CYTOGENETIC ANALYSIS OF A KNOBBED CHROMOSOME 9 IN MAIZE

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The undersigned, appointed by the Dean of the Graduate Faculty, have examined a thesis entitled

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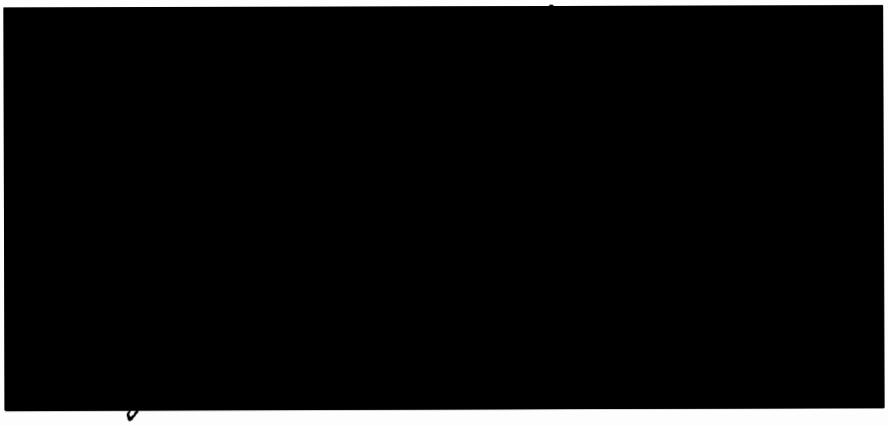

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ABSTRACT

The K^* knob of Mexican origin had a suppressive effect on recombination in the short arm of chromosome 9. It had the capacity to reduce recombination in both the distal and proximal regions or to reduce it only in the distal region (with a concomitant increase in recombination in the proximal region). The suppressive effect was stronger on the female-side than on the male-side. The suppressive effect of the K^* knob was greatest when the chromosome containing it was opposed by a homologue which was knobless. This effect became less pronounced as the size of the opposing knob became larger. It appears that the total amount of knob material present in the bivalent was not a critical factor in this suppressive effect.

In K^*-containing heteromorphs, the effectiveness of the abnormal chromosome 10 in increasing recombination in the distal region was found to be progressively less as the amount of the K^* knob not opposed by knob material in the homologue increased. It was also found that the greater the total amount of heterochromatin in the two knobs of the

bivalent, the less effective was the abnormal chromosome 10 in increasing recombination in the proximal region.

The K^* knob modified the B-chromosome effect on recombination. In megasporocytes of K^*/K^S heteromorphs, although total recombination in the short arm was enhanced in B-chromosome containing individuals over B-less plants, the K^* knob's suppressive effect was still very much in evidence. In these heteromorphs containing the B-chromosomes, recombination was increased in both the proximal and the distal regions. In the K^S/K^S compounds the B-chromosomes did not increase total recombination in the short arm but only effected a shift in recombination from the distal to the proximal region. The K^* knob did not influence the zig-zag effect on recombination induced by the odd-even number of B-chromosomes.

Previous preferential segregation studies have indicated that it is the genes linked to the larger of the two knobs of chromosome 9 bivalents which are preferentially recovered in the eggs. The K^*/K^L study has provided the first exception : genes linked to the smaller K^L knob were preferentially recovered.

No evidence was obtained to substantiate the possibility that the K^*9 knob was functionally similar to the K10 chromosome although the K^*9 and K10 knobs are morphologically quite similar. Thus it is not known whether the K^*9 knob is a transposed K10 knob or not.

ACKNOWLEDGEMENTS

The author is deeply appreciative of the guidance, counsel and assistance enthusiastically provided by Dr. G. Y. Kikudome throughout this work. Special thanks go to Dr. M. G. Neuffer for making some crosses for this experiment in winter time at Florida.

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I. INTRODUCTION

Heitz in 1928 first observed that the darkly stained bodies in telophase nuclei represent specific chromosomal regions which do not unravel during telophase and remain hetero~~chroma~~pycno tic through the cell cycle. He referred to these regions as "heterochromatin"; the regions which do unravel during telophase being referred to as "euchromatin". Since its discovery heterochromatin has been found to be of common occurrence in both plant and animal chromosomes. A number of unique properties has been assigned to heterochromatin. These include : genetic inertness, position effect, high breakability, late replication in the S-phase of the cell cycle, high content of redundant DNA with characteristic base composition, in addition to the capacity to alter the frequency and distribution of recombination and chiasma formation.

In the maize genome the most conspicuous heterochromatic elements are the knobs, present at characteristic positions in distinctive size in normal chromosomes and in abnormal chromosome 10, and the supernumerary or B-chromosomes. Several intriguing phenomena have been ascribed to these heterochromatic elements : neocentric activity, preferential segregation, mitotic nondisjunction, preferential fertilization, chromosomal elimination, insensitivity to paramutation, and alteration of the frequency of crossing over and genetic recombination.

Recently a chromosome 9 of Mexican origin was found to possess an unusually large terminal knob in the short arm. Morphologically it is similar to the one located in the abnormal chromosome 10. The present study was undertaken to ascertain the following : (a) whether this knobbed chromosome 9 is functionally similar to the abnormal chromosome 10 inasmuch as the knob of this chromosome 9 could conceivably be one transposed from the abnormal chromosome 10, (b) the behavior of this knob in the presence of the abnormal chromosome 10, and (c) the influence of this knob on the B-chromosome effect on recombination.

II. REVIEW OF LITERATURE

Recombination and its Modification in Heterochromatic Chromosomal Regions

Painter and Muller (1929, 1932) first constructed a cytological map of the X-chromosome in *Drosophila melanogaster*. When the cytological map was compared to the genetic map, they found that the distances between the genes located near the centric heterochromatic region were relatively much longer cytologically than suggested by the genetic map. The same holds true for chromosome II (Dobzhanshy 1930). This discrepancy between the genetic and cytological maps was explained by Dobzhansky (1930) on the basis of differential crossing over in different chromosomal regions. Rick (1970) made a more extensive and recent comparison of cytological and genetical maps of tomato, maize and *Drosophila*. According to him, with the possible exception of nucleolar chromosomes, there seems to be a general agreement that recombination occurs very infrequently in the proximal heterochromatin flanking the centromere.

This suppression of crossing over in centric heterochromatin could be due to centromere effect or heterochromatin effect or both. Not to be excluded is the possibility that in some species effective pairing for crossing over in the proximal region is not as complete as in the distal region if pairing is initiated at the distal ends and is terminated within a specified time limit (see Darlington

1940). Evidence for the centromere effect comes from Beadle's (1932) data involving a homozygous III-IV translocation and from Mather's (1939) data on homozygous X-chromosome inversions in Drosophila melanogaster. Support for the suppression effect of heterochromatin can be found in the works of Callan and Montalenti (1947) and White (1942, 1951) with the insect Mecostethus. Their data indicate the participation of centric heterochromatin in the localization of proximal chiasma in the proximal euchromatin bordering the centric heterochromatin.

A possible indication that strong heteropycnosis may interfere with chiasma formation in the heterochromatic segment is noted. In tetraploid spermatocytes of several species of grasshopper, while the autosomes do form quadrivalents, the two X-chromosomes which are heteropycnotic do not form bivalents. In oocytes, however, the two X's are not heteropycnotic and do form bivalents (White 1951).

That the recombination frequency in the proximal region is more sensitive to modification by genetic and environmental factors is well documented. Temperature (Plough 1917; Towe and Stadler 1964; Grell and Chandley 1965; Grell 1967), radiation (Muller 1925; Whitinghill 1951), chemicals (Suzuki 1963; Suzuki and Parry 1964; Hayashi and Suzuki 1968), interchromosomal effect of chromosomal aberration (Schultz and Redfield 1951; Roberts 1965), and the abnormal chromosome 10 in maize (Rhoades and Dempsey 1966) are reported to increase recombination in the proximal regions.

The increase in recombination induced in the proximal or centric regions by various agents was generally assumed to be related to the presence of proximal heterochromatin. Various hypotheses based on this assumption have been proposed to account for this increase (see Yost and Benneyan 1957, for review). Two of them may be mentioned here. The recombinagenic agents may directly participate in the recombination processes, e.g. they may have the ability to cause chromosome breakage. The high breakability of heterochromatin and the fusion of the broken ends of chromosomes resulting in viable exchanges could explain the increase in recombination observed in the proximal region (Whitinghill 1955). Another hypothesis, postulated by Suzuki (1963), is based on the assumption that chromosomal regions that are active in the synthesis of mRNA are structurally incapable of undergoing crossing over. The recombinagenic agents are supposed to affect crossing over indirectly by inhibiting the genetic activity of genes in the proximal heterochromatin which normally function at the time of crossing over, thus favoring the occurrence of crossing over.

Mather (1939) concluded from his studies of different homozygous X-chromosome inversions of Drosophila melanogaster that high sensitivity of crossing over to temperature is a property of the heterochromatin. To the contrary, Lawrence (1963) who studied the effect of temperature on crossing over in the X-chromosome of Drosophila found that environmental sensitivity is unrelated to the distribution of

heterochromatin. Thompson (1964) reported that crossing over may be increased by high temperature in proximal regions in which the centromere effect has previously been eliminated -- the inhibitory effect of the centromere has been shown to depend on contiguous centromere pairing. From his study on X-ray induced crossovers in structurally different chromosomes of Drosophila melanogaster, Puro (1969) concluded that the frequency of induced recombination is merely a function of the number of salivary chromosome bands between gene loci and not of hypersensitivity of heterochromatin. The apparent greater increase is due to the fact that heterochromatic region has longer physical length per crossing over unit than does the euchromatic region.

The interchromosomal effect of chromosome aberrations on recombination has been described as being most pronounced in the proximal heterochromatic region. Is this increase really in the heterochromatic region or in the euchromatic region immediately adjacent to the heterochromatin? Data obtained by Roberts (1965) indicate that the effect is pronounced in the proximal euchromatin but absent in the heterochromatin. Grell (1967) reported that temperature-induced crossing over occurred within heterochromatin. On the other hand, Hayashi and Suzuki (1968) found no induction of crossing over within the heterochromatin by temperature. Roberts' and Hayashi and Suzuki's data may indicate the importance of the close proximity of heterochromatin to the euchromatin in which crossing over is increased. It is also

possible that there may be two general classes of recombinagens, those that affect crossing over in the proximal euchromatin and those which also induce recombination in the heterochromatin (Hayashi and Suzuki 1968).

Chromosome Structural Changes Involving Heterochromatin and Interchromosomal Effect on Recombination

Schultz and Redfield (1951) compared the interchromosomal effects of two inversions which were identical in so far as the length of the euchromatic segment involved but differed in the relative amounts of proximal and distal heterochromatin involved. The results led them to conclude that heterochromatic regions of chromosome may play a key role in interchromosomal effect. A more extensive test was undertaken by Suzuki (1963). He tested the effects of eight different X-chromosome inversions on crossing over in chromosome III. In heterozygous condition all eight inversions increased autosomal recombination. In homozygous condition, however, two of the eight inversions failed to induce an interchromosomal effect. These two were wholly euchromatic rearrangements. The common feature of the six inversions that were capable of inducing an interchromosomal effect in the homozygous condition was the proximity of proximal heterochromatin to the distal tip. Suzuki concluded that the causative factor was this new neighboring of distal tip and proximal heterochromatin rather than the reversal in orientation of the euchromatic region *per se*.

Hinton (1965) reported that interchromosomal effect of

translocation heterozygotes involving chromosome II and III in Drosophila depends on the position of the breakpoints. Short interstitial distances (breakpoint in proximal heterochromatin) resulted in a decrease and long interstitial distances in an increase in recombination, whereas translocations with intermediate distances failed to alter the level of recombination. In the grasshopper Cibolacris parviceps, Hewitt (1967) found a chromosomal interchange which considerably raised the chiasma frequency in all of the chromosomes, including the interchange itself. Both of the chromosomes involved in the interchange had pronounced heterochromatic regions. Hence, a possible involvement of heterochromatin in altering recombination was suspected.

Heterochromatic Supernumerary Chromosome Segment and Recombination

In the meadow grasshopper Chorthippus parallelus, certain chromosomes possess heterochromatic supernumerary segments. The presence of such a segment causes a significant elevation of the mean chiasma frequency as compared to the absence of such segment in individuals from the same population (John and Hewitt 1966; Hewitt and John 1968).

The abnormal chromosome 10 in maize differs from the normal one in the chromomere pattern of the distal region of the long arm and by the presence of an extra segment attached to the distal end of the long arm. The extra segment consists of a large heterochromatic knob and proximal and

distal euchromatic regions. Rhoades and Dempsey (1957) first reported that abnormal chromosome 10 (K10) increases crossing over in chromosome 3. This conclusion was confirmed by Kikudome (1959) in chromosome 9 and by Nel (1968) in the proximal a-bt-pr region of chromosome 5. Kikudome's data show that the amount of increase produced by the K10 chromosome is influenced by the size of the knob on chromosome 9. In the presence of the K10 chromosome, recombination in the wd-wx region was increased from 12.7% to 30.3% in K^L/k plants, from 17.7% to 26.8% in K^M/k plants and from 26.9% to 31.5% in K^S/k plants. Rhoades and Dempsey (1966) reported that abnormal chromosome 10 increased crossing over in both normal and structurally rearranged chromosomes 3. The K10 chromosome had little effect, if any, on recombination in the distal lg-a region when both normal homologues of chromosome 3 were either knobbed or knobless, but produced a highly significant increase in the frequency of crossing over in the proximal gl-lg interval. Although the K10 chromosome had little effect on recombination in the distal lg-a region in homomorphic pairs, it tended to reverse the decrease in this region caused by knob heteromorphy.

Additional evidence of the ability of the K10 chromosome to increase crossing over in structurally rearranged chromosomes was provided by the study of plants heterozygous for a reciprocal translocation between chromosomes 6 and 9 (Dempsey and Rhoades 1961). Plants with the K10 chromosome had more genetic crossing over in the sh-wx region than did

normal chromosome 10 sibs and had more chiasmata as indicated both by the higher percentage of Rings of Four (type of quadrivalent) and by the drastic reduction in the frequency of trivalents. In autotetraploid maize, Snope (1967) reported that Chain of Four quadrivalents were reduced from a frequency of 46% in controls with four normal chromosomes 10 to 36%, with a concomitant increase in the frequency of Ring of Four quadrivalents, in plants with one or two K10 chromosomes. This was interpreted as reflecting a K10 chromosome induced increase in chiasma formation.

Heterochromatic Supernumerary or B-chromosomes and Recombination

Influence of the B-chromosomes on recombination has been reported in several species of plants and animals (John and Hewitt 1965; Hewitt and John 1967; Jones and Rees 1967; Cameron and Rees 1967; Rhoades 1968; Ayonoadu and Rees 1968; Hanson 1969; Nel 1969; Zecevic and Paunovic 1969; Barlow and Vosa 1970). The effects in the different species seem to be different. These include increase or decrease in total chiasma or recombination frequency, increase or decrease in the mean variance of chiasma frequency, and change in distribution of chiasma or recombination frequency. With increasing number of B-chromosomes, the effect on recombination can be additive or non-additive. If additive, the increase can be a linear rise or zig-zag rise. The variety of effects of the B-chromosomes on recombination in differ-

ent species studied are listed in Appendix 1.

An "odd-even" or "zig-zag" effect of B's on A-chromosomes' recombination was reported in rye by Jones and Rees (1967). They found that B-chromosomes increased the mean cell variances for within plants chiasma frequency. The mean variances increased with increasing numbers of B's but not in a linear fashion. The effect of a given odd-numbered B-chromosome class was consistently higher than that of even-numbered B-chromosome classes preceding or following it. This "zig-zag" rise effect was also noted by Barlow and Vosa (1970) in Listera ovata where B-chromosomes increase the chiasma frequency in both pollen and egg mother cells.

