STUDIES ON THE PHENOMENON OF PREFERENTIAL SEGREGATION IN MAIZE[1]

GARY Y. KIKUDOME[2]

Department of Botany, University of Illinois, Urbana, Illinois

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RHOADES (1942) demonstrated that in maize plants heterozygous for a type of chromosome 10 known as "abnormal 10", this chromosome undergoes preferential segregation during megasporogenesis. In 1945, LONGLEY reported that the phenomenon of preferential segregation is not confined to the abnormal chromosome 10, but that the chromosome induces preferential segregation of the other chromosomes of the complement if one homologue is knobbed and the other is knobless. However, there was little or no evidence to show whether the size of the knob is an important factor in preferential segregation. Therefore, one of the primary goals of this investigation was to determine the effect on preferential segregation of knobs of different sizes.

Experiments were also conducted to determine the effect of the abnormal chromosome 10 on preferential segregation and recombination in the long arm of chromosome 10, especially in the segment distal to the *r* locus.

MATERIALS AND METHODS

Some strains of maize have a chromosome 9 with a large terminal knob, others have knobs of smaller sizes, and some have a knobless chromosome 9. Knobs of three different sizes were selected for study (see FIGURES 1–3).

The largest knob (K^L) on chromosome 9 is approximately the size of the conspicuous heterochromatic piece found in the abnormal chromosome 10. The "medium"-sized knob (K^M) is one half to two thirds the length of the largest knob, while the smallest knob (K^S) involved in the experiments described below is approximately one fifth the size of the largest knob and about the same dimensions as that found on chromosome 9 in the inbred line, KYS. The knobless (k) chromosome 9 utilized in these experiments is, in reality, deficient for a very short segment of the end of the short arm. According to MCCLINTOCK (1944), the deficiency includes the terminal knob and part of the first adjacent chromomere.

The knobless chromosome described above is an ideal one for these experiments since it can be identified genetically as well as cytologically. When a plant is homozygous for this type of knobless chromosome 9, it is an albino *(wd wd)*, whereas an individual homozygous or heterozygous for the knobbed homologue *(Wd Wd* or *Wd wd)* and having nonmutant alleles in the *Wd* segment is always a green plant.

[1] From a thesis submitted in partial fulfillment of the requirements of the degree of Doctor of Philosophy, University of Illinois.

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

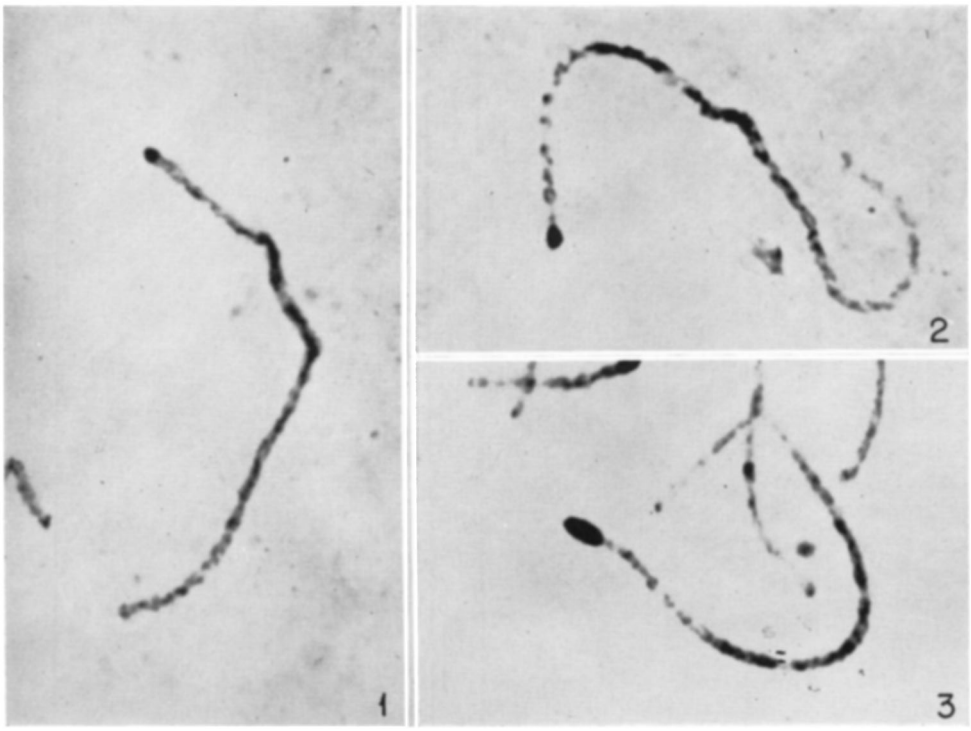

FIGURE 1.—Pachynema of a chromosome 9 bivalent homozygous for the small knob (K^S/K^S). FIGURE 2.—Pachynema of a chromosome 9 bivalent with one homologue having a "medium"-sized knob and the other knobless (K^M/k). FIGURE 3.—Pachynema showing a chromosome 9 bivalent homozygous for the large terminal knob (K^L/K^L).

The following gene symbols are used: A_1, colored aleurone, plant and pericarp; *c*, colorless aleurone; *r*, colorless aleurone and plant; g_1, "golden" plant; Lg_2, liguled leaf; *Py*, nonpigmy plant; sh_1, shrunken endosperm; sr_2, white striations on plant; *wd*, albino plant; *wx*, waxy endosperm; yg_2, yellow-green seedling and plant.

