that the "semisterile" plants showed at diakinesis and metaphase I of meiosis a ring of four chromosomes representing an association of two of the smaller pairs. Linkage tests showed that one of these pairs carries the shrunken-waxy linkage group. MCCLINTOCK (1930) has shown that the second and third smallest chromosomes, 9 and 8, are involved; and, by a study of midprophase stages, that there has been an interchange between them of unequal terminal segments of the long arms, chromosome 8 having lost the larger piece. Her trisomic tests showed that chromosome 9 is the one carrying the waxy linkage group. BEADLE (1932) has used this interchange to mark the maize chromosomes 8 and 9 for a cytogenetical study of the hybrid with Euchlaena. CREIGHTON and MCCLINTOCK (1931) have used it as one of the morphological markers in their demonstration that an actual exchange of pieces of homologous chromosomes accompanies genetic crossing over.

The present paper presents additional cytogenetical data on this interchange. In order to have the genetic data together in this paper, Dr. H. B. CREIGHTON has furnished the data in table 3 which she has collected on crossing over with aurea 1 (au_1) and virescent 1 (v_1).

For convenience the symbol T will be used to represent the complex with respect to the chromosomes involved in the interchange. Different interchanges may be differentiated by adding numerals indicating the chromosomes concerned. The one being considered here will be referred to henceforth as T 8–9. The term "X-normal" has been proposed (BRINK and BURNHAM 1929) for plants homozygous for an interchange; while BLAKESLEE uses the term "Prime type" in Datura. In this paper, no special term will be used, reserving T 8–9 for the interchange complex.

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BREEDING BEHAVIOR OF SEMISTERILE PLANTS

Semisterile plants, when used as the pollen parents in crosses with normals, gave, out of 2812 plants, 47.4 percent that were semisterile, the remainder being normal, where a 1:1 ratio was expected. Where semisterile plants were used as the female parents in crosses with normals, out of 8141 plants, 49.7 percent were semisterile. The small deficiency noted in the former is similar in amount to that observed by BRINK (1925) in crosses using plants heterozygous for *wx* as the pollen parents. In these crosses the waxy gene (*wx*) entered with the T 8–9 complex. The cross in which *wx* was in the normal chromosome has not been tested in sufficient numbers for comparison.

The progeny of a self-pollinated semisterile plant includes normals and semisteriles. Among the normals there should be plants homozygous for the interchange. Since the gene *wx* entered with the T 8–9 complex and is rather closely linked with it, the waxy seeds from such a self-pollinated plant should be homozygous for T 8–9 in most cases. This proved to be the case and facilitated their isolation. The plants of this homozygous stock show no external differences from the semisterile sibs or from normal plants. They have ten bivalents at meiosis and show no more ovule or pollen abortion than do normal plants. When crossed with a standard normal line, all the F_1 plants are semisterile. This serves as the genetic test for the isolation of lines homozygous for an interchange. In the earlier report (BURNHAM 1930), the cross of T 8–9 with T 1–2a (semisterile-1) was found to give plants having two separate rings of four at meiosis and about 79 percent pollen abortion. From this cross, a fertile stock homozgous for both interchanges has been isolated. When crossed with standard normals, all the F_1 plants show the high sterility (79 percent).

Semisterility behaves in linkage tests as though it were at one definite locus in each of the two chromosomes involved in the interchange. These loci must correspond to the points at which the breakages and reattachments originally occurred. Tests for linkage of genes with the points of breakage are made by crossing a semisterile plant or one homozygous for the interchange with a normal stock carrying recessive factors. The semisterile F_1 plants (carrying the interchange) are then back-crossed to the same normal recessive stock. The relative numbers of semisterile and normal plants in each of the resulting genetic classes indicate the strength of the linkage. The classifications for sterility were made by examination under the microscope of tassel samples taken from the plants at the time of pollen shedding. In a semisterile plant approximately half of the grains are aborted. It might seem possible to use ear classifications since the ears on plants heterozygous for T 8–9 are only partially filled. In one experiment

TABLE 1

Summary of linkage from backcrosses involving T 8-9 and factors in chromosomes 8 and 9. The three-point and four-point data are included.