The effect of the B-chromosome on recombination in maize was first investigated by Hanson (1961, 1962, 1969). He found that the recombination frequencies in chromosomes 3 and 9 were enhanced by a higher number of B-chromosomes. The enhancement was accompanied by a decrease in chromosomal interference. In the gl-lg-a-et segment of chromosome 3, recombination was increased in all regions in the presence of B's, but there was a greater increase in the proximal gl-lg region than in the other regions. In the yg-c-sh-wx segment of chromosome 9, recombination was enhanced in the c-sh-wx region, but there was a decrease in the distal yg-c region. Rhoades (1968) studied recombination in an altered chromosome 9 containing a transposed piece of chromosome 3 which was inserted between the markers sh and wx. In plants homozygous for the transposition, recombination in the c-wx

region was not increased because of the presence of the transposed chromosome segment. However, in the presence of low numbers of B-chromosomes, recombination in the c-wx region was drastically increased. The effect of increasing numbers of B's appears to be that of increasing, additively, recombination in the c-wx region and of decreasing, additively, the amount of crossing over in the adjacent but distal yg-c region; an indication of a shift in the distribution of crossing over along the chromosome arm. Nel (1968, 1969) studied the effect of the B-chromosomes on crossing over in chromosome 5. He found that in the a-bt-pr region, crossing over was increased additively with increasing numbers of B's. The effect was more marked in microsporocytes than in megasporocytes. He also obtained recombination data from plants containing both B-chromosomes and abnormal chromosome 10. The results indicate that the combining effect of B's and K10 chromosome was greater than the effect of B's or K10 chromosome alone on the male-side. On the female-side, the effect of K10 chromosome and B's was greater than the effect of B's alone but was approximately of the same magnitude as that for K10 chromosome alone (Nel 1968).

Proposed Mechanisms by which Heterochromatin affect Recombination

Darlington (1937) postulated that precocious condensation or heteropycnosis of homologous chromosomes during meiosis prevents their intimate synapsis. Thus he attempted

to explain the lack of crossing over in heterochromatic segments, in general, and in sex chromosomes, in particular. On the other hand, Suzuki (1963) offered the following hypothesis to explain the dearth of recombination in the heterochromatic regions. Chromosomal segments undergoing genetic activities are structurally incapable of undergoing crossing over and that the genes functioning in meiosis are located in heterochromatic regions.

Several hypotheses have been proposed for the interchromosomal effect of chromosome aberrations on recombination, and most of them presuppose the involvement of heterochromatin. Some models (mechanical) are based on the property of non-homologous pairing of heterochromatin, while others (physiological) are based on position effects which are associated with heterochromatin (see discussion in Lucchesi and Suzuki 1968). Dissatisfied with all these models, Lucchesi and Suzuki postulated that interchromosomal effect on recombination is accomplished through the alteration of the time for pairing and crossing over.

Rhoades and Dempsey (1966) observed in maize that pairing in inversion heterozygotes (In 3b) was more intimate if abnormal chromosome 10 was present. They attributed the increase in recombination observed to this intimate pachytene pairing caused by the abnormal chromosome 10.

B-chromosomes in both Secale and Puschkinia extend the mitotic cycle (Ayonoadu and Rees 1968; Barlow and Vosa 1969) and they extend the duration of pollen development in

Sorghum purpureo-sericeum (Darlington and Thomas 1941). Hence the possibility that B-chromosomes could modify the frequency of recombination by altering the time-course of meiosis was entertained by Barlow and Vosa (1970). According to Barlow and Vosa, B-chromosomes may also be responsible for modification of the genetic component involved in chiasma localization and cause a change in chiasma distribution.

The greater increase of recombination in the proximal region by environmental factors was explained by either a direct effect of recombinagen involving specific breakage in heterochromatin (Whitinghill 1955) or an indirect effect involving the inhibition of genetic activity in heterochromatin by recombinagens (Suzuki 1963).

III. MATERIALS AND METHODS

The chromosome 9 (K^*9) of Mexican origin employed in this study was obtained from H. L. Everett of Cornell University. It possesses a large knob which is morphologically similar to that found on abnormal chromosome 10 (K10). The K^*9 knob is, on the average, 4.4 u long and 1.2 u wide and is larger than the large knob (K^L9) used by Kikudome (1959) in his study of knob size and preferential segregation.

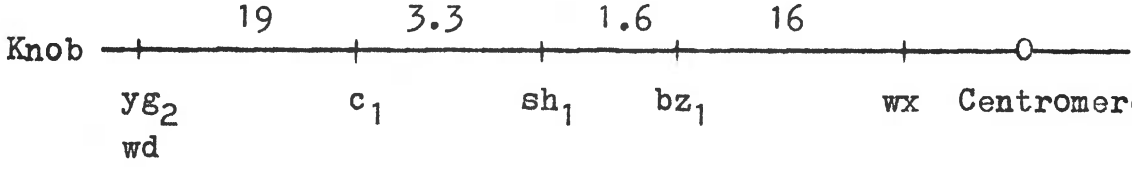
Inasmuch as the K^*9 knob resembled the knob on abnormal chromosome 10 it occurred to us that it could well be a knob which was transposed from an abnormal chromosome 10. If this knob is indeed an abnormal chromosome 10 knob it could conceivably possess the abnormal chromosome 10 attributes. These are : induction of neocentromere formation and preferential segregation of homologous chromosomes with dissimilar knobs, and increase in recombination frequency in other chromosomes.

The first part of this study was therefore designed to test whether the K^*9 knob is functionally similar to the abnormal chromosome 10. The K^*9 chromosome was also examined for its segregation behavior in the presence of abnormal chromosome 10.

The ability of the K^*9 chromosome to cause preferential segregation was tested in three different chromosome 9 heteromorphs : K^*/k, K^*/K^S, and K^*/K^L. The small knob (K^S9)

used in this test has similar dimensions to that found on chromosome 9 of inbred line KYS. The knobless chromosome 9 (k9) is actually deficient for the terminal knob and part of the first adjacent chromomere. The knobs used in this experiment are shown in Plates 1-3.

The following genes on the short arm of chromosome 9 were used for this study of preferential segregation and recombination : wd -- white deficiency, yg_2 -- yellow-green seedling and plant, c_1 -- colorless aleurone, sh_1 -- shrunken endosperm, bz_1 -- bronze-colored aleurone, and wx -- waxy endosperm. The linear order and standard map distances are :



The following test crosses were made to determine if the K^*9 chromosome can cause preferential segregation in chromosome 9 heteromorphs :

1 $\frac{K^* \; + \; c \; +}{k \; wd \; + \; wx}$ X yg c wx

2 $\frac{K^* \; + \; c \; + \; +}{K^S \; yg \; + \; sh \; wx}$ X yg c sh wx

3 $\frac{K^* \; + \; + \; +}{K^L \; yg \; sh \; wx}$ X yg sh wx

Microsporocytes were examined to determine whether the K^*9 chromosome is capable of eliciting neocentromere activity.

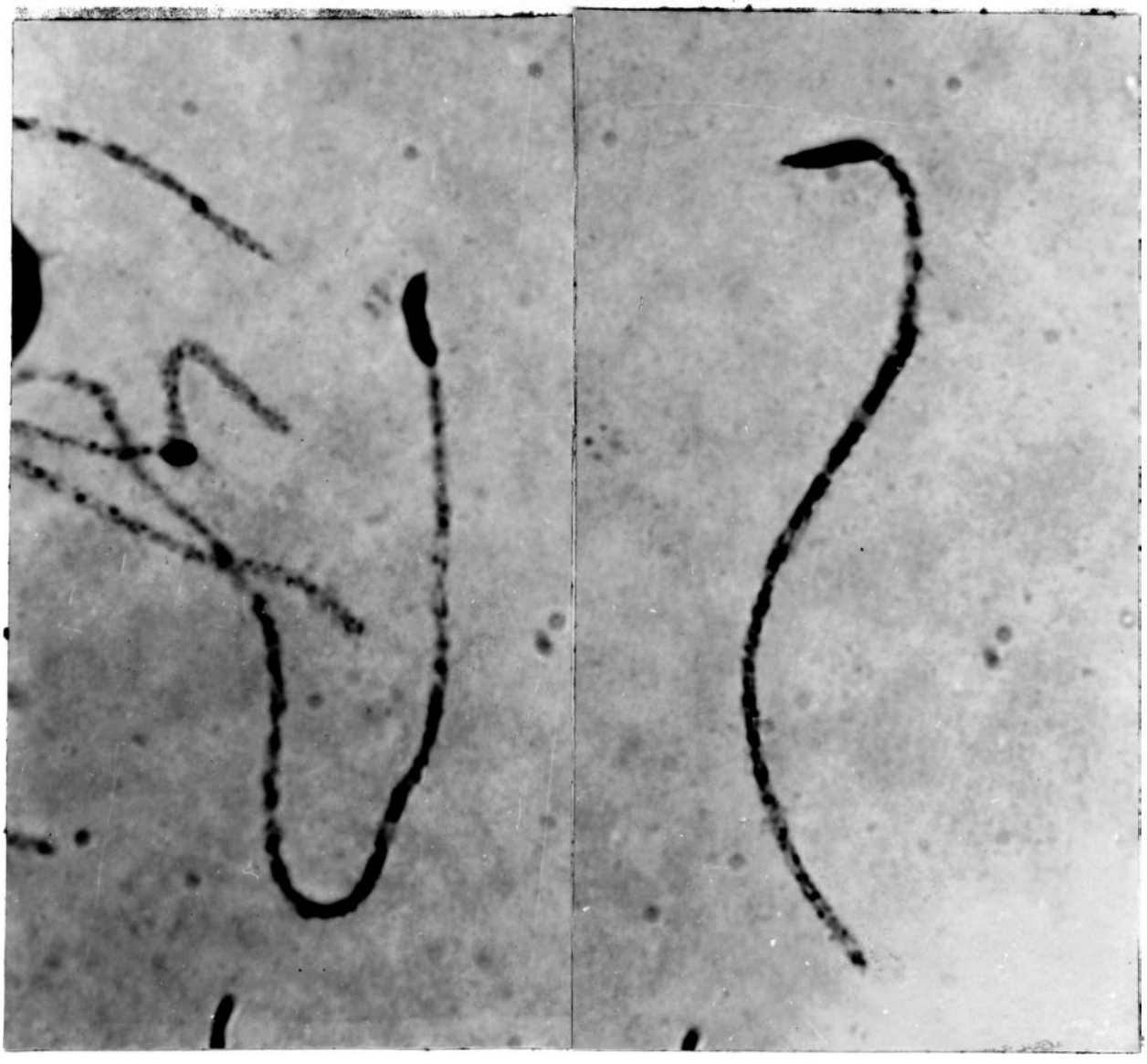


Plate 1 : Figures 1 and 2 -- Chromosome 9 bivalents homomorphic for $K^{*}9/K^{*}9$ at pachytene stage.

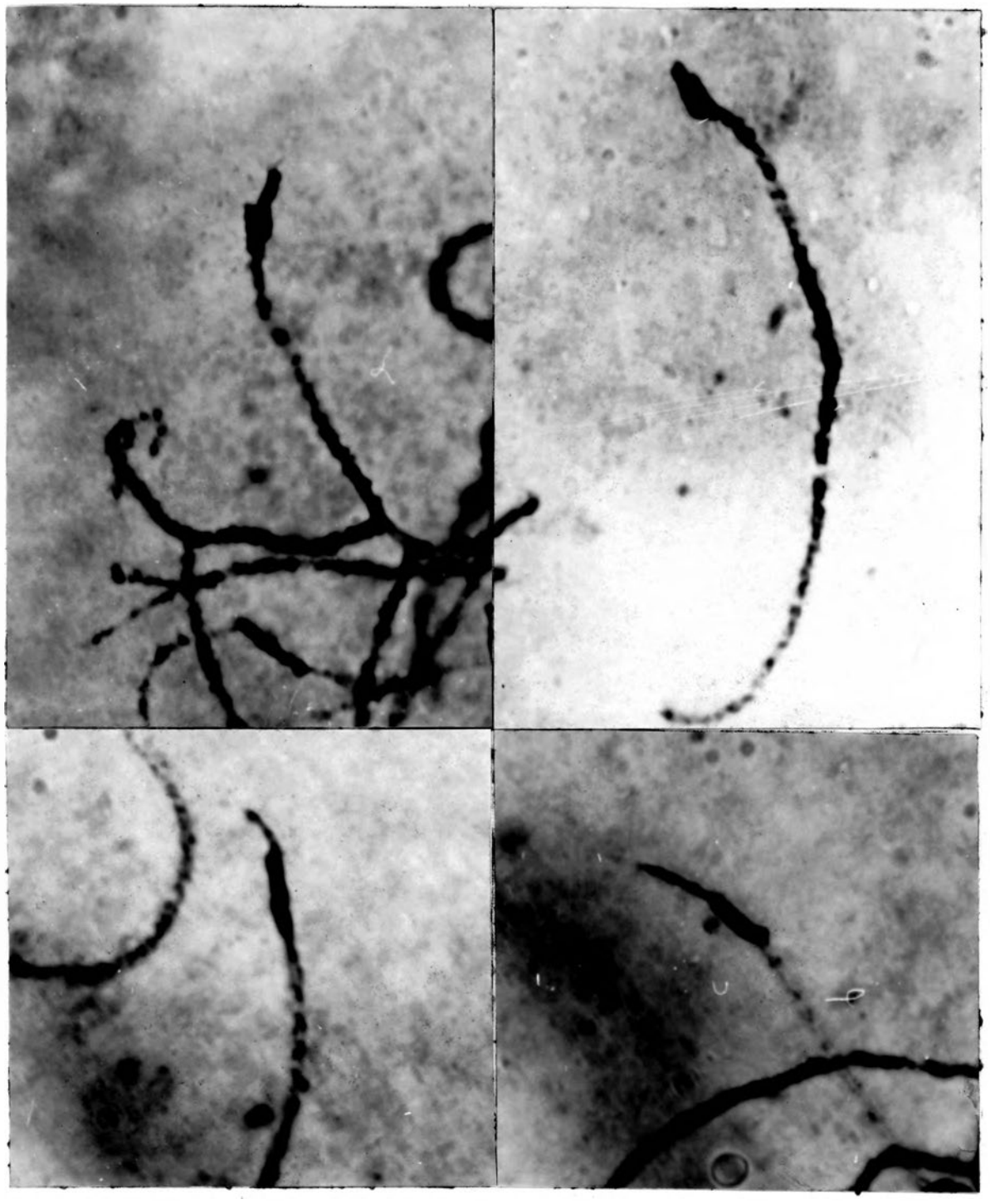


Plate 2 : Figures 1-4 -- Chromosome 9 bivalents heteromorphic for $K^{*}9/K^{L}9$ at pachytene stage.

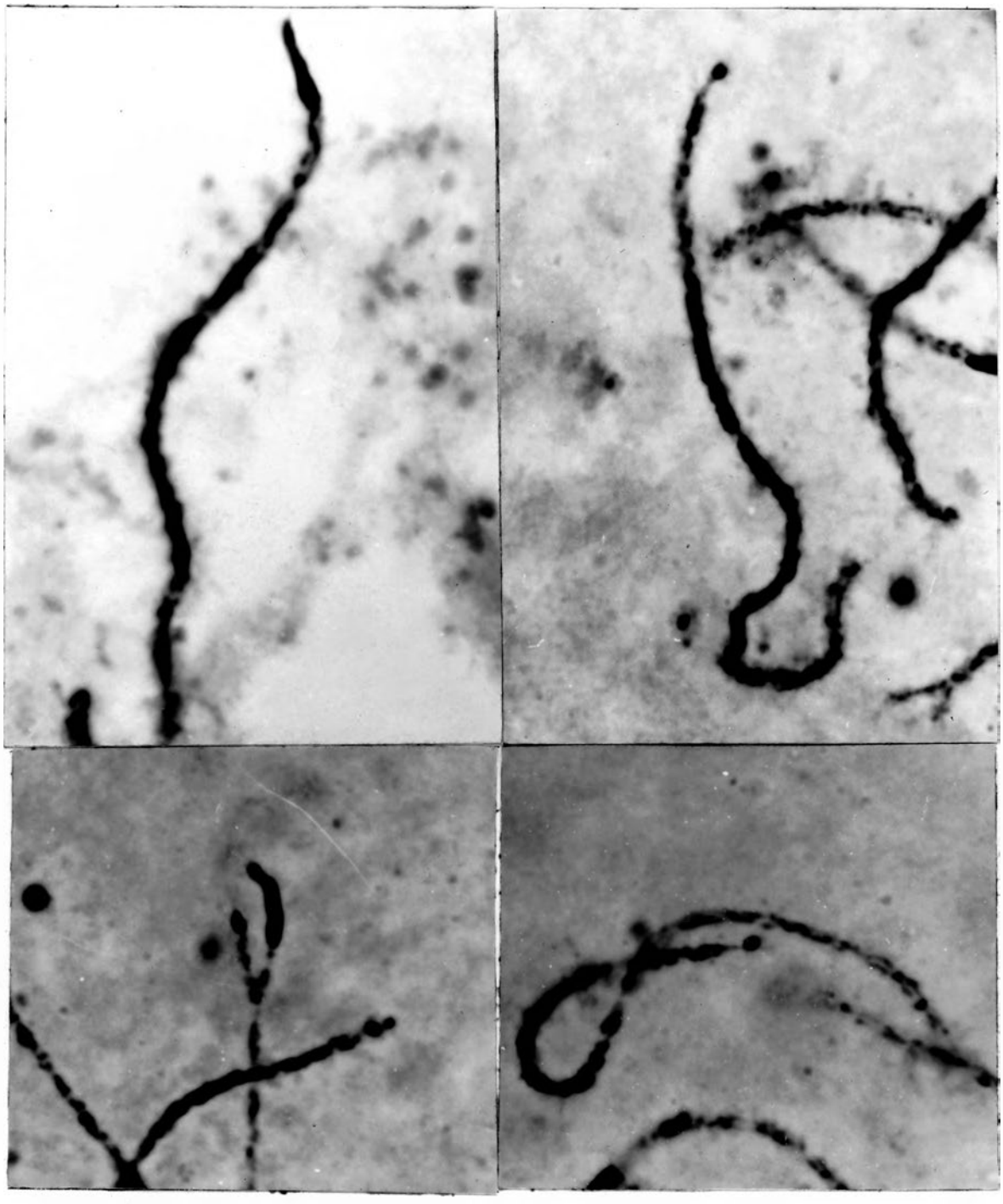
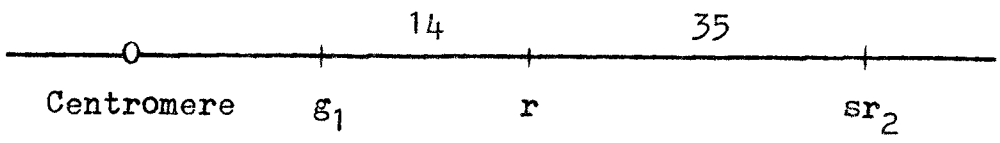


Plate 3 : Figures 1 & 2 (left) -- Chromosome 9 bivalents heteromorphic for $K^{*}9/K^{S}9$ at pachytene stage showing knobs paired and unpaired. Figures 3 & 4 (right) -- Chromosome 9 bivalents heteromorphic for $K^{S}9/k9$ at pachytene stage.