A variety of heterozygotes possessing knobs of different sizes in several combinations were tested. In all cases, the effect of varying knob size on the degree of preferential segregation is revealed by backcross ratios of genes linked to the knobs. In some of the compounds, the knobbed chromosome 9 is opposed by a knobless homologue while in other tests the homologues possess knobs of different sizes. The genes involved in these experiments include *wd*, yg_2, *c*, sh_1, and *wx* in various combinations as indicated. Of these, *wd* is the most distal and *wx* is closest to the centromere. In the study of preferential segregation and recombination in chromosome 10, the g_1, *r*, and sr_2 loci were employed. They are situated in the long arm of chromosome 10, sr_2 being the most distal locus. The abnormal chromosome 10 (K10) is derived from stocks used by RHOADES (1942, 1952) in his studies of preferential segregation.

TABLE 1

Results from backcrosses of K9/k9 compounds

Female parent	+ +	+ *wx*	*wd* +	*wd wx*	Total	Percent *Wd*	Percent *Wx*	Percent recomb.
a. K^S + +/k *wd wx* K10/k10	1372	669	419	992	3452	59.1**	51.9*	31.5
b. K^S + +/k *wd wx* k10/k10	2154	778	763	2035	5730	51.2	50.9	26.9
c. K^M + +/k *wd wx* K10/k10	3994	1543	757	2276	8570	64.6**	55.4**	26.8
d. K^M + +/k *wd wx* k10/k10	2598	582	518	2510	6208	51.2	50.2	17.7
e. K^L + +/k *wd wx* K10/k10	854	380	163	396	1793	68.8**	56.7**	30.3
f. K^L + +/k *wd wx* k10/k10	1555	213	225	1456	3449	51.3	51.6	12.7
g. K^L + *wx*/k *wd* + K10/k10	1442	3463	1726	581	7212	68.0**	56.1** (*wx*)	28.1

* Significant at the 5% level.
** Significant at the 1% level.

TABLE 2

Data obtained from backcrosses of K9/K9 compounds possessing knobs of different sizes

Female parent†	+ + + +	*yg c sh wx*	+ *c sh wx*	*yg* + + +	+ + *sh wx*	*yg c* + +	+ + *c wx*	*yg c sh* +	+ *c sh* +	*yg* + *sh wx*	+ *c sh* +	*yg* + *wx*	+ + *sh* +	*yg c* + *wx*	+ *c* + *wx*	*yg* + *sh* +	Total
a. K^L *sh wx*/K^M + +	1897	.....	.....	.....	2466	.....	435	.....	.....	....	.....	....	727	....	....	....	5525
b. K^M + + + +/K^S *yg c sh wx*																	
1. Pregermination	7030	.....	4763	.....	482	.....	1924	.....	208	....	886	....	20	....	10	....	15323
2. Postgermination	6199	2586	1100	624	366	180	1770	657	20	17	52	32	15	10	....	....	13628
c. K^M + + + +/K^S *yg c sh wx*	1655	704	409	173	128	68	488	192	3	2	18	19	10	4	....	....	3873
d. K^M + + + +/K^S *yg c sh wx*	512	487	114	91	22	29	114	116	2	2	8	5	2	0	....	....	1504
e. K^S *c sh wx*/k + + +‡	1157	.....	1828	.....	41	.....	344	.....	101	....	677	....	2	....	5	....	4155
f. K^M + + +/K^S *c sh wx*	2286	.....	1393	.....	134	.....	827	.....	79	....	423	....	4	....	5	....	5151

† Plants of entries a, b, c, e, and f are K10/k10 while those of entry d are k10/k10.
‡ Sib control cross for entry f.

RESULTS

Studies on preferential segregation in chromosome 9

The degree of preferential segregation of a small knob (K^S) against a knobless homologue was observed in plants heterozygous for abnormal chromosome 10 and in sib plants with only normal chromosome 10. As can be seen in the first two entries of Table 1, the *Wd* and *Wx* alleles linked with the knob were preferentially recovered in the presence of the abnormal chromosome 10 while random segregation for the two loci was observed in the sib control class homozygous for the normal chromosome 10. As previously reported by RHOADES (1942, 1952) using loci on chromosome 10, and by LONGLEY (1945) working with chromosome 9, loci further removed from the knob undergo a lesser degree of preferential segregation than those situated closer to the knob.

Recombination between the *Wd* and *Wx* loci was found to be 31.5 percent in the experimental class, while a value of 26.9 percent was observed in the sib control class. Although the difference between these two recombination values is small, it has been found to be statistically highly significant. The increase in the recombination frequency can be attributed to the presence of the abnormal chromosome 10. This statement finds support in the data of RHOADES and DEMPSEY (1957).

The third and fourth items in Table 1 (c and d) show the results of backcrosses of plants heterozygous for a knobless chromosome 9 and one carrying a medium-sized knob (K^M). The percentage values for the two loci studied deviate significantly from the expected 50 percent in plants with the abnormal chromosome 10, but normal backcross ratios occur in the class homozygous for the normal chromosome 10.

In the presence of the abnormal chromosome 10, a recombination value of 26.8 percent was realized. In the closely related control class, 17.7 percent recombination was found between *wd* and *wx*. This value is significantly lower than that found in plants heterozygous for the abnormal chromosome 10.