GENES	LINKAGE	NUMBER OF INDIVIDUALS					RECOMBINATIONS	
x Y	PHASE	*XY*	*Xy*	*xY*	*xy*	TOTAL	NUMBER	PERCENT
au_1* T 8–9	RB	617	43	53	574	1237	96	7.5
c T 8–9 (♂)	CB	116	54	55	126	351	109	31.1
c T 8–9 (♀)	CB	42	37	32	58	169	69	40.8
total						520	178	34.2
sh T 8–9 (♀)	CB	347	183	121	274	925	304	32.9
v_1* T 8–9	RB	650	17	21	563	1251	38	3.0
wx T 8–9 (♂)	RB	48	430	433	70	981	118	12.0
wx T 8–9 (♀)	RB	136	974	952	170	2232	306	13.7
wx T 8–9 (♂)	CB	99	23	14	63	199	37	18.6
wx T 8–9 (♀)	CB	103	15	17	61	196	32	16.3
total						3608	493	13.7
j T 8–9 (♂)	CB	53	34	50	33	170	84	49.4
j T 8–9 (♀)	CB	92	43	32	77	244	75	30.7
total						414	159	38.4

* Data furnished by H. B. CREIGHTON.

where pericarp was to be noted later, the plants were tagged and numbered and separate classifications for sterility based on the ears and on the pollen were made. Out of 190 plants, 3 of those having normal pollen had been classed as semisterile on the basis of the appearance of the ears. The errors were all in this one direction. Plants having small ears were not included. In this case no weak recessives were segregating. Where they are, the percentage error might be much higher, since such plants have smaller ears and often silk late when there is little pollen being shed. When using the pollen for classification, certain precautions are also necessary. The most important of these seems to be that certain of the linkage testers in use show pollen abortion which is not associated with ovule abortion. In crosses with standard normals, however, this sterility has not proved to be dominant.

TABLE 2

Three-point linkage data from backcrosses involving T 8–9 and factors in chromosomes 8 and 9.

GENETIC CONSTITUTION	PARENTAL COMBINATIONS		RECOMBINATIONS						TOTAL
			REGION 1		REGION 2		REGIONS 1, 2		
C+T	42	58	2	4	35	28	0	0	169
csh+ (♀)	100		6 3.6%		63 37.3%		0		
c++	114	89	45	27	10	14	12	1	312
C wx T (♂)	203		72 27.2%		24 11.9%		13		
+ *wx* T	205	171	82	49	40	17	6	3	573
sh++ (♀)	376		131 24.4%		57 11.5%		9		
++T	77	39	13	13	14	13	3	—	172
sh wx+ (♀)	116		26 16.9%		27 17.4%		3		
++*j*	61	68	12	1	38	23	1	2	206
wx T + (♀)	129		13 7.8%		61 31.1%		3		
++*j*	15	33	6	3	20	36	4	5	122
wx T+ (♂)	48		9 14.8%		56 53.3%		9		

The new two-point data on the linkages obtained with T 8–9 are given in table 1. The combined data for factors in chromosome 9 indicate: 34.2 percent recombination with *c*, 32.9 percent with *sh*, and 13.7 percent with *wx*. The tests with *wx* show apparently significant deviations from equality in the two recombination classes. In the *Wx* class the data show 11.6 percent recombination while in the *wx* class they show 14.8 percent. The small deficiency of semisterile offspring obtained in crosses of normals with the pollen of semisterile plants would explain this difference, but although no deficiency in number of semisterile offspring was found when semisteriles were used as the female parents, the same difference is found in the percentages of recombination in the *Wx* and *wx* classes. However, the data from individual ears are not consistent in showing this. The cause of the difference, if it exists, is not known at present.

The two-point data, and the three-point data in table 2 show that the break lies on the side of *sh* away from *wx;* that is, that the order is: *c–sh–wx*–T 8–9. The earlier data (BURNHAM 1930) from 2n+1 plants which were partially sterile had indicated this as the position of the break. These data show the order of the genes from the point of interchange but do not determine on which side of this point they lie. MCCLINTOCK (1931), by the use of a $2n+1$ or trisomic type carrying only one of the interchanged chromosomes in T 8–9, showed that these genes lie in the part of chromosome 9 possessing the spindle fiber insertion region and not in the piece translocated to chromosome 8. (The term "interchanged" or "translocated piece" is used to designate the piece which does not include the fiber insertion region). This conclusion is also substantiated by my data on a 5–9 interchange (BURNHAM 1934) in which the break in chromosome 9 occurred in the short arm.

The data furnished by CREIGHTON in table 3 show 3 percent of recombination between T 8–9 and v_1, and 7.5 percent between T 8–9 and au_1. These 4-point data also indicate that au_1 and v_1 are between *wx* and the break, with v_1 closer to the break than au_1.