To ascertain whether the $K^{*}9$ chromosome has the ability to enhance crossing over in other chromosomes as does the abnormal chromosome 10, the g_1-r-sr_2 region of normal chromosome 10 (k10) was examined. The genes, g_1 -- "golden" plant, r -- colorless aleurone, and sr_2 -- white striation on plant, are shown in the following map :



Long arm

The recombination frequencies obtained from the following sib crosses were analyzed :

1 $\frac{k10\ +\ +\ +}{k10\ g_1\ r\ sr_2}$; $\frac{K^{*}9}{K^{S}9}$ X $g_1\ r\ sr_2$

2 $\frac{k10\ +\ +\ +}{k10\ g_1\ r\ sr_2}$; $\frac{K^{S}9}{K^{S}9}$ X $g_1\ r\ sr_2$

Experimental results so far reported (e.g. Kikudome 1959) have indicated that in the presence of abnormal chromosome 10 genes linked to the larger of the two knobs are preferentially recovered. Is size of the knob the critical factor in preferential segregation? Could the quality of the knob have a deciding role in preferential segregation as well as size? If the $K^{*}9$ chromosome is capable of inducing preferential segregation, what would be the consequence when abnormal chromosome 10 is present in the same nucleus? To answer these questions the following crosses were made :

1 $\frac{K^{*} + c +}{k \; wd \; + \; wd}$; $\frac{K10}{k10}$ X yg c wx

2 " ; $\frac{k10}{k10}$ X "

3 $\frac{K^{*} + c + +}{K^{S} \; yg \; + \; sh \; wx}$; $\frac{K10}{k10}$ X yg c sh wx

4 " ; $\frac{k10}{k10}$ X "

5 $\frac{K^{*} + + +}{K^{L} \; yg \; sh \; wx}$; $\frac{K10}{k10}$ X yg sh wx

6 " ; $\frac{k10}{k10}$ X "

The abnormal chromosome 10 used in this experiment was derived from the stock of Kikudome (1959) (Plate 4, Figure 1).

The second part of this study was to determine the effect of the K^{*} knob on recombination in the yg-wx region of chromosome 9 and its influence on the abnormal chromosome 10 and the B-chromosome effects on recombination.

1. The effect on recombination in chromosome 9.

The effect of the K^{*} knob on recombination in chromosome 9 was studied in K^{*}/K^{S} and K^{*}/k heteromorphs using K^{S}/K^{S} and K^{S}/k heteromorphs respectively as controls :

1 $\frac{K^{*} + c + +}{K^{S} \; yg \; + \; sh \; wx}$ X yg c sh wx

2 $\frac{K^{S} + c + +}{K^{S} \; yg \; + \; sh \; wx}$ X yg c sh wx

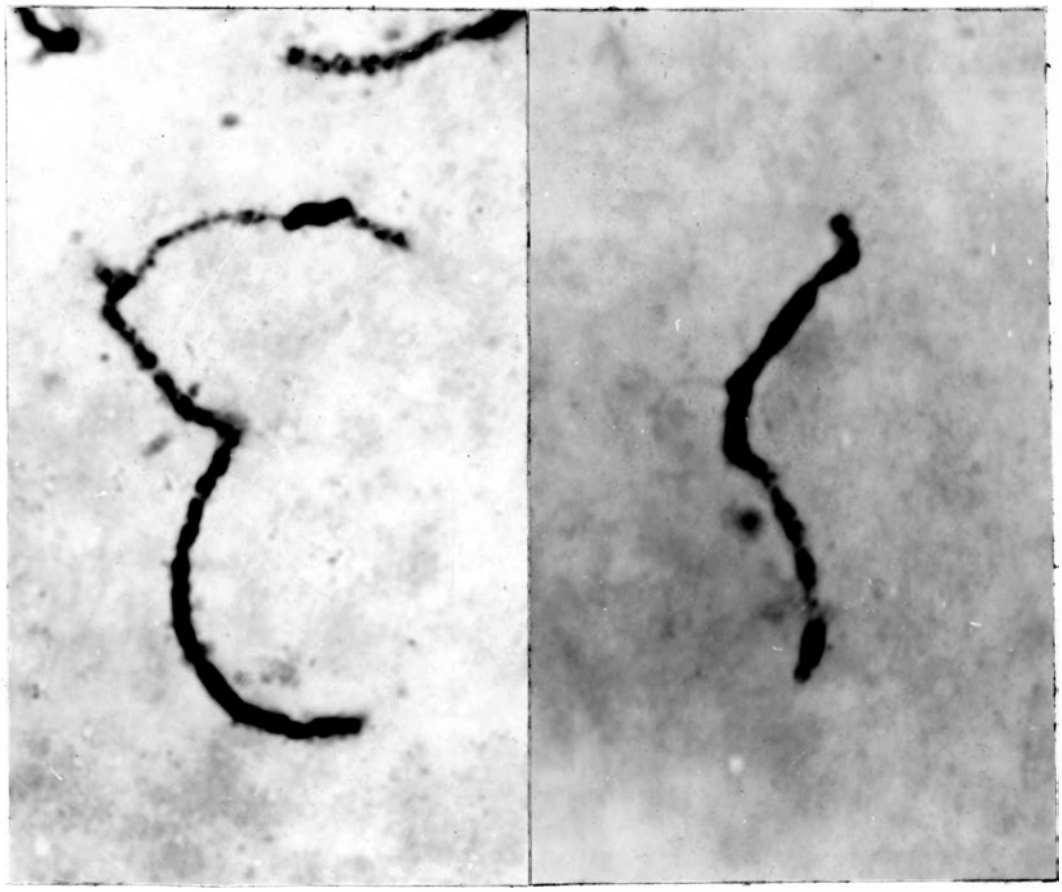


Plate 4 : Figure 1 (left) -- Chromosome 10 bivalent heteromorphic for abnormal chromosome 10 (K10/k10) at pachytene stage. Figure 2 (right) -- B-chromosome bivalent at pachytene stage.

3 $\frac{K^{*} + + + +}{K^{S} yg\ sh\ bz\ wx}$ X yg sh bz wx

4 $\frac{K^{S} + + + +}{K^{S} yg\ sh\ bz\ wx}$ X yg sh bz wx

5 $\frac{K^{*} + c +}{k\ wd\ +\ wx}$ X yg c wx

6 $\frac{K^{S} + c +}{k\ wd\ +\ wx}$ X yg c wx

In order to determine whether it is the total amount of heterochromatin present in the knobs or the difference in the amount of heterochromatin existing between the knobs of the homologues is the determining factor in recombination, the following crosses were made :

1 $\frac{K^{*} + I + +}{K^{L} yg\ c\ sh\ wx}$ X yg C sh wx

2 $\frac{K^{*} + I + +}{K^{S} yg\ c\ sh\ wx}$ X yg C sh wx

2. <u>The effect of the interaction of $K^{*}9$ and K10 chromosomes on recombination.</u>

The abnormal chromosome is known to increase recombination in chromosome 9. If the $K^{*}9$ chromosome also has an influence on recombination, what effect would the presence of both of these chromosomes in the same nucleus have on recombination? To answer this question, recombination frequencies in the <u>yg-wx</u> region of K^{*}/k, K^{*}/K^{S}, and K^{*}/K^{L} heteromorphs in the presence and absence of abnormal chromo-

some 10 obtained from the following crosses were analyzed :

1 $\frac{K^{*} + c +}{k\ wd + wx}$; $\frac{K10}{k10}$ X yg c wx

2 " ; $\frac{k10}{k10}$ X "

3 $\frac{K^{*} + c + +}{K^{S}\ yg + sh\ wx}$; $\frac{K10}{k10}$ X yg c sh wx

4 " $\frac{k10}{k10}$ X "

5 $\frac{K^{*} + + +}{K^{L}\ yg\ sh\ wx}$; $\frac{K10}{k10}$ X yg sh wx

6 " ; $\frac{k10}{k10}$ X "

3. <u>The influence of the $K^{*}9$ chromosome on the B-chromosome-effect on recombination</u>.

The influence of the $K^{*}9$ chromosome on the B-chromosome-effect on recombination was studied by comparing the effect of different numbers of B's on recombination in K^{*}/K^{S} heteromorphs and K^{S}/K^{S} homomorphs. The data were obtained from the following crosses :

1 $\frac{K^{*} + + + +}{K^{S}\ yg\ sh\ bz\ wx}$ + B's (0-5) X yg sh bz wx

2 $\frac{K^{S} + + + +}{K^{S}\ yg\ sh\ bz\ wx}$ + B's (0-5) X yg sh bz wx

The plants employed in the above crosses are partial sibs. The original stock carrying the B-chromosomes was obtained from B. Y. Lin of the University of Wisconsin

(Acc. No. J-1630-10). The B-chromosomes used in this study are shown in Plate 4, Figure 2.

The knob constitutions and the presence of the abnormal chromosome 10 were cytologically determined in sporocytes at pachytene stage. The number of B's was determined somatically, using a slightly modified wheat root tipping technique of G. Kimber (See Appendix 2).

The frequencies of genes obtained from test crosses were tested by the Chi-square method to determine if they significantly deviated from the expectation of 1 : 1 segregation. F-tests were used for data involving the effect of different numbers of B's. When two recombination frequencies were compared, the following formula was used :

$$d = (k_1 - k_2) / [\, k\,(1 - k)\,(1/n_1 + 1/n_2)\,]^{\frac{1}{2}}$$

where :

k_1, k_2 and k = observed crossover frequency of sample 1, 2, and combined sample of 1 and 2 respectively;

n_1 and n_2 = total number of individuals in sample 1 and 2 respectively;

d as normal variable with zero mean and unit standard deviation.

When coincidence values were compared, the following formula was used to calculate the standard error of coincidence (Steven 1936) :

$$E_c = C\,[\,(2C - 1)/n - 1/A - 1/B + 1/D\,]^{\frac{1}{2}}$$

where :

E_c = standard error for coincidence;

C = coefficient of coincidence;

n = total number counted;

A = number of crossovers in the first interval;

B = number of crossovers in the second interval;

D = number of double crossovers.

IV. RESULTS

1. Test for the functional similarity of the K^*9 chromosome to the abnormal chromosome 10.

The ability of the K^*9 chromosome to induce preferential segregation was tested in three chromosome 9 heteromorphs : K^*/k, K^*/K^S, and K^*/K^L. The results are presented in Table 1 (entries 1, 3, 5). In K^*/k heteromorphs, the genes, Yg, c, and Wx, linked to the K^*9 knob were not preferentially recovered in the eggs. The percentages were 49.4, 49.6, and 49.3, respectively. Similarly, the genes in the K^*9 homologue in K^*/K^S individuals were also randomly recovered. The values obtained were : Yg = 50.3%, c = 50.4%, Sh = 50.6%, Wx = 50.7%. The data from K^*/K^L heteromorphs showed the same results (Yg = 49.5%, Sh = 49.9%, Wx = 50.0%). These observations indicate that the K^*9 chromosome is incapable of inducing preferential segregation in chromosome 9.

Pollen mother cells containing one or two K^*9 chromosomes were cytologically examined for signs of neocentromere activity. No such activity was observed.

The results from the experiments to test whether the K^*9 chromosome has the ability to influence recombination in chromosome 10 are presented in Table 2. The recombination percentages in g_1-r, r-sr_2 regions were 13.36 and 33.32 respectively for plants carrying the K^*9 chromosome. These values are not significantly different from those obtained from the sib controls without the K^*9 chromosome (g_1-r =

TABLE 1

THE RECOVERY OF GENES ON CHROMOSOME 9 HETEROMORPHS BEARING THE K^*9 KNOB FOLLOWING TEST CROSSES. THE BRACKETED COMBINATIONS ARE SIBS

	Female parent	Yg %	c %	Sh %	Wx %	Total progeny
1	$\frac{K^* \; + \; c \; +}{k \; wd \; + \; wx}$; $\frac{k10}{k10}$	49.4	49.6		49.3	2438
2	" ; $\frac{K10}{k10}$	64.2**	63.5**		54.4**	6881
3	$\frac{K^* \; + \; c \; + \; +}{K^S \; yg \; + \; sh \; wx}$; $\frac{k10}{k10}$	50.3	50.4	50.6	50.7	9962
4	" ; $\frac{K10}{k10}$	68.6**	66.3**	64.8**	52.5**	7321
5	$\frac{K^* \; + \; + \; +}{K^L \; yg \; sh \; wx}$; $\frac{k10}{k10}$	49.5		49.9	50.0	4335
6	" ; $\frac{K10}{k10}$	45.6**		47.2**	49.3*	12412

* and ** -- significant at 5% and 1% levels, respectively 50% and control values.

The original data are in Appendixes 9, 11, and 13. /from

TABLE 2

THE RECOMBINATION PERCENTAGES IN THE g-r-sr REGION OF CHROMOSOME 10 OBTAINED FROM TESTCROSSES (SIB COMPARISON)

	k10 $\frac{+^1 \quad +^2 \quad +}{g_1 \quad r \quad sr_2}$ ♀ k10	Non-crossovers	Crossovers in region			Total recombination			Total progeny
			1	2	1.2	1	2	Σ	
1	K^S9/K^S9	53.50	13.25	32.73	0.52	13.77	33.25	47.02	3819
2	K^*9/K^S9	54.08	12.60	32.56	0.76	13.36	33.32	46.68	4364

The values in entry 2 are not statistically significant from the control values (entry 1).

The original data are in Appendix 13.

13.77%, $\underline{r}$-$\underline{sr}_2$ = 33.25%).

These experiments, involving tests for the occurrence of preferential segregation, neocentromere formation, and the effect of the K*9 chromosome on recombination in chromosome 10, failed to indicate any functional similarity of the K*9 chromosome to the abnormal chromosome 10. Structurally altered abnormal chromosome 10 has been reported to cause preferential segregation in chromosome 9 but not in chromosome 10 (Emmerling 1959). This raises the possibility that although the K*9 chromosome does not cause preferential segregation in chromosome 9, it does have an effect on other chromosomes. This is an unlikely possibility, since no neocentromere activity was observed in sporocytes containing the K*9 chromosome. Neocentromere activity and preferential segregation induced by the abnormal chromosome 10 are considered to be related. Reduced neocentromere activity accompanied by weaker or no preferential segregation has been reported (Emmerling 1959; Kikudome 1961), but preferential segregation without neocentromere activity has never been observed in maize. The present results permit the statement that either the K*9 knob was not transposed from the abnormal chromosome 10 or it was but its function has been altered after the transposition.