The fifth, sixth, and seventh entries in Table 1 involve compounds heterozygous for a large knob (K^L) on chromosome 9. In plants carrying abnormal chromosome 10, values of 68.8 percent *Wd* and 56.7 percent *Wx* were found when the genes were in coupling and values of 68.0 percent *Wd* and 56.1 percent *wx* when the markers were in repulsion phase. Therefore, it is the knob on chromosome 9, and not the alleles associated with the knob, that is responsible for the preferential segregation of chromosome 9 when the abnormal chromosome 10 is present in the megasporocyte. As expected, the sib control class gave random segregation values for these loci.

In the control class there was 12.7 percent recombination in the *wd-wx* region, while a value of 30.3 percent was found in the sib class heterozygous for the abnormal chromosome 10. A value of 28.1 percent recombination was realized in the repulsion experiment. These values again show the effect of the abnormal chromosome 10 in increasing the frequency of crossing over.

It is important to note that there are marked differences in the control recombination values presented in Table 1. The highest value (26.9 percent) occurred in the data involving the smallest knob and the lowest value (12.7 percent) was associated with the large knob, while an intermediate value (17.7 percent) was found in plants heterozygous for the K^M knob. Thus, in the knobbed-knobless heterozygotes, there is a negative correlation of crossing over and knob size. In a situation where one chromosome is knobbed and the homologue is knobless, the knob might interfere with the intimate pairing and thus cause difficulty in crossing over. The larger the knob, the greater might be the disturbance in synapsis. Working with an interstitial knob on chromosome 3, RHOADES and DEMPSEY (1957) have shown that recombination in the *Lg-A* region is reduced when the knob is heterozygous. Pairing in chromosome 3 bivalents heterozygous for the knob was not as intimate as in those bivalents without the knob. The disturbance in synapsis is not surprising since the knob is interstitial, but in the case of chromosome 9 pairs, where the knob is terminal, interference with pairing seems less likely to occur. No obvious departure from the normal synaptic pattern was observed by the writer in the knob heterozygotes. However, since the sporocytes were not examined specifically for pairing between the homologues of chromosome 9, it is not possible at this time to conclude whether the reduction in recombination is due to a disturbance in synapsis or to some other cause.

In the presence of the abnormal chromosome 10, recombination frequencies are increased and in some cases strikingly so. Apparently, the abnormal chromosome 10 counteracts the suppressive action of the knob on chromosome 9. In the compound where the small knob was opposed by a knobless chromosome 9, the frequency of recombination observed in the experimental class was increased by 17 percent from that observed in the sib control class. When the medium knob was employed, the recombination frequency in the experimental class was approximately 50 percent higher than in the closely related control class. The recombination value of 30.3 percent for the class heterozygous for the abnormal chromosome 10 and for the K^L 9 chromosome is nearly 150 percent greater than the value of 12.7 percent observed in the sib control class. Why the increase in crossing over caused by the abnormal chromosome 10 is greatest when the K^L chromosome is present is difficult to understand. A point of interest is that in all three classes of knob heterozygotes the abnormal chromosome 10 increased the frequency of crossing over from three different control values to a value of about 30 percent.

According to the hypothesis of preferential segregation advanced by RHOADES (1942, 1952), the frequency with which the knobbed chromosome becomes incorporated into the functional basal megaspore would depend on the frequency of preferential segregation following such crossovers. As previously mentioned, the frequency of crossing over in the three groups of knob heterozygotes was about 30 percent. Yet, the segregation values observed for the *Wd* and *Wx* loci are not identical for the three knob groups. The recovery of these dominant alleles was highest in the large knob group and lowest in the small knob group. It would

seem, then, that preferential segregation following such crossovers must occur more frequently with the larger knob and less frequently as the size of the knob decreases. A possible explanation is that the large knob has a greater neocentric activity than the smaller knobs and is thus a more efficient agent in reaching the functional basal megaspore. This point is difficult to demonstrate since chromosome 9 cannot be distinguished from the other chromosomes of the complement at anaphase II of meiosis. It should be pointed out that the comparisons made among the three groups of knob heterozygotes, i.e., K^S/k *vs.* K^M/k, etc., are not among full sibs, but the strains are closely related and it is believed that the comparisons are justified.

LONGLEY (1945) reported that the knobbed chromosome of a pair of chromosomes 9 is preferentially recovered over the knobless homologue even in the absence of the abnormal chromosome 10. He suggested that the abnormal chromosome 10 merely accentuates preferential segregation. The fact that random segregation was observed in the controls of the three knob heterozygotes clearly indicates that the heterozygous knobs on chromosomes 9 do not in themselves determine preferential segregation since this results only when the abnormal chromosome 10 is present. The data of RHOADES and DEMPSEY (1957) lend support to the above statement. There is no convincing evidence that preferential segregation occurs when the abnormal chromosome 10 is not present.

The studies described above deal only with heterozygous bivalents composed of one knobbed and one knobless homologue. To test further the hypothesis that knob size has an effect on preferential segregation, experiments were conducted in which both of the homologues of chromosome 9 had knobs which, however, differed in size. The results of these experiments are given in Tables 2 and 3.