Data from another interchange, T 5–9, show that the genes *sh* and *wx* are in the short arm of chromosome 9 (BURNHAM 1934). From the genetic data, it seems probable that v_1 is in the long arm of that chromosome. Cytologically, in T 8–9 the break in chromosome 9 occurred in the long arm at about three-fifths of the distance from the end to the spindle fiber insertion region. Genetically the break shows about 14 percent of recombination with waxy and about 3 percent with v_1. The $wx-v_1$ interval therefore includes a region fairly close to the break. A few data were gathered on the effect of T 8–9 in herterozygous condition on recombination in this $wx-v_1$ interval. Based on 506 plants (grown only in the seedling stage), the recombination value is 18 percent. The data collected by CREIGHTON based on 1253 plants, given in table 3, show 14 percent. BEADLE (1932) found 8.3 percent for this same interval in a normal stock. Comparison with strictly comparable control material is necessary to determine if the observed increases are significant. The data are at least sufficient to show that T 8–9 causes no great decrease in crossing over in the $wx-v_1$ interval which includes a region close to the break. This failure to show any reduction is correlated with the observation in a later part of this paper that the interchange cross at prophase is very constant in its position, that is, that there is very little association of non-homologous parts. This correlation is to be expected if abnormal association is one of the causes of reduced crossing over in certain interchanges in regions adjacent to the break. Possibly T 8–9 will cause reduction in a very short region adjacent to the break. The tests are being continued.

TABLE 3

Four-point backcross data involving T 8-9 and factors in chromosome 9, furnished by Dr. H. B. Creighton.

GENOTYPE OF HETEROZYGOUS PARENT	PARENTAL COMBINATIONS	RECOMBINATIONS							
		REGION* 1	REGION* 2	REGION* 3	REGIONS 1 AND 2	REGIONS 1 AND 3	REGIONS 2 AND 3	REGIONS 1, 2 AND 3	TOTAL
$\frac{+wx+\text{T } 8\text{-}9}{sh+au_1+}$	407† 338	146† 139	83 60	34 38	11 7	7 2	7 4	3 1	
Total	745	285	143	72	18	9	11	4	1287
		24.6%	13.7%	7.5%	1.4%	0.7%	0.9%	0.3%	
$\frac{+wx+\text{T } 8\text{-}9}{c+v_1+}$	414 336	139 150	80 82	15 15	4 8	3 2	3 0	8 0	
	750	289	162	30	12	5	3	0	1251
		24.5%	14.1%	3.0%	1.0%	0.4%	0.2%		

* The percentages given for each of these regions include the proper double and triple crossovers.

† These figures are arranged systematically as follows: In each column the first number is of the genotype whose first gene is the one at the extreme upper left end. Thus, in the au_1 data for Region 1 there were 146 $++au_1+$ and 139 *sh wx*+T 8-9.

CROSSING OVER BETWEEN THE MAIZE CHROMOSOME 9 AND ITS TEOSINTE HOMOLOGUE

In hybrids between Florida teosinte and maize, EMERSON and BEADLE (1932) found little or no recombination in the $C-wx$ interval. Since T 8–9 is at some distance from *wx*, it was crossed to a stock furnished by BEADLE carrying the $c-Wx$ chromosome of the Florida teosinte. This stock was the fifth or sixth generation resulting from backcrosses to *wx* maize in which the teosinte *Wx* chromosome had been saved in each generation. The results given in table 4 indicate that the amount of crossing over is not the same in different plants. Based on the ratios of *C wx* to *c Wx* (corn:teosinte), the plants appear to fall into two groups; one in which the ratio through the pollen shows only a small deficiency in the *c Wx* or teosinte class (42.8 percent of *c Wx*) and the other in which this class is extremely deficient (only 21.6 percent in the total from three such plants). In both groups, using the same heterozygous plants as the female parents, the ratios are approximately 1:1. In each case the crosses are exact reciprocals between the same two plants. In the group giving approximately normal ratios in reciprocal crosses, the recombination value with T 8–9 is 16.3 and 12.2 percent from the crosses using the heterozygote as the female and as the male respectively. This is to be compared with 4.3 and 2.3 percent in the crosses through female and male respectively in the group giving deficient ratios through the pollen. The difference between the two groups is significant. The differences in crossing over through female and male are not significant.