2. <u>The segregational behavior of the K*9 chromosome in the presence of the abnormal chromosome 10</u>.

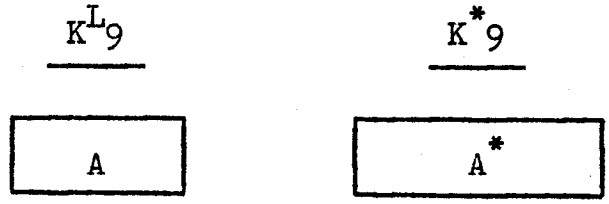
In the presence of the abnormal chromosome 10, genes on

chromosome 9 heteromorphs were differentially recovered in the eggs (Table 1, entries 2, 4, 6). This differential recovery of genes is of two categories depending on the types of knobs present : the preferential recovery of genes on the chromosome carrying the larger knob or preferential recovery of genes on the chromosome carrying the smaller knob. For $K^*9/k9$ heteromorphs, genes on the K^*9 chromosome were preferentially recovered (Table 1, entry 2). The percentages for Yg, c, and Wx alleles on the K^*9 homologue were 64.2, 63.5, 54.4, respectively, and are statistically significant from the expectation of random segregation (50%) and the control values (Table 1, entry 1). For K^*9/K^S9 heteromorphs, genes linked to the larger of the two knobs (K^*9) were preferentially recovered (Table 1, entry 4). The recovery values for the alleles linked to the K^*9 chromosome (Yg = 68.6%, c = 66.3%, Sh = 64.8%, Wx = 52.5%) are all significantly higher than the 50% expectation of random segregation. On the contrary, for K^*9/K^L9 heteromorphs, genes linked to the smaller knob of the two (K^L9) were preferentially recovered : yg = 54.4%, sh = 52.8%, wx = 50.7% (Table 1, entry 6). Statistical comparison of these values with those of the sib control (Table 1, entry 5) and with the 50% expectation value reveals them to be significant deviations (1% level : yg and sh ; 5% level : wx).

A common feature of preferential segregation for all the three heteromorphs is that genes further from the centromere showed a greater degree of preferential segregation

than genes closer to the centromere. This supports the hypothesis of Rhoades that preferential segregation occurs only when heteromorphic dyads are produced as a consequence of crossing over between gene and the centromere.

Observations heretofore (e.g. Kikudome 1959) have indicated that, in the presence of abnormal chromosome 10, genes linked to the larger of the two knobs are preferentially recovered in the eggs. The present results indicate that this is not always true. The preferential recovery of genes linked to the smaller knob, $K^{L}9$, in $K^{*}9/K^{L}9$ heteromorphs in contrast to the results obtained from $K^{*}9/k9$ and $K^{*}9/K^{S}9$ heteromorphs indicates that preferential segregation of genes can not always be predicted solely from the size of the knob. The result suggests the existence of knobs of quality difference in their response to the abnormal chromosome 10 for inducing preferential segregation. If the functional units are not different between the two knobs : $K^{*}9$ and $K^{L}9$, then two other possibilities are conceivable. The different behavior of the two knobs could be due to a difference in the amount of active genetic component in the knob or due to an organizational difference of such components. The three possibilities are diagrammatically shown as follows :



1. Difference in the quality of genetic material.

2. Difference in the quantity of the active genetic components in the knob.

3. Difference in the organization of the active genetic components.

At the moment which, if any, of these alternatives is valid remains unknown.

3. <u>The effect of the K^*9 knob on recombination in chromosome 9</u>.

Sib comparison and partial sib comparison of the recombination frequencies in K^*9/K^S9 heteromorphs and K^S9/K^S9 homomorphs are presented in Tables 3 and 4. These tables contain replications which permit confirmation of one experiment by the other. The results obtained are essentially similar. For illustration, an example is given as follows (Table 4, entries 1-4) :

+	+	+	+
yg	sh	bz	wx
1	2	3	

	Region	K^S/K^S	K^*/K^S
♀	1	23.86	8.35**
	2	2.47	1.61**
	3	15.72	19.10**
	Total	42.05	29.05**
		**	**
		**	**
	Total	46.15	48.47
♂	1	25.58	20.48**
	2	2.46	2.82
	3	18.11	25.17**

** -- significant at the 1% level from K^S/K^S for same region or from that indicated by line.

As can be seen from the illustration, recombination frequencies were reduced, at times strikingly so, in the distal region (Region 1 : yg-sh) with a concomitant increase in the proximal region (Region 3 : bz-wx) in K^*/K^S heteromorphs as compared to the K^S/K^S homomorphs. Although this effect was found in both micro- and megasporocytes, the degrees of the effect, however, were different. In megasporocytes recombination frequency was drastically reduced in the distal region from 23.86% (K^S/K^S) to 8.35% (K^*/K^S) but concomitantly recombination frequency in the proximal region was increased from 15.72% (K^S/K^S) to 19.10% (K^*/K^S). In microsporocytes, the decrease in the distal region was

TABLE 3

CROSSOVER PERCENTAGES IN K^S/K^S HOMOMORPHS AND K^*/K^S HETEROMORPHS. THE BRACKETED COMBINATIONS ARE SIBS

+ c + + / yg + sh wx (1 2 3)		Recombination 1	2	3	Total	Total progeny
1 K^S/K^S	♀	17.25	4.81	17.96	40.02	4220
2 K^*/K^S	♀	4.63**	1.56**	18.91	25.10**	3199
3 K^S/K^S	♂	17.38	4.92	24.12	46.42	2682
4 K^*/K^S	♂	13.83**	4.06	31.48**	49.38*	2487
		(yg-sh)				
5 K^S/K^S	♀	22.06		17.64	39.70	2896
6 K^*/K^S	♀	7.86**		18.50	26.36**	2887
7 K^S/K^S	♀	25.39		15.95	41.34	3041
8 K^*/K^S	♀	7.80**		22.14**	29.94**	3333

* and ** -- significant at 5% and 1% levels, respectively from its sib control value of same sex.

The original data are in Appendixes 9-10 and 13-14.

The coincidence values (regions 1 and 3) for entries :

1 = 0.05 2 = 0.10 3 = 0.33 4 = 0.25

5 = 0.12 6 = 0.07 7 = 0.11 8 = 0.07

TABLE 4

CROSSOVER PERCENTAGES IN K^S/K^S HOMOMORPHS AND K^*/K^S HETEROMORPHS. THE BRACKETED COMBINATIONS ARE PARTLY SIBS

+ + + + / yg sh bz wx / 1 2 3		Recombination 1	2	3	Total	Total progeny
1 K^S/K^S	♀	23.86	2.47	15.72	42.05	7934
2 K^*/K^S	♀	8.35**	1.61**	19.10**	29.05**	6038
3 K^S/K^S	♂	25.58	2.46	18.11	46.15	2479
4 K^*/K^S	♂	20.48**	2.82	25.17**	48.47	3369
		(sh-wx)#				
5 K^S/K^S	♀	23.64	17.93		41.56	1523
6 K^*/K^S	♀	8.29**	21.17*		29.46**	1762
7 K^S/K^S	♂	26.61	2.17	19.15	47.93	5066
8 K^*/K^S	♂	21.49**	1.82	24.23**	47.54	5048

* and ** -- significant at 5% and 1% levels, respectively from K^S/K^S control of same sex.

-- classification of bz on the female-side was difficult, in this particular cross, so the bz locus was ignored.

The original data are in Appendixes 13-17 and 19.

The coincidence values (regions 1 and 3) for entries :

1 = 0.07 2 = 0.10 3 = 0.47 4 = 0.44

5 = 0.11 6 = 0.23 7 = 0.46 8 = 0.46

from 25.58% to 20.48% and the increase in the proximal region was from 18.11% to 25.17%.

On the female-side, the greater decrease in recombination in the distal region (- 15.51) was not fully compensated by the increase in the proximal region (+ 3.38). Therefore the K^*9 strikingly decreased the total recombination in the short arm of chromosome 9 (from 42.05% in K^S/K^S to 29.05% in K^*/K^S).

On the male-side, the reduction in recombination in the distal region was not as great as on the female-side (- 5.10 on male-side vs. - 15.51 on female-side). The reduction was compensated by the concomitant increase in the proximal region (+ 7.06). Therefore the K^*9 did not influence the total recombination in the yg-wx region (46.15% in K^S/K^S and 48.47% in K^*/K^S) but caused a shift in recombination from the distal to the proximal region.

The region between the proximal and the distal region was differentially affected by the K^*9 knob on the male-side and on the female-side. In the given example, recombination in the sh-bz region was decreased on the female-side (from 2.47% in K^S/K^S to 1.61% in K^*/K^S) but was slightly increased, although statistically not significant, on the male-side (2.46% for K^S/K^S and 2.82% for K^*/K^S). Another experiment (Table 3, entries 1-4) showed that recombination in the c-sh region was decreased from 4.81% (K^S/K^S) to 1.56% (K^*/K^S) on the female-side but not affected on the male-side (4.92% for K^S/K^S and 4.06% for K^*/K^S).

Coincidence values obtained for entries in Tables 3 and 4 reveal that the K^*9 knob has no discernible effect on the chromosomal interference in both the micro- and megasporocytes. The values obtained for the example given on page 34 are :

	Female-side	Male-side
K^*/K^S	0.10	0.44
K^S/K^S	0.07	0.47

The obtained values also reveal that chromosomal interference is lower on the male-side than on the female-side.

The effect of the K^* knob on recombination in $K^*9/k9$ heteromorphs was studied in the wd-c-wx region. The comparison of the recombination values obtained for K^*/k and K^S/k sibs used as female parents (Table 5, entries 1 and 2) shows that the K^* knob significantly decreased recombination frequencies in both the proximal and the distal regions (K^*/k : wd-c = 1.48%, c-wx = 10.55%; K^S/k : wd-c = 5.58%, c-wx = 24.30%). Comparable result was obtained from a non-sib comparison (Table 5, entries 5 and 6). The recombination values for wd-c, c-wx regions were 1.63% and 10.11%, respectively, for K^*/k. These are significantly lower than the 6.70% and 21.52% for wd-sh and sh-wx regions, respectively, found in K^S/k individuals. Although the regions employed in the non-sib comparison analysis are not identical to those used for the sib-comparison, the results obtained in both analyses are strikingly similar. Thus, the non-sib values can be said to confirm the observations made in the

TABLE 5

TEST CROSS VALUES FOR $K^*9/k9$ AND $K^S9/k9$ HETEROMORPHS. THE BRACKETED COMBINATIONS ARE SIBS

$\frac{K^* \text{ or } K^S \; + \; c \; +}{k \; wd \; + \; wx}$		Recombination			Total progeny
		1	2	Total	
1 K^S/k	♀	5.58	24.30	29.88	4951
2 K^*/k	♀	1.48**	10.55**	12.02**	5281
3 K^*/k	♀	1.40	13.29	14.69	2438
4 K^*/k	♂	9.04**	31.17**	40.21**	2621
5 K^*/k	♀	1.63	10.11	11.74	3125
$\frac{K^S \; + \; sh \; wx}{k \; wd \; + \; +}$		wd-sh	sh-wx		
6 K^S/k	♀	6.70**	21.52**	28.22**	3342

** -- significant at the 1% level from its sib. Entries 5 and 6 are compared irrespective of a short c-sh region difference.

The original data are in Appendixes 11-12.

The coincidence values for entries :

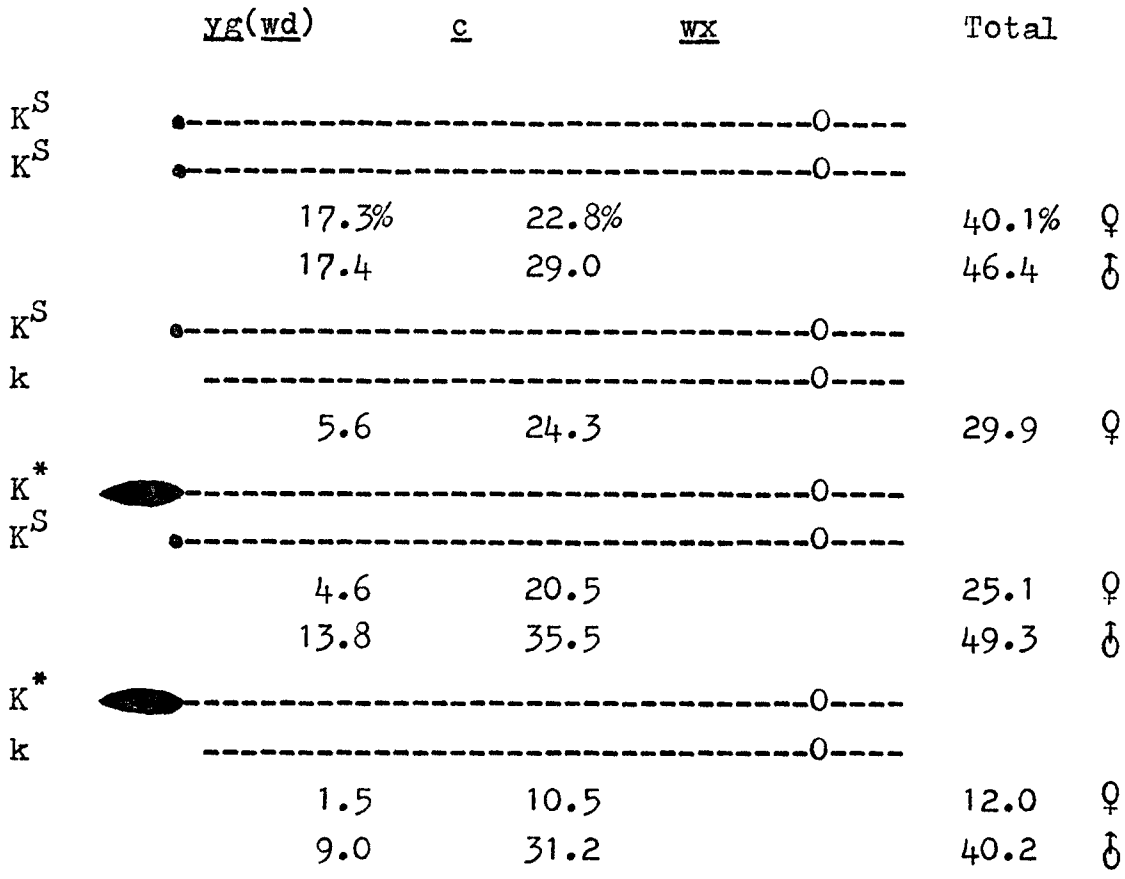
1 = 0.25 2 = 0.70 3 = 1.77 4 = 0.53

5 = 0.97 6 = 0.33

sib-comparison.

Reduction in recombination by the K^* knob in K^*/k heteromorphs in both the proximal and distal regions was found only on the female-side. On the male-side, however, recombination was reduced in the distal wd-c region (9.04%) but not in the proximal c-wx region (31.17%) (Table 5, entries 3 and 4). Total recombination in the wd-wx region in K^*/k heteromorphs was much higher on the male-side (40.21 %) than on the female-side (14.69%).

When the recombination values obtained on the female-side in K^S/K^S, K^*/K^S, and K^*/k individuals for the various subregions of the overall yg(wd)-wx region are compared (see Tables 3-5), it becomes apparent that the K^* knob has a tremendous suppressive effect over a long region of the short arm of chromosome 9. It is especially noticeable when this knob is not opposed by another knob in the homologue. On the male-side, the suppressive effect of the K^* knob is limited to the distal yg-c region. These results are shown in the following :



When the recombination values found in K^S/K^S, K^S/k, and K^*/k constitutions (see Tables 3 and 5) are compared one finds that the values found in the distal region for the heteromorphs are considerably lower than that found in the homomorph. Furthermore, the recombination values obtained for the distal and proximal regions in K^*/k heteromorphs are significantly lower than those obtained for the K^S/k heteromorphs. These observations further emphasize the ability of the K^* knob to suppress recombination.

In maize there is evidence for recombination differences associated with sex. Rhoades (1941) has suggested that only those regions adjacent to the centromeres show this differ-

ence. The data obtained for K^S/K^S homomorphs appear to support his contention, but those obtained for K^* knob bearing heteromorphs do not lend support. In these heteromorphs the recombination values for both the proximal and the distal regions on the male-side were found to be significantly higher than those obtained on the female-side (for K^*/K^S heteromorphs see Table 3 entries 2 and 4, distal yg-c : 4.63% in ♀, 13.83% in ♂ ; proximal sh-wx : 18.91% in ♀, 31.48% in ♂, for K^*/k heteromorphs see Table 5 entries 3 and 4, distal wd-c : 1.40% in ♀, 9.04% in ♂ ; proximal c-wx : 13.29% in ♀, 31.17% in ♂).