When plants of the constitution K^L9 *wx*/K^M9 *Wx*, K10/k10 were used as female parents in backcrosses to k9 *wx*, k10 plants, the recessive allele *wx* was recovered more frequently than the dominant *Wx* allele. In a total of 7682 kernels, 4151 (54 percent) were found to have the *wx* type of endosperm starch. Since the *wx* locus is some distance from the knob and would therefore give a value for the recovery of the K^L9 chromosome which is less accurate than values obtained with loci closer to the knob, the following experiment was carried out.

The K^L chromosome was genetically marked with the sh_1 as well as the *wx* alleles, while the K^M chromosome carried the dominant alleles of these two genes. On the linkage map (see RHOADES 1955) the locus of the sh_1 gene is 29 while that of the *wx* gene is 59. Since the sh_1 locus is much closer to the knob (position 0) than the proximal *wx* locus, it is a more efficient marker in determining preferential segregation. In the backcross, 57.8 percent of the kernels were "shrunken" (sh_1) and 52.5 percent of the kernels were "waxy" (*wx*) (Table 3-a). Chi-square tests show that the deviations from the expected 50 percent value are highly significant.

A comparison of segregation at the *wx* locus in K^L/K^M and K^L/k heterozygotes shows that the allele located on the K^L chromosome was recovered with a higher frequency from the K^L/k compounds than from K^L/K^M plants. A *t* test indicates

TABLE 3

Summary of results from backcrosses of K9/K9 compounds possessing knobs of different sizes

Female parent†	Total	Percentage Yg_2	C	Sh_1	Wx	Recombination percentage yg-c	c-sh	sh-wx	yg-sh	c-wx	yg-wx
a. K^L *sh wx*/K^M + +	5525	...	...	57.8** (*sh*)	52.5** (*wx*)	...	..	21.0	...	...	...
b. K^M + + + +/K^S *yg c sh wx*											
1. Pregermination	15323	...	61.7**	59.9**	53.1**	...	4.7	18.5	...	22.8	...
2. Postgermination	13628	65.0**	61.7**	59.9**	53.1**	13.5	4.5	18.6	17.5	22.7	34.5
c. K^M + + + +/K^S *yg c sh wx*	3873	70.0**	63.9**	62.2**	54.7**	16.1	5.6	18.9	21.4	23.7	37.7
d. K^M + + + +/K^S *yg c sh wx*	1504	51.5	49.7	50.1	50.5	14.9	3.8	16.3	18.0	19.8	32.3
e. K^S *c sh wx*/k + + +‡	4155	...	62.8** (*c*)	61.3** (*sh*)	53.4** (*wx*)	...	3.6	24.7	...	28.0	...
f. K^M + + +/K^S *c sh wx*	5151	...	63.1**	62.1**	54.2**	...	4.3	24.4	...	28.4	...

** Significant at the 1% level.
† Plants of entries a, b, c, e, and f are K10/k10 while those of entry d are k10/k10.
‡ Sib control cross for entry f.

TABLE 4

Results from backcrosses of g_1 + + *K10/*+ r sr_2 *k10 and* g_1 + + *k10/*+ r sr_2 *k10 compounds made in two different seasons*

Female parent	g_1 ++	+ r sr_2	g_1 r sr_2	+++	g_1 + sr_2	+ r +	++ sr_2	g_1 r +	Total	Percentage g_1	R	Sr_2
a. K10/k10†	564	196	33	87	3	5	0	0	888	67.6**	73.6**	73.9**
b. k10/k10†	268	263	59	62	183	169	9	5	1018	50.6	51.3	49.5
c. K10/k10‡	1907	870	159	323	6	17	1	1	3284	63.1**	68.1**	68.4**
d. k10/k10‡	632	589	122	105	344	404	8	9	2213	50.0	49.2	52.0

** Significant at the 1% level.
† Backcrosses made in Winter.
‡ Backcrosses made in Summer.

that this difference is significant at the one percent level. This finding could be interpreted to mean that K^L is incompletely dominant to K^M in producing preferential segregation or that competition exists between the two knobs. Although the K^L/k and K^L/K^M plants were not sibs and therefore not strictly comparable, they possess many genes in common since both were derived from crosses involving the same K^L/K^L stock.

The only data at all comparable to those described above come from LONGLEY's (1945) work on chromosome 6. LONGLEY studied preferential segregation of a pair of chromosomes differing morphologically in that one possessed three knobs and the other only two. In addition, there was a size difference in one of the knobs common to both chromosomes. The larger knob was located on the homologue with the three knobs as was the dominant *Py* allele. In this study the *Py* allele was recovered with a frequency of 67.2 percent. Since both homologues differ in two respects, it is very difficult to determine whether the knob-knobless condition or the large knob-small knob condition (or possibly both) is responsible for the preferential recovery of the *Py* allele. Furthermore, the location of the *Py* locus in relation to the knobs is not known.

The behavior of combinations of knobs in preferential segregation has been more precisely studied in tests involving a marker situated close to the knob as well as three more distant loci. The chromosome 9 with the larger of the two knobs (K^M) carried the dominant alleles Yg_2, *C*, Sh_1, and *Wx*. According to the best available linkage map (RHOADES 1955), the yg_2 locus is located seven map units from the terminal knob, while *c*, sh_1, and *wx* are located 26, 29, and 59 map units from the knob, respectively. The chromosome with the smaller knob (K^S) carried the recessive alleles of the genes listed above.