Backcross data from this cross of T 8–9 with Florida teosinte have been reported by BEADLE (1932). The F_1 plants used in his experiments were sibs of the ones used in mine. He reported a recombination value of 12.3 percent which agrees fairly well with my results from the group giving more nearly normal ratios through the pollen. The explanation of the low values in the other group is not known. Germination was low in both groups but was lower in the group giving the more nearly normal ratios of *C wx*:*c Wx* through the pollen. It is possible that the entire original teosinte *Wx* chromosome crossed over with its maize homologue only rarely. Since none of the long arm of chromosome 9 was marked genetically, part of this arm may have been replaced by a maize segment in the backcrosses by which the stock used here was established. Such crossovers might have occurred at different points in that arm, resulting in substitution of different amounts of the maize chromosome. Since two types appeared in this one cross, one such change would have had to occur in the immediate parent of the cross with T 8–9. In addition, to explain the low ratios through the pollen, a factor slowing up pollen-tube growth must be assumed to have been located in the part of the teosinte 9 chromosome be-

tween waxy (*wx*) and T 8–9. The loss of this section should result in a stock giving normal ratios through the pollen, and the recombination percent between T 8–9 and *wx* should be nearer normal. The tests need to be repeated in a cross of T 8–9 with pure Florida teosinte.

LINKAGE TESTS FOR CHROMOSOME 8

The determination of the linkage group carried by chromosome 8 which also would be expected to show linkage with T 8–9 was aided by McCLINTOCK'S unpublished results on the association of several different trisomics with their linkage groups. Her tests finally showed that chromosome 8 carried either the sugary (*su*)—Tunicate (*Tu*) group or else it was the one for which no linkages had been found. (In the earlier note [BURNHAM 1930] aberrant ratios for red aleurone (*pr*) were reported from a trisomic plant carrying T 8–9 which suggested that this group might be involved. Direct tests showed that the *pr* group is not linked with T 8–9). Backcross tests of T 8–9 with *su* and with *Tu* showed independence, indicating that chromosome 8 must carry the missing linkage group. Japonica (*j*), an unplaced gene, was tested with T 8–9. This character was described as a Japanese strain and illustrated by a colored plate under the name *Zea japonica* by VAN HOUTTE in 1865–1867. The tests for linkage of *j* with T 8–9 (table 1) give conflicting data. When the semisterile plants were used as female parents, the progeny show linkage of *j* with T 8–9; when used as pollen parents they do not. This difference may not be very significant, since the crosses are not between the same stocks. A similar result has been reported, however, by ANDERSON (1934) and by RHOADES (1933). The combined data show 38.4 percent of recombination between *j* and T 8–9. Trisomic tests with *j* were made to determine whether it is in chromosome 9 or in chromosome 8. The test with chromosome 9 (2n+1, simplex for *j*, selfed) gave 209 *J*:73 *j*, a close fit to the 3:1 (70.5 *j* expected) from disomic inheritance. The trisomic test with chromosome 8 [+*j*(2n)×++*j* (2*n*+1)] gave 137 *J*:23 *j* in the *R* aleurone class and 83 *J*:13 *j* in the *r* aleurone class. In these cultures, the plants were tillered and the classification for japonica was clearcut in both aleurone classes. These numbers are close to the 5:1 ratio expected from trisomic inheritance and indicate that *j* is in chromosome 8. McCLINTOCK (1933) also has shown cytologically by means of deficiencies that *j* is in chromosome 8 and that its locus is toward the end of the long arm. Japonica must therefore lie in the piece of chromosome 8 which was translocated to chromosome 9.

Tests for linkage of T 8–9 with chocolate (*Ch*) which ANDERSON and EMERSON (1931) suggested might be located in chromosome 8 gave negative results, there being 222 parental:223 new combinations. The tests of *Ch* with *j* gave 225 parental:227 new combinations. In addition, tri-

somic tests with chromosome 8 (normal $ch \times 2n+1$ simplex for *Ch*) gave 77 chocolate:72 normal; a 1:1 or disomic ratio where 1:2 was expected

TABLE 4

Data from backcrosses to cwx of plants heterozygous for the teosinte cWx chromosome (9) and for the maize Cwx T 8–9 chromosome.

PLANT NUMBER	EAR RATIO			CLASS. FOR POLLEN STERILITY				
	C wx (CORN)	*c Wx* (TEOS)	PERCENT *Wx*	*C wx*		*c Wx*		PERCENT RECOMBINATION BETWEEN *wx* AND T 8–9
				SEMI-STERILE	NORMAL	SEMI-STERILE	NORMAL	
HETEROZYGOUS PLANT USED AS ♀								
2434–9	92	90	49.5	33	—	—	16	0
2465–5	25	24	49.0	16	—	2	8	7.7
608–10*	77	111	59.0	61	2	5	69	5.1
subtotal	194	225	53.7	110	2	7	93	4.2
2465–6	58	72	55.4	22	6	2	19	16.3
2464–4*								
subtotal								
HETEROZYGOUS PLANT USED AS ♂								
2434–9	133	56	29.6	52	1	2	17	4.2
2465–5	144	20	12.2	27	—	1	2	3.3
608–10*	141	39	21.7	89	1	—	19	0.9
subtotal	418	115	21.6	168	2	3	38	2.4
2465–6	111	83	42.8	19	5	3	20	17.0
2464–4*	99	74	42.8	41	2	3	24	7.1
subtotal	210	157	42.8	60	7	6	44	11.1

* Only segregating for $C-c$.

from trisomic inheritance. Therefore chocolate is not in chromosome 8. Similar trisomic tests of chocolate with chromosome 7 indicate it is not in that chromosome. Also backcross tests for linkage of chocolate with brown midrib 2 (bm_2) (32 parental:36 new combinations) indicate independence with this end of linkage group 1 which previously had not been tested with chocolate.