The data entered in Table 6 lend support to the notion that whenever there is more of the K^*9 knob present the greater is the suppressive effect on recombination. Both sib comparisons do not argue in favor of the possibility that total volume of heterochromatin is critical in recombination suppression.

4. The effect of the interaction of K^*9 and K10 chromosomes on recombination.

The recombination frequencies in the wd-c-wx region of $K^*9/k9$ heteromorphs in the presence and absence of the K10 chromosome are presented in Table 7. In megasporocytes, the recombination in the wd-c region was increased by the K10 chromosome from 1.4% to 2.8%. The increase in the c-wx region was from 13.3% to 28.9%. For both regions, the increase in recombination was doubled when the abnormal

TABLE 6

TEST CROSS VALUES FOR CHROMOSOME 9 HETEROMORPHS
THE BRACKETED COMBINATIONS ARE SIBS

$\frac{+ \quad + \quad + \quad +}{yg \quad c \quad sh \quad wx}$ ♀ 1 2 3	Recombination 1	2	3	Total	Total progeny
1 K^*/K^L	11.92	2.88	18.42	33.22	4554
2 K^*/K^S	5.73**	2.14*	17.67	25.54**	4154
	(yg-sh)				
3 K^*/K^L	15.89		19.06	34.95	2524
4 K^L/K^S	14.10		22.80	36.90	2057

* and ** -- significant at the 5% and 1% levels, respectively from its sib.

The original data are in Appendixes 9-10 and 13-14.

TABLE 7

RECOMBINATION PERCENTAGES IN K^*/k HETEROMORPHS WITH AND WITHOUT K10 CHROMOSOME. THE RESULTS ARE FROM RECIPROCAL TEST CROSSES OF SIB PLANTS

$\frac{K^* + c +}{k\ wd + wx}$ 1 2	Recombination			Total progeny
	1	2	Total	
1 k10/k10 ♀	1.4	13.3	14.7	2438
2 K10/k10 ♀	2.8**	28.9**	31.7**	6881
	+1.4	+15.6	+17.0	
3 k10/k10 ♂	9.0	31.2	40.2	2621
4 K10/k10 ♂	9.0	35.2**	44.2**	4798
	+0.0	+ 4.0	+ 4.0	

** -- significant at the 1% level from k10/k10 of same sex.

The original data are in Appendixes 11-12.

The coincidence values for entries :

1 = 1.77 2 = 0.29 3 = 0.53 4 = 0.58

chromosome 10 was present, but the amount of increase was greater in the proximal c-wx region (+ 15.6%) than in the distal wd-c region (+ 1.4%). The K10 chromosome's effect on recombination was weaker in microsporocytes. Recombination in the distal wd-c region was not altered and only a 4% increase was realized in the proximal c-wx region. The total increase in recombination induced by K10 chromosome in the wd-wx region was 17% on female-side and 4% on male-side.

The effect of the K10 chromosome on recombination in $K^{*}9/K^{S}9$ heteromorphs is presented in Table 8. In megasporocytes, recombination was enhanced by K10 chromosome from 9.9% to 12.9% in the yg-sh region and from 20.3% to 30.0% in the sh-wx region. The total increase in the yg-wx region was 12.7%, the proximal sh-wx region experiencing 9.7% increase. In microsporocytes, the K10 chromosome did not increase recombination in the distal yg-sh region (24.9% for k10/k10, 22.8% for K10/k10), and increased it only 3.2% in the proximal sh-wx region (from 24.5% to 27.7%). Total recombination was not significantly changed (49.4% for k10/k10, 50.5% for K10/k10). In spite of the enhancing effect of K10 chromosome on recombination in the distal region on the female-side, the suppressive effect of $K^{*}9$ is still quite significant. The standard map distance for yg-c region is 19. This was reduced to 6.5% in K^{*}/K^{S} heteromorphs. In the presence of the K10 chromosome, it was enhanced to only 7.9%. In conformity with observations made in K^{*}/k individuals, the K10 chromosome and K^{*} knob effects

TABLE 8

RECOMBINATION PERCENTAGES IN K^*/K^S HETEROMORPHS WITH AND WITHOUT K10 CHROMOSOME. THE RESULTS ARE FROM RECIPROCAL TEST CROSSES OF SIB PLANTS

	$\frac{K^*}{K^S}$	+ c + + / yg + sh wx (1 2 3)		Recombination				Total progeny
				1	2	3	Total	
1	k10/k10	♀		6.5	3.4	20.3	30.2	9962
2	K10/k10	♀		7.9**	5.0**	30.0**	42.9**	7321
				+1.4	+1.6	+9.7	+12.7	
3	k10/k10	♂		19.8	5.1	24.5	49.4	3072
4	K10/k10	♂		17.2*	5.6	27.7**	50.5	2533
				-2.6	+0.5	+3.2	+1.1	

* and ** -- significant at the 5% and 1% levels respectively from k10/k10 of same sex.

The original data are in Appendixes 9-10.

The coincidence values (regions 1 and 3) for entries :

1 = 0.20 2 = 0.22 3 = 0.46 4 = 0.49

are weaker on the male-side.

In K^*9/K^L9 heteromorphs (Table 9) the K10 chromosome again had a weaker effect on recombination on the male-side. In megasporocytes, recombination was increased from 15.2% to 19.0% in yg-sh region, and from 17.3% to 21.8% in sh-wx region; the amount of increase, unlike in K^*/k and K^*/K^S heteromorphs, was only slightly higher in the proximal region (+ 4.5%) than in the distal region (+ 3.8%). In microsporocytes, the K10 chromosome's effect was negligible in the proximal sh-wx region (24.7% for k10/k10, 25.1% for K10/k10) and only significant at the 5% level for the distal yg-sh region (19.5% for k10/k10, 22.3% for K10/k10). The total increase in recombination of 3.2% was not statistically significant.

The summary of the effect of the K10 chromosome on recombination in the three heteromorphs, K^*/k, K^*/K^S, and K^*/K^L, is presented in Table 10. In general, the K10 chromosome's effect was primarily found in megasporocytes and restricted to the proximal region. In megasporocytes, the increase in recombination in the yg-wx region was greater when the knob size difference was greater (K^*/K^L : +8.3% ; K^*/K^S : +12.7% ; K^*/k : +17.0%). This result is comparable to that found by Kikudome (1959) in his comparison of the K^L/k, K^M/k, and K^S/k heteromorphs. In Kikudome's data, however, the K10 chromosome increased the recombination in the wd-wx region of K^L/k, K^M/k, and K^S/k heteromorphs to about the same level (30%). The present results seem to

TABLE 9

RECOMBINATION PERCENTAGES IN K^*/K^L HETEROMORPHS WITH AND WITHOUT K10 CHROMOSOME. THE RESULTS ARE FROM RECIPROCAL TEST CROSSES OF SIB PLANTS

$\frac{K^*}{K^L}$ + + + yg sh wx 1 2		Recombination 1	Recombination 2	Recombination Total	Total progeny
1 k10/k10	♀	15.2	17.3	32.5	4335
2 K10/k10	♀	19.0**	21.8**	40.8**	12412
		+3.8	+4.5	+8.3	
3 k10/k10	♂	19.5	24.7	44.2	1281
4 K10/k10	♂	22.3*	25.1	47.4	2495
		+2.8	+0.4	+3.2	

* and ** -- significant at 5% and 1% levels respectively from k10/k10 of the same sex.

The original data are in Appendixes 13-14.

The coincidence values for entries :

1 = 0.13 2 = 0.17 3 = 0.47 4 = 0.52

TABLE 10

COMPARISON OF K10 CHROMOSOME INCREASED RECOMBINATION IN DIFFERENT HETEROMORPHS

	Knob constitution		K10 chromosome increased recombination				
			distal region		proximal region		Total
			yg-c	yg-sh	sh-wx	c-wx	(yg-wx)
1	$K^{*}9/K^{L}$	♀		+3.8	+4.5		+8.3
		♂		+2.8	+0.4		+3.2
2	$K^{*}9/K^{S}9$	♀	+1.4	+3.0	+9.7	+11.3	+12.7
		♂	-2.6	-2.1	+3.2	+3.7	+1.1
3	$K^{*}9/k9$	♀	+1.4			+15.6	+17.0
		♂	+0.0			+4.0	+4.0

indicate that the K10 chromosome enhances recombination in the yg(wd)-wx region in K^*/K^L and K^*/K^S heteromorphs to a higher level (about 40%) than in K^*/k heteromorphs (about 30%). The data further reveal that the amount of recombination increased by the K10 chromosome in the proximal region is influenced by the distal knob constitutions : the greater the difference of the knobs the greater the amount of recombination increased by the K10 chromosome in the proximal region (sh-wx region : K^*/K^L +4.5%, K^*/K^S +9.7% ; c-wx region : K^*/K^S +11.3%, K^*/k +15.6%).

A comparison of coincidence values obtained for these three heteromorphs in the presence and absence of K10 chromosome (Table 11) shows that the abnormal chromosome 10 did not significantly influence chromosomal interference except in megasporocytes of K^*/k individuals. The coincidence values were higher on the male-side in K^*/K^S and K^*/K^L heteromorphs than on the female-side in plants with or without the K10 chromosome. For K^*/k, this was true when the K10 chromosome was present (0.29 in ♀ and 0.58 in ♂), but in the absence of K10 chromosome, the value was lower on the male-side (0.53) than on the female-side (1.77). The negative interference value may be due to a greater sampling variation occurred as a consequence of reduced recombination in the distal wd-c region of K^*/k heteromorphs.

TABLE 11

COEFFICIENT OF COINCIDENCE VALUES FOR CHROMOSOME 9 HETEROMORPHS IN THE PRESENCE AND ABSENCE OF THE ABNORMAL CHROMOSOME 10

Knob constitution (regions)	k10/k10 ♀	K10/k10 ♀	k10/k10 ♂	K10/k10 ♂
K^*/K^L (yg-sh, sh-wx)	0.13	0.17	0.47	0.52
K^*/K^S (yg-c, sh-wx)	0.20	0.22	0.46	0.49
K^*/k (wd-c, c-wx)	1.77	0.29**	0.53	0.58

** -- significant at the 1% level from k10/k10 of same sex.

5. The influence of the K^*9 chromosome on the B-chromosome-effect on recombination.

The influence of the K^*9 chromosome on the B-chromosome effect on recombination was studied by comparing recombination in K^*/K^S heteromorphs and K^S/K^S homomorphs (Tables 12 and 13, the values in Table 12 are from combined data of the two replications in Appendixes 15-18).

F-tests of the recombination percentages obtained on the female-side (Appendixes 3-6) show that the variations in recombination values due to the different numbers of B-chromosomes were significant at the 5% level for the proximal (bz-wx) and the distal (yg-sh) regions in both K^*/K^S heteromorphs and K^S/K^S homomorphs. The variations in the replication were not significant except in the proximal region of K^*/K^S heteromorphs which was significant at the 5% level. This indicates that the two replications are essentially similar.

The data in Tables 12 and 13 are graphically presented in Figures 1 and 2, respectively. The graphs for the two replications of female-side data (Appendixes 16 and 18) are shown in Appendixes 7 and 8.

In K^S/K^S homomorphs employed as females (Table 12 and Figure 1), an odd number of B-chromosomes increased recombination in the proximal bz-wx region and concomitantly decreased recombination in the distal yg-sh region. An even number of B-chromosomes, on the other hand, seems to reestablish the values found in the OB class. Although the

TABLE 12

RECOMBINATION VALUES OBTAINED FROM TESTCROSSES OF PLANTS WITH DIFFERENT COMBINATIONS OF KNOBS AND NUMBER OF B-CHROMOSOMES

+ + + + / yg sh bz wx	♀	yg - sh	sh - bz	bz - wx	Total	Total Progeny
1	K^S/K^S	23.86 %	2.47 %	15.72 %	42.05 %	7934
0 B						
2	K^*/K^S	8.35	1.61	19.10	29.05	6038
3	K^S/K^S	22.08*	2.32	17.27*	41.66	5658
1 B						
4	K^*/K^S	10.92**	1.69	21.39**	34.00**	9539
5	K^S/K^S	24.17	2.12	16.23	42.52	7444
2 B						
6	K^*/K^S	8.12	1.43	21.05*	30.60	5121
7	K^S/K^S	21.77*	1.84	17.44*	41.06	2494
3 B						
8	K^*/K^S	10.41**	2.05	23.34**	35.80**	4430
9	K^S/K^S	23.47	1.86*	16.07	41.40	5587
4 B						
10	K^*/K^S	8.97	1.48	21.94**	32.39**	4202
11	K^S/K^S	23.69	1.85	18.33*	43.87	971
5 B						
12	K^*/K^S	12.63**	1.86	22.77**	37.27**	966

* and ** denote deviations, significant at the 5% and 1% levels respectively, from the " OB" class of the same knob constitution.

The original data are in Appendixes 15-18.

TABLE 13

RECOMBINATION VALUES OBTAINED FROM TESTCROSSES OF PLANTS WITH DIFFERENT COMBINATIONS OF KNOBS AND NUMBER OF B-CHROMOSOMES

+ yg	+ sh	+ bz	+ wx	♂	yg - sh	sh - bz	bz - wx	Total	Total Prpgeny
1				K^S/K^S	25.58 %	2.46 %	18.11 %	46.15 %	2479
2		0 B		K^*/K^S	20.48	2.82	25.17	48.47	3369
3				K^S/K^S	23.32	1.79	20.56*	46.67	2344
4		1 B		K^*/K^S	18.16*	1.84*	25.30	45.30*	2285
5				K^S/K^S	26.50	1.44**	17.64	45.59	2494
6		2 B		K^*/K^S	15.94**	1.88*	25.43	43.26**	2973
7				K^S/K^S	24.94	1.70	17.23	43.87	2530
8		3 B		K^*/K^S	19.23	1.96*	25.48	46.66	2814
9				K^S/K^S	24.48	1.74*	17.81	44.03	3734
10		4 B		K^*/K^S	18.48	2.44	24.11	45.03*	1970
11				K^S/K^S	25.83	1.84	19.80	47.48	1409
12		5 B		K^*/K^S	16.67**	1.80*	23.94	42.41**	1278

* and ** denote deviations, significant at the 5% and 1% levels respectively, from the "0 B" class of the same knob constitution.

The original data are in Appendixes 19-20.

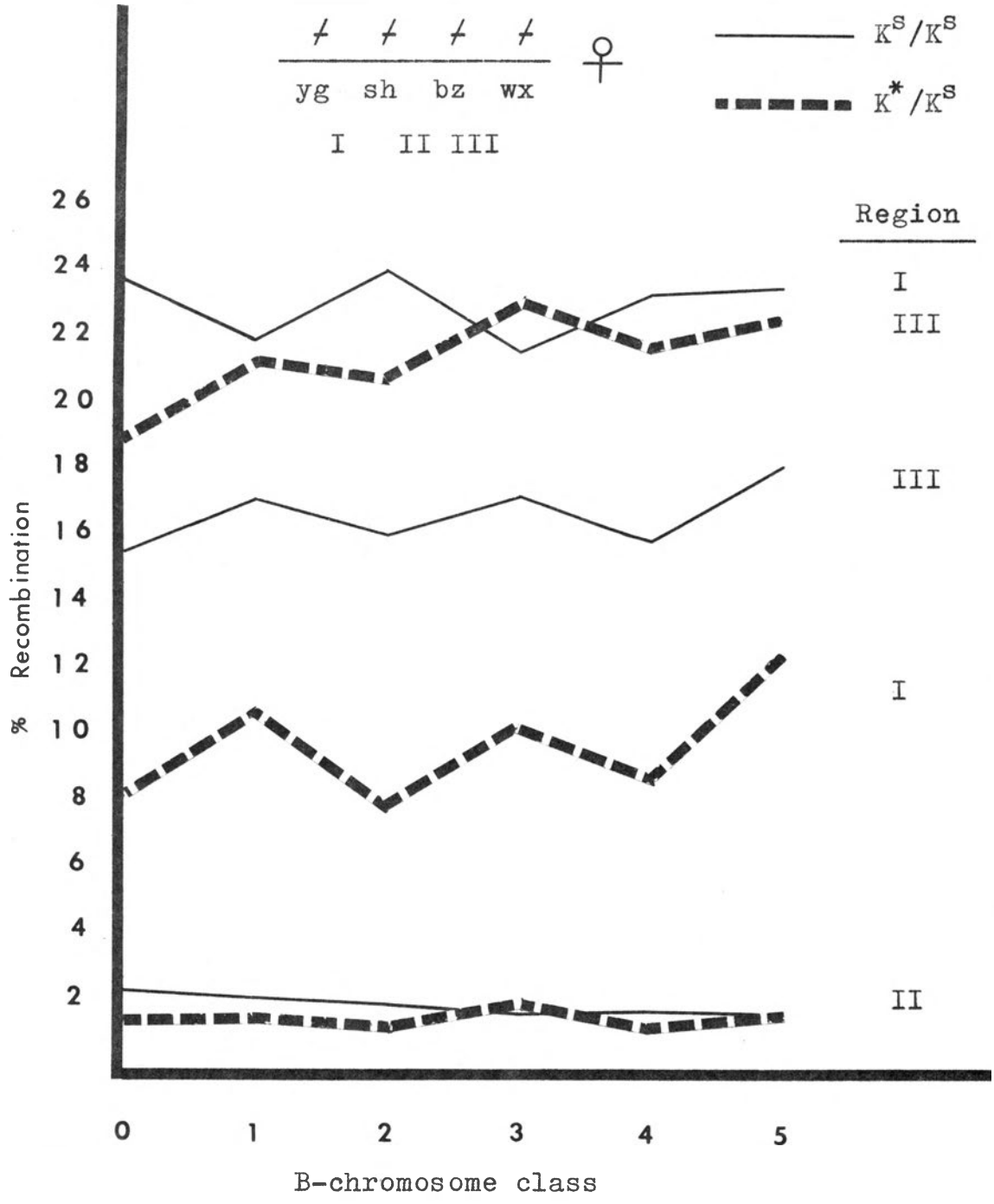


Figure 1 Recombination percentages in K^S/K^S homomorphs and K^*/K^S heteromorphs with different number of B's.