In the backcrosses involving the abnormal chromosome 10 (Tables 2 and 3-b, c, and f) deviations from a 1:1 ratio were found for all four genes, with the genes on the K^M chromosome being recovered more frequently. The genes nearest the knob showed the highest frequencies of preferential segregation. Normal backcross ratios occurred in the backcrosses of plants with normal chromosome 10 (Tables 2 and 3-d).

In Table 3-c the percentage of plants of the Yg_2 phenotype was 70.0 percent, a value similar to that found for the *Wd* phenotype in plants of K^L9 *Wd*/k9 *wd*, K10/k10 constitution (Table 1). The degree of preferential segregation as followed by these pseudoallelic genes is expected to be greater in the K^L/k compounds than in the K^M/K^S plants because the difference between the two homologues in knob size is greater in the former than in the latter compound. The unexpected similarity in these values may be due to environmental differences since the data of the K^L/k compounds came from backcrosses made in the summer of 1957 while the K^M/K^S plants of Table 3 were grown in the winter of 1958. The value for preferential segregation of the Yg_2 locus found in the other K^M/K^S family (Table 3-b) was somewhat less than that given in Table 3-c. The backcrosses of entries b, e, and f of Table 3 were made during the summer of 1957.

The segregation values observed for the K^M/K^S compounds (Table 3-b) are

comparable to those of the K^M/k compounds (Table 1). The K^S and k chromosomes are recovered from these compounds with approximately equal frequencies, although in K^S/k plants the K^S chromosome is favored. It would seem that the larger knob is dominant to the smaller one in K^M/K^S heterozygotes.

More critical evidence on interaction of knobs of different sizes comes from the data in Table 3-e and f where sib comparisons of K^M/K^S and K^S/k heterozygotes are available. Here the frequencies with which the alleles in the larger knobbed chromosomes were recovered are essentially the same. Since it has been found that larger knobs give higher values of preferential segregation than do smaller ones, it would be expected that values from the K^M/K^S compounds would be greater than those from K^S/k heterozygotes if K^M were completely dominant. Such, however, was not the case and the data suggest that K^M is incompletely dominant to K^S.

The recombination frequencies presented in entries c and d of Table 3 indicate that plants carrying the abnormal chromosome 10 undergo a somewhat higher degree of crossing over than those with normal chromosomes 10. The following recombination values were obtained in the control (k10/k10) class and in sibs heterozygous for abnormal chromosome 10:

	k10/k10	K10/k10	Difference
yg–c:	14.9%	16.1%	1.2%
c–sh:	3.8	5.6	1.8
sh–wx:	16.3	18.9	2.6
yg–sh:	18.0	21.4	3.4
c–wx:	19.8	23.7	3.9
yg–wx:	32.3	37.7	5.4

A total of 40.6 percent recombination (sum of the frequencies in the *yg-c*, *c-sh*, and *sh-wx* regions) was observed in the experimental class and a value of 35.0 percent recombination was observed in the sib control class. This is in agreement with the data previously cited.

Studies on recombination and preferential segregation in chromosome 10 in the presence of the abnormal knob

In 1942, RHOADES reported that approximately one percent crossing over occurs between the *r* locus and the extra piece which identifies the abnormal chromosome 10. E. G. ANDERSON (see RHOADES 1952) had found between four and five percent recombination between *r* and a distally placed break in the long arm of the normal chromosome 10 in a translocation heterozygote. Both ANDERSON and RHOADES pointed out the possibility of the existence of a considerable amount of crossing over in the segment distal to the *r* locus in normal chromosome 10. JOACHIM and BURNHAM (1955) found that the "striate" (or Waseca Stripe) gene was 21.4 to 31.0 recombination units distal to the *r* locus. JOACHIM (1956) confirmed this earlier finding and found recombination ranging from 25.1 to 31.5 percent be-

tween *r* and sr_2. She also verified their earlier finding that the order of g_1, *r*, and sr_2 on the long arm of chromosome 10 is as here reported.

In the present experiment, the abnormal chromosome 10 was marked with the recessive allele g_1 and the dominant alleles *R* and Sr_2, and the normal chromosome 10 was marked with the dominant allele G_1 and the recessive alleles *r* and sr_2. Female parents of such a constitution and controls possessing two normal chromosomes 10 were backcrossed to male parents homozygous for the three recessive alleles. The results obtained in the summer and winter seasons of 1957 are given separately in Table 4. The plants used as females in both instances were from kernels taken from the same ear. Comparisons of K10/k10 with k10/k10 plants in both sets of data show significant deviations from 50 percent for the three loci in the K10/k10 classes only. The highest values are obtained for the Sr_2 locus, which is closest to the knob.

The following recombination values were found in the class heterozygous for the abnormal chromosome 10 and in the sib control class:

	Winter		Summer	
	K10/k10	k10/k10	K10/k10	k10/k10
g–r:	13.5%	13.3%	14.7%	11.0%
r–sr:	0.9	36.0	0.8	34.6
g–sr:	14.4	46.5	15.4	44.1
Double crossovers:	0.0	1.4	0.1	0.8

As shown above, there is a drastic reduction in the recombination frequency when the abnormal chromosome 10 is present in the heterozygous condition. In its absence a considerable amount of recombination occurs in the *r*-sr_2 region. The reduction observed in the experimental class is not surprising since there is non-homology in chromosome structure between the abnormal chromosome 10 and the normal one in the distal one sixth segment of the long arm of normal chromosome 10. In spite of the non-homology and absence of crossing over in this segment, pairing, as determined cytologically, is intimate. Although there is a slightly higher frequency of crossing over in the *g*-*r* region in plants with the abnormal chromosome 10 than in those with the normal chromosome, the difference is not statistically significant.