LINKAGE TESTS WITH FACTORS IN OTHER CHROMOSOMES

Considerable data were accumulated in tests of this interchange with genes in other linkage groups. At the beginning, nothing was known about the association of the linkage groups with particular chromosomes. In all cases, the data are from backcrosses. It is sufficient here merely to enumerate the factors that were tested without finding linkage: *chromosome* 1: Pericarp (*P*), fine striped (f_1), brachytic (*br*), anther ear (*an*), and brown midrib (bm_2); *chromosome* 3: anthocyanin (A_1), tassel-seed (ts_4), and dwarf

(d_1); *chromosome* 4: sugary (*su*), and Tunicate (*Tu*); *chromosome* 5: virescent (v_2), red aleurone (*pr*), and brown midrib (bm_1); *chromosome* 6: yellow (*Y*); *chromosome* 7: ramosa (*ra*), glossy (gl_1), virescent (v_5); and *chromosome* 10: aleurone color (*R*). Chromosome 2 was later eliminated by the cytological observation that T 1–2, when crossed with T 8–9, gives two separate rings of 4 chromosomes each (BURNHAM 1930).

Tests showing independence also were obtained with the gene albescent (*al*) which is supposed to be in chromosome 6. Other tests of *al* with purple (*Pl*) and of *al* with T 1–6b in which the break is in the satellite proper show no linkage. The gene *al* appears not to be in chromosome 6 unless the satellite shows a special crossover relation with the remainder of the chromosome, across the nucleole attachment.

In addition, the unplaced genes brown midrib-3 (bm_3), golden (g_2), and tasselseed (Ts_3) were tested with T 8–9 without finding linkage.

CYTOLOGICAL STUDIES

For cytological study T 8–9 was crossed with a stock which is homozygous for a large deeply-staining terminal knob on the end of the short arm of chromosome 9. Counts were made on the relative frequencies of rings and "chains" at diakinesis. "Chains" or strings of four chromosomes are infrequent, out of 108 clear figures, there were 7 such configurations, the remainder being rings. BEADLE (1932) observed 3 per cent of chains in similar material. At this stage, with the presence of the terminal knob on chromosome 9, together with the small differences in lengths, it was possible to determine where lack of association had probably occurred to break the ring into a "chain." In five of the seven "chains" observed, the short interchanged chromosome 8 was at the open end; probably indicating lack of association between the normal 9 and the piece of 9 which was translocated to 8. In the two remaining "chains" the normal 8 was at the open end probably indicating lack of association between the long arm of the normal 8 and the piece of 8 which was translocated to 9. The data are not sufficient to indicate the relative frequencies of the two types of lack of association, but do show that both may occur. At mid-prophase, MCCLINTOCK (1930) has shown that the interchange complex gives a cross-shaped figure when heterozygous, and that unequal terminal pieces have been interchanged. Chromosome 8 has lost more than it received, making it now much shorter than the normal 8; even a little shorter than chromosome 10 which in normal stocks is the shortest of the haploid set. The cross-shaped figure at prophase indicates that the interchanged pieces became attached at the broken ends. In two interchanges which have been reported (BURNHAM 1932, 1934), considerable variability in the position of the center of the cross has been found. Close association of the chromo-

somes was found in all the positions. Since the original breaks must have occurred at only one point in each of the two chromosomes, in every position except the one in which the center of the cross is at these points there must be association of non-homologous parts. In plants heterozygous for T 8–9, the position of the cross is very constant. Occasional figures show the cross at a markedly different position as is shown by the measurements of mid-prophase figures given in table 5. One of the 8 figures is of that

TABLE 5

Data from measurements of camera lucida drawings of mid-prophase meiotic configurations in plants heterozygous for T 8–9 (measurements in mm—magnification: ×4200).