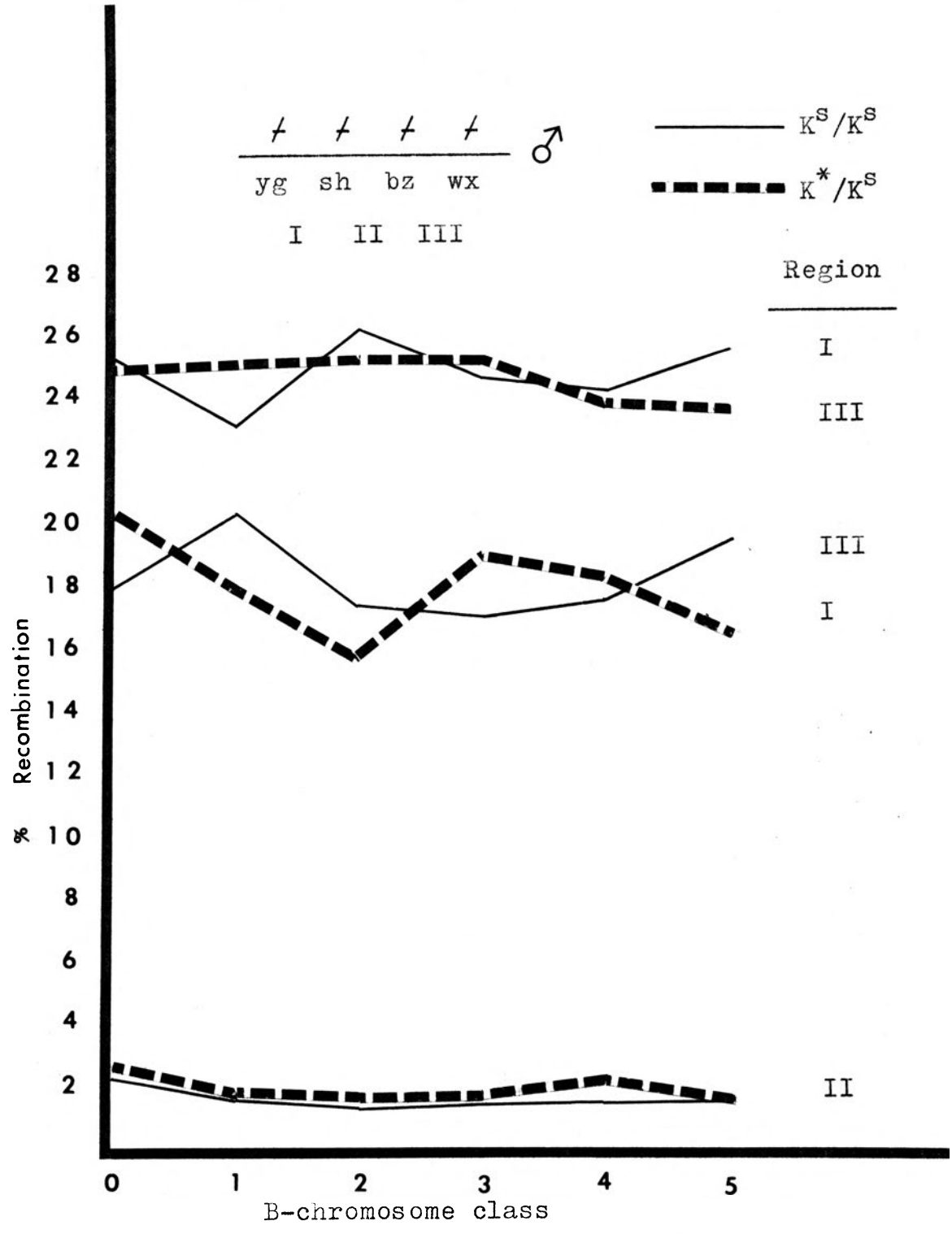


Figure 2 Recombination percentages in K^S/K^S homomorphs and K^*/K^S heteromorphs with different number of B's.

the total recombination in the yg-wx region was not changed by the odd or even numbers of B's, a shift in recombination from the distal to the proximal region occurred whenever odd numbers of B's were involved.

In K^*/K^S heteromorphs (Table 12 and Figure 1), recombination was increased by the B's in both the proximal bz-wx and the distal yg-sh regions. The boost was more pronounced with an odd number of B-chromosomes. When the values obtained for K^*/K^S females are compared with those for K^S/K^S females, the suppression effect of the K^*9 knob on the distal region and total recombination is clearly evident.

In the intermediate (sh-bz) region, recombination was not significantly affected by the B's in both the K^S/K^S homomorphs and K^*/K^S heteromorphs.

The "odd-even" or "zig-zag" effect (see Figure 1), however, was not clearly expressed in the recombination data obtained on the male-side (Table 13 and Figure 2). The failure to find a clear-cut "zig-zag" pattern on the male-side could be due to a differential effect of the B's on mega- and microsporocytes or to the smallness of the samples obtained.

In both mega- and microsporocytes, recombination in all B-chromosome classes was higher in the yg-sh region than in the bz-wx region in K^S/K^S homomorphs, but the reverse was observed in K^*/K^S heteromorphs. The degree of difference in the recombination values between the two distal regions

is considerably greater on the female-side than on the male-side. The proximal region values appear not to show this degree of difference. In general, for microsporocytes, recombination in the yg-sh region of K^S/K^S homomorphs ($\bar{X}$ = 25.1%) approximately equals that in the bz-wx region of K^*/K^S heteromorphs ($\bar{X}$ = 24.9%), while recombination in the bz-wx region of K^S/K^S homomorphs ($\bar{X}$ = 18.5%) approximately matches that in the yg-sh region of K^*/K^S heteromorphs ($\bar{X}$ = 18.2%). In megasporocytes, recombination in the yg-sh region of K^*/K^S heteromorphs ($\bar{X}$ = 9.9%) was much lower than that in the bz-wx region of K^S/K^S homomorphs ($\bar{X}$ = 16.8%). Thus there is an overall reduction in total recombination in the yg-wx region of K^*/K^S heteromorphs as compared to K^S/K^S homomorphs.

Recombination in the intermediate (sh-bz) region was not significantly affected by the B-chromosomes in both mega- and microsporocytes. The data suggest that the K^*9 knob increased recombination in this region on the male-side and decreased it on the female-side.

Hanson (1969) reported that higher numbers of B's (6-9) increased recombination in the short arm of chromosome 9. The increase was accompanied by a decrease in chromosomal interference. The present study indicates that the effect of fewer number of B's (1-5) was primarily on single exchanges (Appendixes 16 and 18). The coincidence values for yg-sh and bz-wx regions in K^S/K^S homomorphs and K^*/K^S heteromorphs with different numbers of B's are presented in Table

14. An increase in the coincidence value associated with the presence of B's was noted in K^S/K^S homomorphs only on the female-side. The coincidence values for K^*/K^S heteromorphs were not significantly affected by the presence of B's on both male- and female-sides. The coincidence values calculated for K^*/K^S heteromorphs were not significantly different from those obtained for K^S/K^S homomorphs with different numbers of B's when the sex compared was the same. The coincidence values were significantly higher for microsporocytes than for megasporocytes in both knob classes. (average for male $K^S/K^S = 0.45$, $K^*/K^S = 0.39$; for female $K^S/K^S = 0.15$, $K^*/K^S = 0.11$).

TABLE 14

COEFFICIENT OF COINCIDENCE VALUES FOR THE yg-sh AND bz-wx REGIONS IN K^S/K^S HOMOMORPHS AND K^*/K^S HETEROMORPHS AND K^*/K^S HETEROMORPHS WITH DIFFERENT NUMBERS OF B's.

No. of B's	K^S/K^S ♀	K^*/K^S ♀	K^S/K^S ♂	K^*/K^S ♂
0 B	0.07	0.10	0.47	0.44
1 B	0.11**	0.08	0.47	0.35*
2 B	0.14**	0.15	0.44	0.46
3 B	0.18**	0.08	0.51	0.43
4 B	0.09	0.18	0.36	0.39
5 B	0.29**	0.07*	0.47	0.28**
Average	0.15	0.11	0.45	0.39

* and ** -- significant at the 5% and 1% levels respectively from the 0 B class.

V. GENERAL DISCUSSION

It is evident from the present study that the K^* knob of chromosome 9 has a suppressive effect on recombination in the short arm. Whether this suppressive effect is confined to a short region or is effective over a longer region is dependent upon the size of the opposing knob found in the homologue. Suppression is greatest when the chromosome bearing the K^* knob is paired with a homologue without a knob and becomes less pronounced as the size of the opposing knob becomes larger. This type of suppressive effect on recombination is stronger on the female-side than on the male-side. The data obtained from the chromosome 10 study indicate that this particular knob of chromosome 9 is incapable of effecting a change in the frequency of recombination in other chromosomes. In chromosome 9, the K^* knob is capable of modifying the abnormal chromosome 10 and B-chromosome effects on recombination.

The results from the study of the segregational behavior of the K^*9 chromosome in the presence of abnormal chromosome 10 suggest that knob material (heterochromatin) in maize does not have a uniform structure and function. The notion that different heterochromatic elements are not similar in structure and function is also indicated by other observations. The nucleolar organizer is often a knob forming or heterochromatic region. It contains cistrons for rRNA and is indispensible for its synthesis (Brown and

Gurdon 1964). Pardue and Gall (1970) have shown that constitutive heterochromatin located around the centromere regions contains satellite DNA, whereas other types of heterochromatin do not. Vosa (1970) found that heterochromatin from different species can be classified into four categories according to its positive or negative response in cold treatment and fluorescent dye staining. All of these observations suggest that heterochromatins need not be identical in structure and function.

In his study of knobbed-knobless chromosome 9 heteromorphs, Kikudome (1959) found that the greater the size of the knob, the greater the degree of suppression of recombination. It appears from his study that there is a higher degree of suppression when there is greater volume of heterochromatin. The present study does not lend credence to this hypothesis. Rather, it appears that in both instances it is not the total amount of heterochromatin which is critical but the amount of heterochromatin of a given knob not opposed by the heterochromatin present in the opposing knob. The following hypothesis is offered to explain the results of the two studies. This hypothesis is based on the assumption that knobs or heterochromatin contain genes controlling the recombination processes and different knobs can contain different alleles of these genes. It is postulated that only those genes in the knob which are in the hemizygous state are capable of exerting their suppressive effect on recombination. According to this hypothesis, then, the

greater the number of unpaired genes, the greater the suppressive effect.

If the knob does not contain recombinational genes, the effect of the knob on recombination may be explained on a mechanical basis. In this hypothesis it is postulated that the greater the unpaired knob region, the greater the interference on effective chromosome pairing and hence on crossing over. This interference would be the result of the difficulty encountered by the homologues possessing knobs of different sizes in the pairing act. Furthermore this effective pairing or the pairing associated with crossing over precedes the pairing observable in pachynema. The data obtained from the various knob-knob and knob-knobless combinations are in agreement with the hypothesis.

Recombination studies in chromosome 3 and 5 of maize show that the abnormal chromosome 10 increases crossing over primarily in the proximal regions. Those studies cited in the literature review section, dealing with induction of recombination by various genetic and environmental factors, also reveal that the primary increase is in the proximal regions of the chromosomes examined. The K^* knob effect, on the contrary, is seen to affect the distal region rather than the proximal region, and is one of decreasing recombination. The data obtained in the study of K^*9-K10 chromosome interaction involving the K^*/k, K^*/K^S, and K^*/K^L heteromorphs appear to indicate that the effectiveness of the K10 chromosome in increasing recombination in the distal region

becomes progressively less as the amount of the K^* knob not opposed by knob material in the homologue increases. It was also found that the greater the total amount of heterochromatin in the two knobs, the less effective is the K10 chromosome in increasing recombination in the proximal region. If the hypothesis proposed by Rhoades and Dempsey in 1966 that the abnormal chromosome 10 brings about an increase in recombination through the very intimate pairing of the members of a bivalent is valid, then the observed failure of the abnormal chromosome 10 to overcome the suppressive effect of the K^* knob on recombination in the distal region of chromosome 9 requires an explanation. The failure of abnormal chromosome 10 could be due to its inability to bring about the degree of effective pairing necessary for optimum recombination or could be due to some factor other than intimate pairing.

Hanson observed in his 1969 study that B-chromosomes enhanced recombination in the proximal regions but decreased it in regions adjacent to the knob. He postulated that his results occurred as a consequence of an interaction between knobs and B's. Evidence has been obtained to indicate that the K^*9 knob can influence the B-chromosome effect on recombination. In the absence of the K^* knob, B-chromosomes did indeed increase recombination in the proximal region but decreased it in the region adjacent to the knob. In the presence of this K^* knob, recombination in both the proximal and distal regions was increased by the B's. When the total

recombination values in the B-containing and B-less K^S/K^S compounds are examined, one finds that they are essentially the same. However, when a similar comparison is made for compounds containing the K^*9 chromosome, the total recombination found for the B-less compounds is less than that found for the B-containing ones. Thus in K^S/K^S classes, the B-effect appears to involve a shift in the distribution of crossing over. In the heteromorphs no such shift is seen.

The observations made in the K^*-containing individuals can best be explained on the basis of a K^*-B-interaction. In one respect the interaction can be viewed as a partial nullification of the K^* knob effect by the B-chromosomes. This partial nullification may be due to the ability of the B-chromosomes to improve effective pairing in K^*-containing bivalents. Accordingly one is forced to state that in other knob-containing chromosome 9 bivalents, e.g. K^S/K^S, the B-chromosome is incapable of improving effective pairing.

The "odd-even" or "zig-zag" phenomenon found in this experiment with different numbers of B's did not offer any clues as to the role of the K^*9 knob in this effect.

Kirk and Jones (1970) found in rye that the relative amount of total nuclear protein and nuclear RNA decreased with increasing number of B's but not in a linear fashion. The values were consistently lower for odd numbered B-classes of plants. Histone protein was found to increase as the number of B's increased, the values were consistently higher for odd-numbered B-classes. A negative correlation was found

between histone and total nuclear protein, and histone and nuclear RNA amounts. This may be interpreted as that the B-chromosomes have the ability to inhibit the genetic activity. Suzuki (1963) postulated that the inhibition of genetic activity in the proximal heterochromatic region causes the increase in recombination in that region. From the results of Kirk and Jones and the postulation of Suzuki, an explanation can be offered for the B-chromosome effect observed in this study. According to Suzuki, the proximal heterochromatic region may contain genes that function during meiosis. The inhibition of the activity of those genes will account for the recombination increase observed in the proximal region of both K^S/K^S homomorphs and K^*/K^S heteromorphs. In K^*/K^S heteromorphs, if the genetic activity of K^* knob that causes the recombination suppression is inhibited by B's, the recombination in the distal region is expected to be enhanced as was found in this experiment. When the recombination in the proximal region is facilitated the time course for recombination in the distal region may be changed. This change may explain the concomitant decrease observed in the distal region of K^S/K^S homomorphs.