The frequencies with which the three genes were recovered in the summer experiment (Table 4-c) are somewhat lower than those found in the winter (Table 4-a) as shown by the following comparison:

	K10/k10	
	Summer	Winter
g_1	67.6%	63.1%
R	73.3	68.1
Sr_2	73.9	68.4

Since the F_1 kernels used in the two sets of experiments were from the same ear, the differences cannot be attributed to genetic variations but must be due to

environmental differences in the two growing seasons. The recombination frequencies obtained in the two seasons, however, are quite similar as shown below:

	K10/k10	
	Summer	Winter
g–r	13.5%	14.7%
r–sr	0.9	0.8
g–sr	14.4	15.4
Double crossovers	0.0	0.1

The only obvious difference between the two experimental classes is the absence of double crossovers in the summer group. The recombination values in the sets of data are not significantly different, but the frequency of preferential segregation is higher in one than in the other. If the hypothesis is accepted that preferential segregation occurs only when appropriate crossovers produce heteromorphic dyads, it would seem that similar crossover frequencies should result in like frequencies of preferential segregation. Since this was not the case, it follows that preferential segregation does not invariably occur when heteromorphic dyads are present. It would not be surprising to find variation in the behavior of heteromorphic dyads, possibly dependent on the amount of neocentric activity, associated with changes in the growing season.

Of the 25 crossovers in the *r-sr* region (Table 4-c), 17 were of G_1-*r*-Sr_2 constitution and one was of the g_1-*r*-Sr_2 constitution. These crossover plants were analyzed cytologically to determine where the exchange occurred. All were heterozygous for the abnormal chromosome 10 and possessed the three small chromomeres which are found in the proximal section of the foreign piece. Thus, crossovers between *r* and sr_2 occur to the left of the three chromomeres (see Figure 4). This is in agreement with RHOADES' observation (1942) that crossovers between the *R* locus and the foreign segment occur in a region proximal to the three chromomeres. Crossing over, apparently, is restricted to regions which are truly homologous.

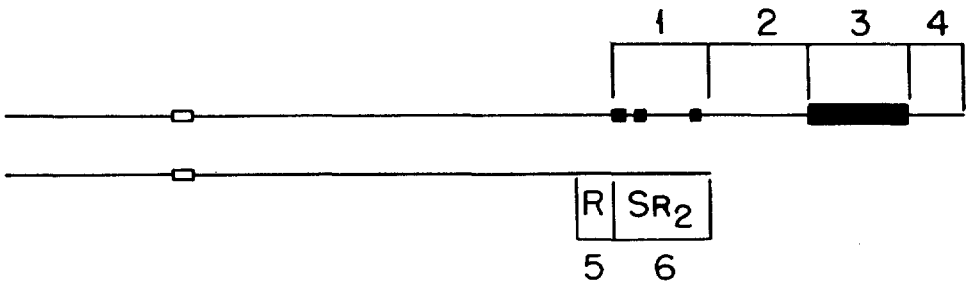

FIGURE 4.—Diagram of a chromosome 10 bivalent heterozygous for the abnormal chromosome 10 showing approximate locations of *R* and Sr_2. 1 = distal one sixth segment in which crossing over is inhibited, 2 = euchromatic segment, 3 = heterochromatic segment, 4 = distal euchromatic segment, 5 = region in which *r*-sr_2 crossovers occur, and 6 = region containing the Sr_2 locus.

It has been shown that exchanges in the *r-sr* region are drastically reduced in K10/k10 heterozygotes and that they probably do not occur in the distal one sixth segment of 10L. These observations have some bearing on the cytological position of the Sr_2 locus. Two possibilities exist: (1) either Sr_2 is found to the left of the distal one sixth segment or (2) it is located within this region. The available evidence favors the latter position although no certain conclusion can be reached at present. Studies with deficiencies for the *R* locus (STADLER 1933) have shown that *R* is placed very close to the distal one sixth segment. Since, in normal chromosome 10 bivalents, Sr_2 lies at least 35 units beyond *R*, it is logical to assume that it is located within the distal one sixth segment. Moreover, the restriction of crossing over between *R* and Sr_2 to the segment to the left of the three chromomeres readily accounts for the low percentage of recombination for this region in K10/k10 plants. It is likely that the structural dissimilarity existing between the distal one sixth segment of normal chromosome 10 and the corresponding segment of abnormal chromosome 10 is responsible for the great reduction in recombination in the *r-sr* region. If the morphologically different segment of the abnormal chromosome 10 did not contain loci normally present in chromosome 10, stocks homozygous for abnormal chromosome 10 would be deficient for the loci in the distal segment. Deficiencies in this segment are not tolerated in the male gametophyte as has been shown by translocation studies (ANDERSON, unpublished data).