SHORT ARM	LONG ARM			RATIO OF ARMS (LONG/SHORT)
	INSERTION TO "CROSS"	"CROSS" TO END OF ARM	PERCENT INTERCHANGED	
CHROMOSOME 8				
52	43	152	77.9	3.8
53	73	90	55.2	3.1
93	42	178	80.9	2.4
44	23	151	86.8	4.0
92	34	192	85.0	2.5
—	13	79	85.9	—
72	42	179	81.0	3.1
—	—	—	—	—
Average, excluding the second figure.			82.9	3.2
CHROMOSOME 9				
73	78	89	53.3	2.3
53	115	48	29.4	3.1
99	72	125	63.5	2.0
—	39	65	62.5	—
75	69	104	60.1	2.3
—	27	39	59.1	—
—	—	—	—	—
115	64	128	66.7	1.7
Average, excluding the second figure			60.9	2.1

type. The average for the other seven gives about 83 percent of the long arm of chromosome 8 and 60 percent of the long arm of chromosome 9 as the pieces which were interchanged (this description should be less confusing than the use of the terms "right" and "left" end). These values are practically the same as those diagrammed by McCLINTOCK (1930) for this interchange. The corresponding values for the off-type figure are 55 and 29 percent respectively; that is, when the center of the cross is out nearer the end of the long arm of chromosome 9, it is also nearer the end of the

long arm of chromosome 8 by a similar amount. Such a result could not have arisen by mere stretching. The data in table 5 include only those figures in which the entire chromosome could be followed with certainty and do not indicate relative frequencies. Many figures were found and studied, from which it was concluded that the center of the cross is very constant in its position. In two interchanges, T 5–9 and T 2–6, which were studied cytologically and genetically the position of the center of the interchange cross at prophase was extremely variable (BURNHAM 1932, 1934). In each case this was associated with greatly reduced crossing over (BURNHAM 1934). In the T 8–9 reported here where there was very little variability, the $wx-v_1$ interval shows no reduction in crossing over. If the association of non-homologous parts, brought about by variability in the position of the center of the cross, prevents crossing over in these regions, the above correlations are expected, that is, great variability with greatly reduced crossing over and little variability with no reduction or reduction in a very short interval.

In the literature, DOBZHANSKY (1931) has emphasized competition in synapsis as a cause for the decreased crossing over observed in regions adjacent to translocations. No plausible mechanism for this competition has been offered. The variability in the position of the center of the cross suggests several possible mechanisms. MCCLINTOCK (1933) has reported evidence in maize that at meiosis synapsis has a tendency to begin at the ends of the chromosomes. There is no evidence to indicate that this process is initiated in different chromosomes at the same time, nor that it proceeds in all at the same rate. Where the chromosomes are in normal pairs, differences in these processes would still give normal synapsis. Where an interchange is present, a difference in either or both of these would affect the position of the center of the cross. Such processes might be expected to be variable, and would result in variable positions of the cross.

DEGREE OF STERILITY

Counts to determine the amount of pollen abortion were made by thoroughly teasing out about one-third of an anther in a drop of iodine-KI solution and counting the grains on the entire slide. The normal and aborted classes of grains are distinct, the aborted ones being practically devoid of starch, and somewhat smaller. Counts on twenty-five plants heterozygous for T 8–9 gave 25,135 pollen grains, of which on the average 59 percent were aborted. There was considerable variation, the standard deviation being 5.8 percent. The amount reported in the earlier paper (BURNHAM 1930) was 57 percent. Counts have not been made, but the ears show a comparable proportion of aborted ovules. In two or three plants out of the total number which have been examined for sterility during the

course of the work there were no visibly aborted grains, but 55 to 60 percent were small and well-filled with starch. Progeny were not grown from these to determine if they were heterozygous for T 8–9.

One culture was found to have plants with 67–69 percent aborted pollen in addition to plants with the usual 57–60 percent. McCLINTOCK (1933) has found an inversion in the short arm of chromosome 8 in certain of the maize stocks. She has observed irregularities in meiosis, and pollen sterility resulting from crossing over in the inverted region. Plants from the culture giving the higher sterility were examined cytologically at mid-prophase, diakinesis, and anaphase of meiosis with special reference to chromosomes 8 and 9. There was no evidence that an inversion was present. There may have been one in another chromosome or some factor affected distribution in the ring. This suggests the possibility that the plus deviation from 50 percent sterility observed in most plants heterozygous for T 8–9 may be due to the presence of an inversion. Several different cultures have been studied cytologically at prophase, none of which has shown an inversion. Nor have any cultures been found which show only 50 percent sterility.