Most of the known cases in plants and animals where recombination takes place in both male and female show higher crossing over in females. Maize is one of the few exceptions in hermaphroditic plants as are some newts in the sexually differentiated animals (Ved Brat 1964). The present results show that recombination is always higher on the male-side

and that the effect of the K^*9 knob is to accentuate this male : female difference. In contrast, the abnormal chromosome 10 has the effect of increasing recombination. This effect is greater on the female-side than on the male-side. The observation also reveals that there is a region in chromosome 9 that is differentially affected by the K^* knob in micro- and megasporocytes. Several explanations could be considered. One is that genes in the K^* knob are differentially activated in micro- and megasporocytes. This type of phenomenon has been reported in maize (Schwartz 1965; Kermicle 1970). Another explanation is based on the assumption that there may be a limited time for effective pairing to occur in certain chromosomal regions and that the K^* knob effectively reduces this time period on the female-side. Thirdly, if the time available for effective pairing is not altered, the male : female difference induced by the K^* knob may be due to greater suppression of effective pairing process in the female than in the male.

This study of a particular knob on chromosome 9 and those involving other knobs clearly indicate that knobs can be utilized in practical ways. When knobs of given quality as well as other similar heterochromatic region are used in certain combinations, linkages can either be loosened or tightened as desired. Furthermore, recombination can be altered in specific regions of chromosomes, depending on the type of knob or heterochromatin used.

Knobs in normal chromosomes and the abnormal chromosome 10, and the B-chromosomes are the prominent heterochromatic elements in maize. Their existence in a given race seems to be interrelated. Through preferential segregation, the abnormal chromosome 10 tends to increase the knob numbers. On the other hand, by the mechanism of chromosome elimination (Rhoades et al. 1967), B-chromosomes tend to decrease the number of knobs. The knob size and number could also be changed by mutation and unequal crossing over as suggested by Longley and Kato (1965). Both preferential segregation and chromosome elimination of knobbed chromosomes are effective mechanisms in genic selection. The ability of these heterochromatic elements to influence the recombination indicates that they are important in providing the variability and stability to the population and therefore are important for the adaptation of the species of maize.

VI. SUMMARY

1. The K^* knob of Mexican origin had a suppressive effect on recombination in the short arm of chromosome 9. It had the capacity to reduce recombination in both the distal and proximal regions as in K^*/k heteromorphs, or to reduce it only in the distal region as in K^*/K^S individuals (with a concomitant increase in recombination in the proximal region). The suppressive effect was stronger on the female-side than on the male-side.

2. The suppressive effect of the K^* knob was greatest when the chromosome containing it was opposed by a homologue which was knobless. This effect became less pronounced as the size of the opposing knob became larger. It appears that the total amount of knob material present in the bivalent was not a critical factor in this suppressive effect.

3. Whereas the abnormal chromosome 10 and the B-chromosomes effect alteration in recombination primarily in the proximal regions of chromosomes, the K^* knob mainly effected alteration in the distal region of chromosome 9.

4. In K^*-containing heteromorphs, the effectiveness of the abnormal chromosome 10 in increasing recombination in the distal region was found to be progressively less as the amount of the K^* knob not opposed by knob material in the homologue increased. It was also found that the greater the total amount of heterochromatin in the two knobs of the bivalent, the less effective was the abnormal chromosome 10 in increasing recombination in the proximal region.

5. The K^* knob modified the B-chromosomes effect on recombination. In megasporocytes of K^*/K^S heteromorphs, although total recombination in the short arm was enhanced in B-chromosome containing individuals over B-less plants, the K^* knob's suppressive effect was still very much in evidence. In these heteromorphs containing the B-chromosomes, recombination was increased in both the proximal and the distal regions. In the K^S/K^S compounds the B-chromosomes did not increase total recombination in the short arm but only effected a shift in recombination from the distal to the proximal region.

6. No indication was obtained that the K^* knob influenced the zig-zag effect on recombination induced by the odd-even number of B-chromosomes.

7. The K^* knob, like the abnormal chromosome 10 and the B-chromosomes, did not affect chromosomal interference in the short arm of chromosome 9. The coincidence values were found to be higher on the male-side than on the female-side.

8. Previous preferential segregation studies have indicated that it is the genes linked to the larger of the two knobs of chromosome 9 bivalents which are preferentially recovered in the eggs. The K^*/K^L study has provided the first exception : genes linked to the smaller K^L knob were preferentially recovered.

9. No evidence was obtained to substantiate the possibility that the K^*9 knob was functionally similar to the K10 chromosome although the K^*9 and K10 knobs are morpho-

logically quite similar. Thus it is not known whether the K^*9 knob is a transposed K10 knob or not.

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APPENDIX

APPENDIX 1. Recombination effects of B-chromosomes in different species.

Species	Effect	Pattern of effect with increasing number of B's.	Reference
1. *Myrmeleotettix maculatus*	Increase in cell chiasma frequency and variance.	Non-additive	Hewitt and John 1967
2. *Puschkinia libanotica*	Increase in chiasma frequency; decrease in variance of both overall chiasma frequency and that of individual bivalents; shift of chiasmata from terminal to proximal in PMC's.	Additive; linear	Barlow and Vosa 1970
3. *Listera ovata*	Increase in chiasma frequency in PMC's and EMC's.	Additive; zig-zag rise	Vosa and Barlow 1970 (cited in above paper)
4. *Festuca mairei*	Increase in chiasma frequency and variance of chiasma frequency	Additive; linear	Malik and Tripathi

APPENDIX 1. Continued.

	within PMC's.		1970
5. *Lolium perenne*	Decrease in mean cell chiasma frequency; increase in variance for bivalent chiasma frequency within PMC's.	Additive	Cameron and Rees 1967
6. *Secale cereale* (wild rye)	Increase in mean cell chiasma frequency.	Additive; linear	Zecevic and Paunovic 1969
7. *Secale* (*S.cereale* X *S.vavilovii*)	Increase in variance for within PMC's and within plant chiasma frequency; increase in asymmetry of chiasma distribution between chr. arms within bivalent.	Additive; zig-zag rise	Jones and Rees 1967
8. *Zea mays*	Increase in crossing over; shift in crossing over along chromosome arm.	Additive	Hanson 1969 Rhoades 1968 Nel 1969
	Increase mean chiasma frequency; influence chiasma distribution in PMC's.		Ayonoadu & Rees 1968

APPENDIX 2. Techniques for the examination of somatic chromosomes in root-tips of maize.

GERMINATION -- Germinate kernels on moist filter paper in Petri dishes at 25° C.

PRETREATMENT -- Take two roots approximately 1 cm long from each seedling and place them in tubes containing a freshly prepared saturated solution of 1-bromonaphthalene in tap water. Number each tube so that it may be related to the corresponding seedling. The seedlings are then planted out. Leave uncorked tubes containing roots and 1-bromonaphthalene solution for 3.5 hours.

FIXATION -- Pour off 1-bromonaphthalene solution and replace with glacial acetic acid. Cork tubes and leave for half an hour or preferably overnight.

STAINING -- Pour off glacial acetic acid and replace with 60° C 1N Hcl for 15 minutes at 60° C. The tubes are uncorked during the hydrolysis. Pour off the hydrochroric acid and replace with leuco-basic fuchsin. Recork the tubes and leave for half an hour for the roots to stain.

PREPARATION OF SLIDES -- Cut off the stained meristematic tip of the root and place in a drop of propionic orcein on a slide. Cover with cover-glass and tap cover-glass to spread the material. Place the slide and cover-glass under a layer of filter paper and press the cover-glass firmly onto the slide.

Appendix 3 F-test of the yg-sh region recombination values in K^S/K^S homomorphs with different number of B's.

Replication	B-chromosome class					Total
	0	1	2	3	4	
1	29.5 (24.27)	28.4 (22.61)	29.8 (24.76)	27.8 (21.76)	28.9 (23.42)	144.4
2	29.1 (23.56)	27.6 (21.52)	28.9 (23.41)	27.9 (21.82)	29.1 (23.57)	142.6
Total	58.6	56.0	58.7	55.7	58.0	287.0

Preliminary calculations

(1) Type of Total	(2) Total of squares	(3) No. of items squared	(4) Observation per squared item	(5) Total of squares per obs.
Grand	82369.00	1	10	8236.90
Replication	41186.12	2	5	8237.22
B-chr.class	16482.14	5	2	8241.07
Observation	8241.90	10	1	8241.90

Analysis of variance

Source of variation	Sum of squares	Degree of freedom	Mean square	F
Replication	0.32	1	0.32	2.51
B-chr.class	4.17	4	1.04	8.18*
Error	0.51	4	0.13	
Total	5.00	9		

* -- significant at 5% level.

Appendix 4 F-test of the bz-wx region recombination values in K^S/K^S homomorphs with different number of B's.

Replication	B-chromosome class					Total
	0	1	2	3	4	
1	23.3	24.5	23.8	24.5	23.4	119.5
	(15.69)	(17.19)	(16.26)	(17.07)	(15.80)	
2	23.4	24.6	23.8	25.5	24.1	121.4
	(15.74)	(17.34)	(16.18)	(18.49)	(16.73)	
Total	46.7	49.1	47.6	50.0	47.5	240.9

Preliminary calculations

(1) Type of Total	(2) Total of squares	(3) No. of items squared	(4) Observation per squared item	(5) Total of squares per obs.
Grand	58032.81	1	10	5803.28
Replication	29018.21	2	5	5803.64
B-chr.class	11613.71	5	2	5806.85
Observation	5807.61	10	1	5807.61

Analysis of variance

Source of variation	Sum of squares	Degree of freedom	Mean square	F
Replication	0.36	1	0.36	3.60
B-chr.class	3.57	4	0.89	8.93*
Error	0.40	4	0.10	
Total	4.33	9		

* -- significant at 5% level.

Appendix 5 F-test of the yg-sh region recombination values in K^*/K^S heteromorphs with different number of B's.

Replication	B-chromosome class					Total
	0	1	2	3	4	
1	16.6 (8.20)	18.4 (10.04)	16.1 (7.84)	18.5 (10.10)	17.5 (8.98)	87.1
2	17.0 (8.61)	19.9 (11.52)	17.6 (9.09)	19.3 (10.90)	17.4 (8.95)	91.2
Total	33.6	38.3	33.7	37.8	34.9	178.3

Preliminary calculations

(1) Type of Total	(2) Total of squares	(3) No. of items squared	(4) Observation per squared item	(5) Total of squares per obs.
Grand	31790.89	1	10	3179.09
Replication	15903.85	2	5	3180.77
B-chr.class	6378.39	5	2	3189.20
Observation	3191.85	10	1	3191.85

Analysis of variance

Source of variation	Sum of squares	Degree of freedom	Mean square	F
Replication	1.68	1	1.68	6.93
B-chr.class	10.11	4	2.53	10.42*
Error	0.97	4	0.24	
Total	12.76	9		

* -- significant at 5% level.

Appendix 6 F-test of the bz-wx region recombination values in K^*/K^S heteromorphs with different number of B's.

Replication	B-chromosome class					Total
	0	1	2	3	4	
1	26.6	28.3	27.3	29.0	28.6	139.8
	(20.08)	(22.49)	(21.41)	(23.47)	(22.95)	
2	24.7	27.0	26.4	28.7	26.4	133.2
	(17.39)	(20.64)	(19.83)	(23.14)	(19.82)	
Total	51.3	55.3	53.7	57.7	55.0	273.0

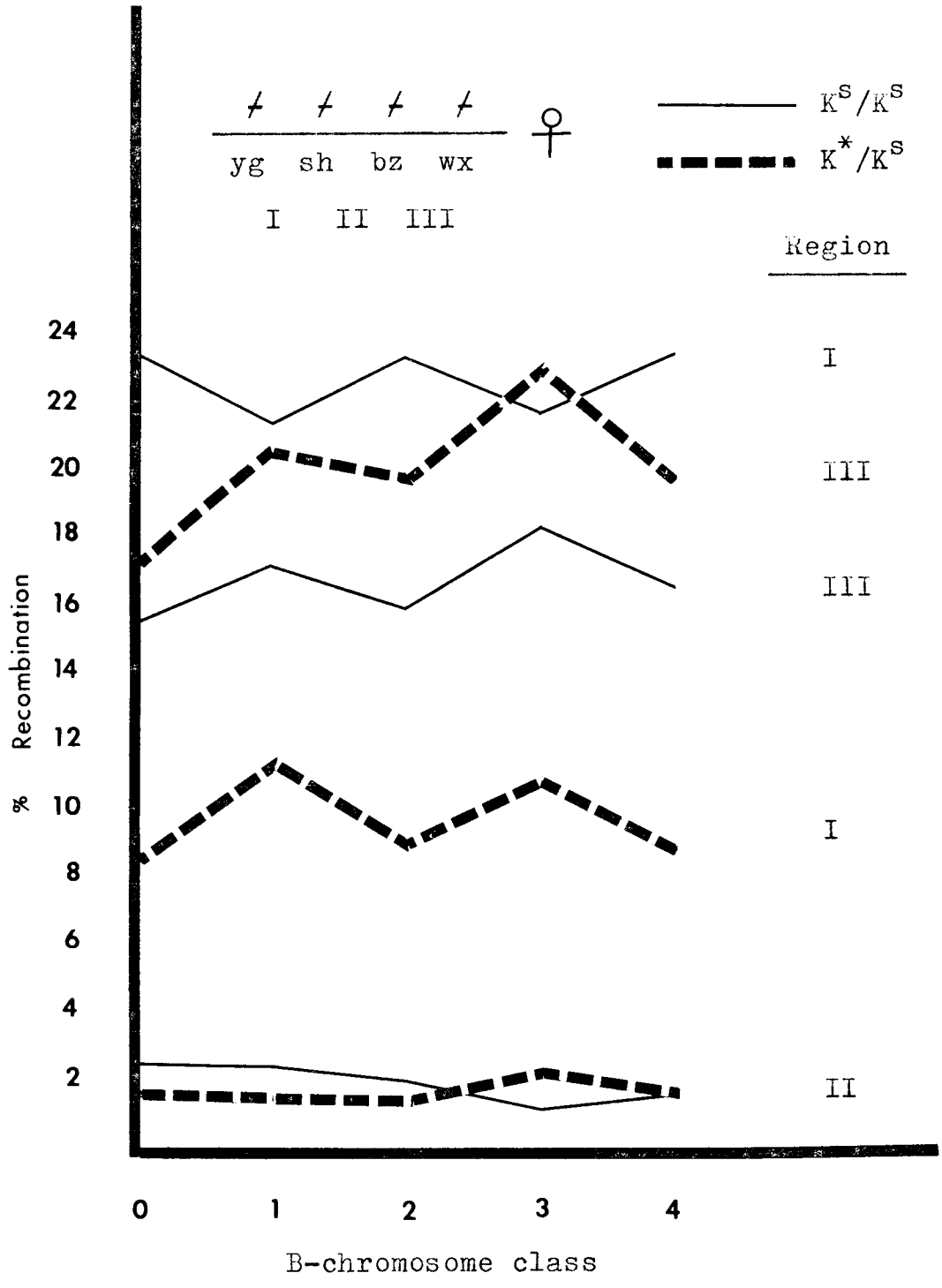
Preliminary calculations

(1) Type of Total	(2) Total of squares	(3) No. of items squared	(4) Observation per squared item	(5) Total of squares per obs.
Grand	74529.00	1	10	7452.90
Replication	37286.28	2	5	7457.25
B-chr.class	14927.76	5	2	7463.88
Observation	7369.40	10	1	7469.40