GENERAL DISCUSSION

There is little doubt that considerable variation exists in the processes leading to preferential segregation in different organisms. In *Drosophila melanogaster*, for example, NOVITSKI (1951), NOVITSKI and SANDLER (1956), and ZIMMERING (1955), working with heteromorphic bivalents, have shown that the chromosome recovered with the higher frequency is always the shorter of the two homologues. In maize the reverse is true. Yet, the formation of heteromorphic dyads as a consequence of crossing over is a necessary prerequisite to preferential segregation in both species. Neocentromeres, which are held responsible for preferential segregation in maize, have not been reported in Drosophila. According to NOVITSKI (personal communication), the non random orientation of the chromatids, which persists through the meiotic divisions, is established at anaphase I. It is believed that the shorter chromatids of heteromorphic dyads are directed toward the poles because they offer less mechanical resistance to the poleward movement.

Another indication that preferential segregation in maize and Drosophila may be determined by divergent mechanisms comes from the study of ZIMMERING (1955). He found that complementary crossover chromatids in certain translocation heterozygotes are not recovered from females with equal frequencies while the noncrossover chromatids occur in the expected ratio of 1:1. The data from maize, on the other hand, show deviations from a 1:1 ratio in all classes, crossover and noncrossover (Table 5). It is not surprising to find preferential segregation among the noncrossover chromatids even though only bivalents with

TABLE 5

Comparisons of the ratios obtained in the noncrossover and crossover classes for all knob combinations studied. In each instance, the value shown is the frequency of the reciprocal class containing the genetic marker closest to the "preferred" knob

	Combination			Class involving a crossover in region								
				Non	*yg-c*	*c-sh*	*sh-wx*	*yg-c+c-sh*	*yg-c+sh-wx*	*c-sh+sh-wx*	*yg-wx*	*wd-wx*
a.	K^L/k	*K*10/k10	(Table 1-g)	0.67	...	...	...	...	...	...	...	0.71
b.	K^L/k	*K*10/k10	(Table 1-e)	0.68	...	...	...	...	...	...	...	0.70
c.	K^L/k	k10/k10	(Table 1-f)	0.51	...	...	...	...	...	...	...	0.49
d.	K^L/K^M	*K*10/k10	(Table 2-a)	0.57	...	...	0.63	...	...	...	...	...
e.	K^M/k	*K*10/k10	(Table 1-c)	0.64	...	...	...	...	...	...	...	0.67
f.	K^M/k	k10/k10	(Table 1-d)	0.51	...	...	...	...	...	...	...	0.53
g.	K^M/K^S	*K*10/k10	(Table 2-f)	0.62	...	0.63	0.66	...	...	0.56	...	...
h.	K^M/K^S	*K*10/k10	(Table 2-c)	0.70	0.70	0.65	0.72	0.60	0.49	0.71	0.70	...
i.	K^M/K^S	k10/k10	(Table 2-d)	0.51	0.56	0.43	0.50	0.50	0.62	1.00	0.51	...
j.	K^M/K^S	*K*10/k10	(Table 2-b)	0.71	0.64	0.67	0.73	0.54	0.62	0.60	0.70	...
k.	K^S/k	*K*10/k10	(Table 2-e)	0.61	...	0.71	0.66	...	...	0.71	...	...
l.	K^S/k	*K*10/k10	(Table 1-a)	0.58	...	...	...	...	...	...	...	0.62
m.	K^S/k	k10/k10	(Table 1-b)	0.51	...	...	...	...	...	...	...	0.51
n.		*K*10/k10	(Table 4-a)	0.74	0.73 (*g-r*)		0.62 (*r-sr*)	... (*g-r* + *r-sr*)		...	...	...
o.		k10/k10	(Table 4-b)	0.50	0.53		0.49	0.64		...	...	...
p.		*K*10/k10	(Table 4-c)	0.61	0.67		0.74	0.50		...	...	...
q.		k10/k10	(Table 4-d)	0.52	0.46		0.54	0.47		...	...	...

a crossover between the knob and the centromere undergo this phenomenon. Knobbed crossover and knobbed noncrossover chromatids are recovered with equal frequencies and in excess of the knobless strands from bivalents with chiasmata. If the proportion of such bivalents is relatively high, the 1:1 ratio of K:k strands from noncrossover bivalents would be altered by the large contribution of knobbed noncrossover strands from bivalents with chiasmata. In Drosophila translocation studies the appropriate chiasma occurs with a frequency of 12–15 percent and therefore has little influence on the ratio of long to short chromatids in the noncrossover class.

In the chromosome 9 studies of maize it is possible to estimate the expected departure from a 1:1 ratio within each crossover and noncrossover class. If it is assumed that preferential segregation of the knobbed chromatid occurs in 70 percent of the heteromorphic dyads and if reasonable chiasma frequencies are adopted (e.g., Table 5-k: 14 percent, one chiasma in the *wx* centromere region; 49 percent, one chiasma between *sh* and *wx;* 7 percent, one chiasma in the *c-sh* region; 31 percent, no chiasma), values are obtained which agree well with those shown in Table 5.