The amount of pollen abortion (59 percent) is significantly in excess of 50 percent. McCLINTOCK (1930) found from cytological observations at the first microspore division in this interchange that any two chromosomes of the ring of 4 may pass together to one pole at anaphase I of meiosis; that is, that six classes of spores are formed. She has suggested that in half the cases, alternate distribution occurs, while the other half includes the two types of adjacent ones. Absolutely random 2 by 2 distribution in the ring of four should give 50 percent of spore abortion; since for each of the two "open" orientations, there should be a corresponding zigzag arrangement occurring with equal frequency. The former give aborted spores while the latter give normal ones.

An attempt was made in this material to determine the relative frequencies of the two types of non-disjunction, that is, 1, non-disjunction of the interchanged pieces and 2, non-disjunction of the non-interchanged pieces. Since only the alternate distributions, that is, zigzag arrangements, give normal spores, it is necessary to study only the "open" rings at metaphase. These are much easier to observe than the twisted or zigzag ones, but after considerable study it was concluded that the differences in chromosome lengths were not sufficient to permit identification in all cases of the two types of "open" ring orientation which lead to the two types of non-disjunction. Since the observed amount of abortion is 59 percent in place of 50 percent, it seems probable that the distribution is not a random one.

In Drosophila interchanges involving small pieces, DOBZHANSKY (1933) found that the type of distribution which resulted in non-disjunction of the greater amount of chromatin was less frequent. He also found that

when the breaks were nearly median, distribution of the four chromosomes was approximately random, showing that the spindle fiber is not the all-important factor determining chromosome disjunction. It may be that the spindle fiber is a region at which the forces first begin to act.

TRISOMIC INDIVIDUALS DERIVED FROM PLANTS HETEROZYGOUS FOR T 8–9

In the preliminary paper (BURNHAM 1930), results were reported from two 21-chromosome, low sterile plants which appeared in the progeny of plants heterozygous for T 8–9. Both gave ratios for factors in chromosome 9 approaching those from trisomic inheritance. One apparently was trisomic for chromosome 8 (27 percent sterile) and the other for 9 (33 percent sterile). The apparent trisomic ratios obtained from the former were due to the fact that *sh* on chromosome 9 shows about 33 percent of recombination with the interchange point. Later generations from the plant trisomic for chromosome 9 and heterozygous for T 8–9 have been studied. The probable genetic make-up of the three chromosomes is: (a) *C Sh wx* T 8–9, (b) *C Sh Wx*+, (c) *c sh wx*+. This plant was crossed to a *c sh wx*+ ♂, and the resulting low sterile (2n+1) plants were used both as the ♂ and the ♀ parents in crosses with *c sh wx*+. The low sterile, 21-chromosome plants may include four types: 1, a type which carries both interchanged chromosomes (T 8–9) and has two normal number 8 chromosomes and one normal 9 (that is, trisomic for chromosome 8); 2, one which has both interchanged chromosomes but has two normal number 9 chromosomes and one normal 8 (that is, trisomic for chromosome 9); 3, one which has a normal 2n complement but in addition has one of the interchanged chromosomes (that is, trisomic for a part of 8 and a part of 9) and 4, one which has a normal complement but in addition has the other interchanged chromosome. These last two types correspond to BLAKESLEE'S "tertiary" types in Datura. For each interchange, it should thus be possible to isolate two such "tertiary" types. These may be obtained in subsequent generations from the cross of the interchange with the corresponding normal or "primary" trisomic stocks, or from 3–1 disjunction in a plant which carries the interchange. When either trisomic type 1 or 2 which carries both interchanged chromosomes is used as the pollen parent, the progeny should consist of normals and "semisterile" plants (heterozygous for T 8–9) with possibly an occasional low sterile as a result of the functioning of n+1 pollen. The data from such crosses are given in table 6. Only 17.9 percent (16.4 when the data are corrected for differential germination of *Sh* and *sh* seeds) of the offspring came from pollen carrying T 8–9. If disjunction were at random in the trivalent, 1/3 of the spores should carry T 8–9. This indicates that the two normal members of the trivalent disjoin more often than they

TABLE 6

Data from crosses of normal ♀ × trisomics carrying interchanged chromosomes from T 8–9. In the first group, those carrying both; in the second, those carrying only one of the interchanged chromosomes.