The effect of heterochromatin on segregation in Drosophila has been studied by STURTEVANT (1936) using the essentially heterochromatic chromosome IV. It was possible to identify 26 kinds of chromosome IV by testing individual chromosomes from different sources in triplo-IV females. The chromosomes IV were found to differ in their propensity to segregate with a specific member of the trivalent. In maize, differences in preferential segregation are probably associated with quantitative rather than qualitative differences in heterochromatin though the latter possibility cannot be disproven. Although knobs are sites of neocentric activity, RHOADES (1952) has shown that they do not form neocentromeres unless a true centromere is present on the chromosome. He believes that a substance from the true centromere accumulates near the knob, which either behaves as a physical barrier or actively attracts and absorbs this material. It is conceivable that a larger knob may be more effective as a block to the "flow" of the centric substance and therefore neocentric activity is increased. It is obvious that knobs play a vital role in preferential segregation, but the means by which they respond to the introduction of the abnormal chromosome 10 are not clear.

It has been demonstrated by RHOADES and confirmed by the writer that crossing over is increased in the presence of the abnormal chromosome 10. Apparently this effect is independent of preferential segregation and the production of neocentromeres since recombination is increased in bivalents which possess no knobs (RHOADES and DEMPSEY 1957) and therefore are not undergoing preferential segregation. REES (1955) has shown the existence of genes in the rye genome which influence the rate of crossing over as determined by the frequency of chiasma formation. It is possible that genetic factors controlling crossing over are situated in the non-homologous distal segment of the abnormal chromosome 10. In addition to the increased recombination induced by the abnormal chromosome 10, the data on chromosome 9 indicate that the extent of increase is greatly

influenced by the size of the knob on chromosome 9. Following introduction of the abnormal chromosome 10, the *wd-wx* recombination was altered from 12.7 percent to 30.3 percent in K^L/k plants, from 17.7 percent to 26.8 percent in K^M/k plants, and from 26.9 percent to 31.5 percent in K^S/k compounds. It is difficult to understand why there should be such a great variation in the extent to which crossing over is increased, unless the 30 percent recombination achieved in each case represents a maximum level which cannot be exceeded.

The frequency with which the knobbed homologue in a heteromorphic bivalent reaches the basal megaspore in plants carrying the abnormal chromosome 10 depends on two variables: (1) the frequency of crossing over between the knob and the centromere and (2) the frequency of preferential segregation following such a crossover. It has been shown that the percent of crossing over in chromosome 9 is increased in the presence of the abnormal chromosome 10 and that the amount of the increase is influenced by the size of the knob (K9). The frequency of preferential segregation is also variable, as can be seen in the experiments where recombination is constant. For example, the recovery of the K9 chromosome is increased in K^L/k compounds over K^S/k compounds in the presence of the abnormal chromosome 10. Since the *wd-wx* recombination is about 30 percent in both cases, it seems probable that there is a higher degree of preferential segregation in the first class of plants. A second example where different degrees of preferential segregation can be observed comes from the chromosome 10 data. Different frequencies of recovery of the *R* and Sr_2 alleles were obtained from plants grown in the field and in the greenhouse. Since the recombination values were the same for the two experiments, it has been concluded that differences exist in the behavior of the chromosomes following such crossing over, perhaps, related to the extent of neocentric activity. Thus, preferential segregation can be influenced by amounts of heterochromatin present on the bivalent in question and probably also by environmental factors associated with different growing seasons.

SUMMARY

Since the investigations of RHOADES and LONGLEY with abnormal chromosome 10 in maize have demonstrated the active participation of knobs in the phenomenon of preferential segregation, it was deemed of interest to ascertain the effect of knob size on the degree of preferential segregation. For such a study, three stocks differing in the size of the terminal knob on the short arm of chromosome 9 were utilized. Compounds were derived in which the knobbed chromosome 9 was opposed by a knobless homologue, while in other plants the homologues possessed knobs of different sizes. In all cases, the effect of varying knob size was determined by backcross ratios of genes linked to the knobs. Experiments were also conducted to determine the extent of preferential segregation and recombination among loci situated in the long arm of chromosome 10, specifically in the segment distal to the *r* locus. In the above experiments, the abnormal chromosome

10 utilized was derived from stocks used by RHOADES in his experiments on preferential segregation.

The results of the investigation reveal the following points:

1. The degree of preferential segregation of a knobbed chromosome 9 over a knobless one becomes progressively greater with increase in knob size.

2. The incidence of crossing over in chromosome 9 is increased in the presence of the abnormal chromosome 10 and the amount of increase is influenced by the size of the knob on chromosome 9. The data are in agreement with the findings of RHOADES and DEMPSEY that crossing over is increased in the presence of the abnormal chromosome 10.

3. In the three kinds of knobbed-knobless heterozygotes, the abnormal chromosome 10 increases the frequency of crossing over in the *wd-wx* region to approximately the same level (30 percent) irrespective of knob size.

4. In the knobbed-knobless heterozygotes homozygous for normal chromosome 10, there is a negative correlation between crossing over and knob size.

5. When members of a pair of chromosomes 9 have knobs of different sizes, the chromosome with the larger knob is recovered more frequently. There is evidence of competition or incomplete dominance between knobs of different sizes.

6. Evidence supports the contention that preferential segregation can occur only in the presence of the abnormal chromosome 10.

7. Crossing over between *r* and the most distal marker, sr_2, in the long arm of chromosome 10 is drastically reduced in bivalents heterozygous for the abnormal chromosome 10. Crossovers between these two genes in plants heterozygous for the abnormal chromosome 10 always occur to the left of the three chromomeres of the abnormal chromosome 10, indicating that no crossing over takes place in the non-homologous regions of the two homologues.

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