CROSS	POLLEN STERILITY OF THE PARENT TRISOMIC PLANT		PROGENY THROUGH POLLEN			
	NUMBER OF GRAINS	PERCENT ABORTED	LOW STERILE	NORMAL	T 8–9 (SEMISTERILE)	PERCENT T 8–9
N×460-1*	1392	25.7	7	246	41	14.3
N×460–9	1608	26.5	—	131	35	21.1
N×460–20	3173	28.0	—	174	33	15.9
N×461–23*	1500	29.8	1	210	56	21.1
N×465–3*	1747	29.8	1	171	38	18.2
subtotal			9	932	203	17.9
N×460–5*	2169	8.9	1	125	—	—
N×461–4*	2693	10.8	2	355	1	—
subtotal			3	480	1	—

* These plants were checked by root tip counts and were found to have 21 chromosomes (2n+1).

should on the basis of chance, at least at the interchange locus in the chromosome. If they disjoined from each other in every case, there would be no semisteriles among the 2n offspring. If the three chromosomes are represented as: (a), (b), (c), of which (a) carries the translocation while (b) and (c) are normal, and if the proportions among the three types of synapsis are assumed to be 4 bc:1ab:1ac, about 16.7 percent of the 2n spores will carry T 8–9. This is very near to the observed percent, but disjunction is probably complicated by the fact that most of the figures are chains of 5 chromosomes. Pollen sterility counts were made on 87 of the "semisterile" plants obtained from the above crosses to determine if they were similar to the original plants heterozygous for T 8–9. Plotting their frequencies by 2 percent intervals shows a slightly bimodal distribution, one mode falling at 58 to 60 percent while the other falls at 62 to 64 percent. Although the difference is not based on large enough numbers, it suggests that there is a group showing higher pollen sterility. The presence of an inversion is one possible explanation for this group. The plants in the other group show a degree of pollen sterility similar to that in the original plants.

The two types of trisomics which carry one of the two interchanged chromosomes as the extra one should give entirely different results. Through the pollen, only normal progeny should appear except where an n+1 grain functions to give a "low sterile" plant. Two such 21-chromosome plants which showed very low pollen sterility (8 to 11 per cent), and which gave abnormal or trisomic ratios for shrunken (*sh*) were tested (last two lines in table 6). There were 480 normal offspring and only one "semi-

sterile." The latter probably was a stray pollination. It will be noted in table 6 that a few "low sterile" plants also appeared, about 0.74 percent. Pollen counts made on these plants determined for certain that they were "low sterile." They undoubtedly arose from the functioning of n+1 pollen. No data have been obtained on the frequency of n+1 through the pollen of normal trisomics of either 8 or 9 for comparison.

Abnormal ratios for factors in chromosome 9 were obtained from many of the trisomic plants. One plant which showed 11 percent of pollen abortion gave only 11.3 percent of *Sh* seeds through the pollen in a backcross to *sh*. By assuming the correct amount of preferential pairing and lagging, such ratios can be explained, but until more is known about the actual distribution there is little value in such an attempt.

SUMMARY

1. The interchange between chromosomes 8 and 9 (T 8–9), referred to earlier as "semisterile-2," shows 13.7 percent of recombination with waxy (*wx*), 32.9 percent with shrunken (*sh*), and 34.2 percent with colored aleurone (*C*). CREIGHTON's data which are included show 7.5 percent with aurea$_1$ (*au*$_1$), and 3 percent with virescent$_1$ (*v*$_1$). The order is *C*−*sh*−*wx*− T 8–9 with *au*$_1$ and *v*$_1$ also probably between *wx* and T 8–9.

2. No reduction in crossing over was found in any of the regions studied. Part of the *wx*−*v*$_1$ interval is probably in the arm in which the break occurred.

3. The gene for japonica stripe (*j*) is in chromosome 8, and shows 38 percent of recombination with T 8–9. That for chocolate pericarp (*Ch*) is not in this chromosome.

4. Crossing over with the Florida teosinte chromosome homologous with the maize chromosome 9 was observed in the *wx*−T 8–9 interval as was reported by BEADLE, but the amount varied. This seems to be associated with some factor upsetting genetic ratios through the pollen.

5. Pollen counts show about 59 percent of aborted pollen in plants heterozygous for T 8–9. There is a comparable degree of ovule abortion in such plants. Plants homozygous for the interchange show normal fertility.

6. Counts at diakinesis in plants heterozygous for T 8–9 showed about 7 percent of chain configurations, the remainder being rings of four chromosomes.

7. Mid-prophase figures show that the position of the interchange "cross" is very constant. Only occasional figures show it in a widely different position. It is pointed out that in the two chromosomes involved in an interchange, a variation in time of initiation of synapsis or a variation in

its rate of progress along the chromosomes would bring about variability in the position of the "cross."

8. Genetic tests of a plant which was trisomic for chromosome 9 and which carried T 8–9 (a chain of five chromosomes) showed that synapsis must have been preferential between the two normal homologues of the group.

9. Tests through the pollen or trisomics carrying only one of the interchanged chromosomes gave mostly normal offspring and no "semisteriles." There was a small percentage of 2n+1 low sterile plants.

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