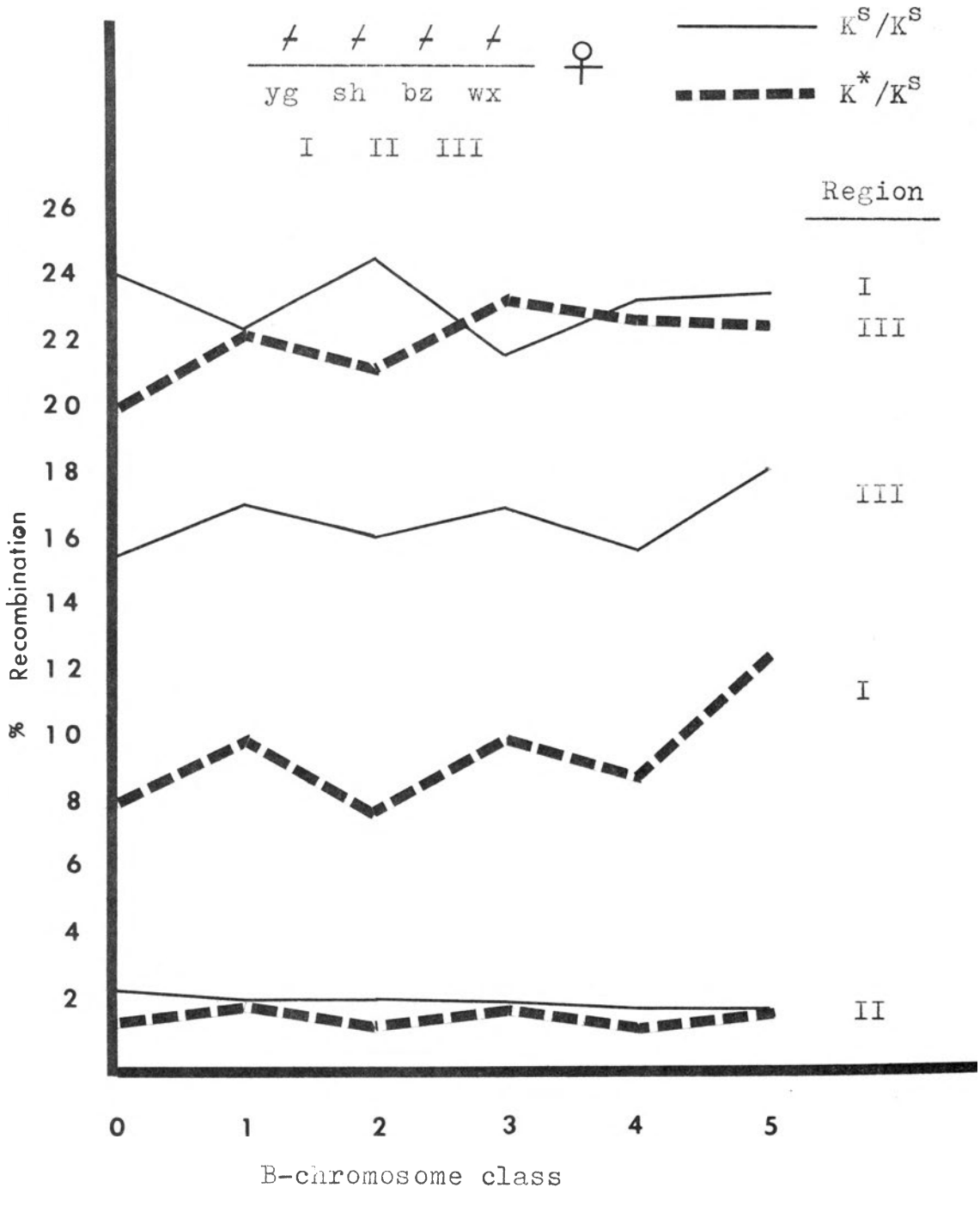
Analysis of variance

Source of variation	Sum of squares	Degree of freedom	Mean square	F
Replication	4.35	1	4.35	14.87*
B-chr.class	10.98	4	2.75	9.39*
Error	1.17	4	0.29	
Total	16.50	9		

* -- significant at 5% level.



Appendix 7 Recombination percentages in K^S/K^S homomorphs and K^*/K^S heteromorphs with different number of B's.



Appendix 8 Recombination percentages in K^S/K^S homomorphs and K^*/K^S heteromorphs with different number of B's.

Appendix 9 Recombination data from test crosses of plants with different combinations of knobs and abnormal chromosome 10. The bracketed combinations are sibs.

		Gametic classes																
+ c + + / yg + sh wx (1 2 3)		(0) + c + +	(0) yg + wx	(1) + sh wx	(1) yg c +	(2) c + wx	(2) yg + +	(3) c + wx	(3) yg + sh +	(12) + + +	(12) yg c sh wx	(13) + + wx	(13) yg c +	(23) c sh +	(23) yg + wx	(123) + + wx	(123) yg c sh +	Σ
1. K^S9/K^S9	♀	1290	1258	332	385	102	90	365	381	1	4	4	2	3	3	0	0	4220
2. K^*9/K^S9	♀	1164	1236	72	72	22	27	309	293	0	1	2	1	0	0	0	0	3199
3. K^S9/K^S9	♂	772	720	178	245	49	66	311	287	3	2	17	20	8	3	1	0	2682
4. K^*9/K^S9	♂	657	637	137	174	47	46	398	356	2	4	12	15	1	1	0	0	2487
5. K^*9/K^L9	♀	1575	1476	266	268	62	64	450	383	0	4	4	1	1	0	0	0	4554
6. K^*9/K^S9	♀	1584	1521	119	110	53	32	384	340	1	0	2	5	0	2	0	1	4154
7. K^*9/K^S9 ; k10/k10	♀	3549	3453	302	310	149	168	987	993	5	4	15	11	7	9	0	0	9962
8. K^*9/K^S9 ; K10/k10	♀	2893	1350	351	186	226	116	1519	615	3	2	20	18	12	10	0	0	7321
9. K^*9/K^S9 ; k10/k10	♂	898	753	237	288	54	76	329	341	9	3	44	25	5	8	2	0	3072
10. K^*9/K^S9 ; K10/k10	♂	718	613	174	195	66	56	312	320	3	5	33	26	4	8	0	0	2533

APPENDIX 10 Recombination values from test crosses of plants with different combinations of knobs and abnormal chromosome 10. The bracketed combinations are sibs.

	+ c + + / yg 1 + 2 sh 3 wx		Non crossovers	Crossovers in region							Total recombination			
				(1)	(2)	(3)	(12)	(13)	(23)	(123)	(1)	(2)	(3)	Σ
1	$K^{S}9/K^{S}9$	♀	60.38	16.99	4.55	17.68	0.12	0.14	0.14	0.00	17.25	4.81	17.96	40.02
2	$K^{*}9/K^{S}9$	♀	75.02**	4.50**	1.53**	18.82	0.03	0.09	0.00*	0.00	4.63**	1.56**	18.91	25.10**
3	$K^{S}9/K^{S}9$	♂	55.63	15.77	4.29	22.30	0.19	1.38	0.41	0.04	17.38	4.92	24.12	46.42
4	$K^{*}9/K^{S}9$	♂	52.03**	12.51**	3.74	30.32**	0.24	1.09	0.08**	0.00	13.83**	4.06	31.48**	49.38*
5	$K^{*}9/K^{L}9$	♀	67.00	11.73	2.77	18.29	0.09	0.11	0.02	0.00	11.92	2.88	18.42	33.22
6	$K^{*}9/K^{S}9$	♀	74.75**	5.51**	2.05*	17.43	0.02	0.17	0.05	0.02	5.73**	2.14*	17.67	25.54**
7	$K^{*}9/K^{S}9$; k10/k10	♀	70.29	6.14	3.18	19.88	0.09	0.26	0.16	0.00	6.50	3.43	20.30	30.23
8	$K^{*}9/K^{S}9$; K10/k10	♀	57.96**	7.33**	4.67**	29.15**	0.07	0.52**	0.30	0.00	7.92**	5.04**	29.97**	42.93**
9	$K^{*}9/K^{S}9$; k10/k10	♂	53.74	17.09	4.23	21.81	0.39	2.25	0.42	0.07	19.79	5.11	24.54	49.45
10	$K^{*}9/K^{S}9$; K10/k10	♂	52.55	14.57**	4.82	24.95**	0.32	2.33	0.47	0.00	17.21*	5.61	27.75**	50.57

* and ** -- significant at 5% and 1% levels respectively from its sib of same sex.
The recombination values were calculated from data in Appendix 9 .

APPENDIX 11 Recombination data from test crosses of plants with different combinations of knobs and abnormal chromosome 10. The bracketed combinations are sibs.

K^s or K^* + c + / k wd + wx (1 2)	Gametic classes (0) + / c / +	(0) wd / + / wx	(1) + / + / wx	(1) wd / c / +	(2) + / c / wx	(2) wd / + / +	(12) + / + / +	(12) wd / c / wx	Total
1. K^s9/k9 ♀	1763	1726	127	132	592	594	10	7	4951
2. K^*9/k9 ♀	2307	2345	37	35	258	293	3	3	5281
3. K^*9/k9 ; k10/k10 ♀	1029	1059	11	15	161	155	3	5	2438
4. K^*9/k9 ; K10/k10 ♀	2997	1722	112	65	1300	669	10	6	6881
5. K^*9/k9 ; k10/k10 ♂	868	738	82	116	358	420	18	21	2621
6. K^*9/k9 ; K10/k10 ♂	1425	1341	176	167	782	819	48	40	4798
7. K^*9/k9 ♀	1400	1363	18	28	146	165	2	3	3125
K^s + sh wx / k wd + + (1 2)	(0) + / sh / wx	(0) wd / + / +	(1) + / + / +	(1) wd / sh / wx	(2) + / sh / +	(2) wd / + / wx	(12) + / + / wx	(12) wd / sh / +	
8. K^s9/k9 ♀	1193	1222	108	100	324	379	7	9	3342

APPENDIX 12 Recombination values from test crosses of plants with different combinations of knobs and abnormal chromosome 10. The bracketed combinations are sibs.

	$\frac{K^S \text{ or } K^*}{k}$	$\frac{+ \quad c \quad +}{wd_1 \quad + \quad wx_2}$	Non crossovers	Crossovers in region			Total recombination		
				(1)	(2)	(12)	(1)	(2)	$\bar{-}$
1	$K^S9/k9$ ♀		70.47	5.23	23.96	0.34	5.58	24.30	29.88
2	$K^*9/k9$ ♀		88.09**	1.36**	10.43**	0.11*	1.48**	10.55**	12.02**
3	$K^*9/k9$; k10/k10 ♀		85.64	1.07	12.96	0.33	1.40	13.29	14.69
4	$K^*9/k9$; K10/k10 ♀		68.58**	2.57**	28.62**	0.23	2.80**	28.85**	31.65**
5	$K^*9/k9$; k10/k10 ♂		61.27	7.55	29.68	1.49	9.04	31.17	40.21
6	$K^*9/k9$; K10/k10 ♂		57.65**	7.15	33.37**	1.83	8.98	35.20**	44.18**
7	$K^*9/k9$ ♀		88.42	1.47	9.95	0.16	1.63	10.11	11.74
	$\frac{K^S}{k}$	$\frac{+ \quad sh \quad wx}{wd_1 \quad + \quad +_2}$							
8	$K^S9/k9$ ♀		72.26	6.22	21.04	0.48	6.70	21.52	28.22

* and ** -- significant at 5% and 1% levels respectively from its sib of same sex.

The recombination values were calculated from data in Appendix 11 .

APPENDIX 13 Recombination data from test crosses of plants with different combinations of knobs and abnormal chromosome 10. The bracketed combinations are sibs.

	Gametic classes								
+ + + / yg sh wx (1 2)	(0) + + +	(0) yg sh wx	(1) + sh wx	(1) yg + +	(2) + + wx	(2) yg sh +	(12) + sh +	(12) yg + wx	Total
1. K^*9/K^L9 ; k10/k10 ♀	1457	1484	312	333	366	368	9	6	4335
2. K^*9/K^L9 ; K10/k10 ♀	3411	4027	1046	1226	1170	1445	39	48	12412
3. K^*9/K^L9 ; k10/k10 ♂	428	316	100	121	133	154	15	14	1281
4. K^*9/K^L9 ; K10/k10 ♂	753	632	221	264	295	258	37	35	2495
5. K^*9/K^L9	823	834	186	200	233	233	8	7	2524
6. K^L9/K^s9	678	628	144	138	230	231	3	5	2057
7. K^s9/K^s9	900	860	298	327	278	219	5	9	2896
8. K^*9/K^s9 ♀	1118	1011	108	116	266	265	2	1	2887
9. K^s9/K^s9	943	854	384	375	241	231	7	6	3041
10. K^*9/K^s9	1165	1174	119	137	388	346	0	4	3333
11. K^s9/K^s9	448	449	190	163	140	126	4	3	1523
12. K^*9/K^s9	630	620	69	70	180	186	2	5	1762
k10 + 1 + 2 + / k10 g_1 r sr_2	(0) + + +	(0) g r sr	(1) + r sr	(1) g + +	(2) + + sr	(2) g r +	(12) + r +	(12) g + sr	
13. K^s9/K^s9 ♀	1061	982	242	264	586	664	7	13	3819
14. K^*9/K^s9 ♀	1262	1098	277	273	645	776	22	11	4364

APPENDIX 14 Recombination values from test crosses of plants with different combinations of knobs and abnormal chromosome 10. The bracketed combinations are sibs.

	$\frac{+ \quad + \quad +}{yg_1 \quad sh_2 \quad wx}$		Non crossovers	Crossovers in region			Total recombination		
				(1)	(2)	(12)	(1)	(2)	$\frac{-}{-}$
1	K^*9/K^L9 ; k10/k10 ♀		67.84	14.88	16.93	0.35	15.23	17.28	32.51
2	K^*9/K^L9 ; K10/k10 ♀		59.93**	18.31**	21.07**	0.70*	19.01**	21.77**	40.78**
3	K^*9/K^L9 ; k10/k10 ♂		58.08	17.25	22.40	2.26	19.52	24.67	44.18
4	K^*9/K^L9 ; K10/k10 ♂		55.51	19.44	22.16	2.89	22.33*	25.05	47.38
5	K^*9/K^L9		65.65	15.29	18.46	0.59	15.89	19.06	34.95
6	K^L9/K^S9		63.49	13.71	22.41**	0.39	14.10	22.80**	36.90
7	K^S9/K^S9		60.77	21.58	17.16	0.48	22.06	17.64	39.70
8	K^*9/K^S9	♀	73.74**	7.76**	18.39	0.10**	7.86**	18.50	26.36**
9	K^S9/K^S9		59.09	24.96	15.52	0.43	25.39	15.95	41.34
10	K^*9/K^S9		70.18**	7.68**	22.02**	0.12*	7.80**	22.14**	29.94**
11	K^S9/K^S9		58.89	23.18	17.47	0.46	23.64	17.93	41.56
12	K^*9/K^S9		70.94**	7.89**	20.77*	0.40	8.29**	21.17*	29.46**
13	K^S9/K^S9	$[\frac{+ \; 1_+ \; 2_+}{g \quad r \quad sr}]$ ♀	53.50	13.25	32.73	0.52	13.77	33.25	47.02
14	K^*9/K^S9		54.08	12.60	32.56	0.76	13.36	33.32	46.68

* and ** -- significant at 5% and 1% levels respectively from its sib of same sex.

The recombination values were calculated from data in Appendix 13 .

APPENDIX 15 Recombination data from test crosses of plants with different combinations of knobs and number of B-chromosomes.

					Gametic classes														
					(0)	(0)	(1)	(1)	(2)	(2)	(3)	(3)	(12)	(12)	(13)	(13)	(23)	(23)	(123)	(123)	
				♀	+ + + +	yg sh bz wx	+ sh bz wx	yg + + +	+ + bz wx	yg sh + +	+ + wx	yg sh bz +	+ sh + +	yg + bz wx	+ sh bz +	yg + wx	+ + bz +	yg sh + wx	+ sh + wx	+ + bz +	Total
	yg	sh	bz	wx	1	2	3														
1				K^S/K^S	1330	1334	534	531	55	60	341	366	1	0	5	4	0	2	0	0	4563
2	0 B			K^*/K^S	821	775	91	98	18	21	197	186	0	0	1	0	0	0	0	0	2208
3				K^S/K^S	803	836	299	290	38	30	254	219	0	1	5	3	1	0	0	0	2779
4	1 B			K^*/K^S	1968	1819	323	314	44	40	593	564	1	2	5	9	0	1	1	0	5684
5				K^S/K^S	958	967	361	383	41	24	238	271	0	1	11	8	0	0	0	0	3263
6	2 B			K^*/K^S	391	416	49	53	9	8	116	110	0	0	1	2	0	0	0	0	1155
7				K^S/K^S	174	215	71	70	2	6	57	62	0	0	2	1	0	0	0	0	660
8	3 B			K^*/K^S	529	568	86	96	16	24	205	186	0	0	3	2	0	1	0	0	1716
9				K^S/K^S	471	487	192	188	12	12	149	119	0	2	3	1	2	0	0	0	1638
10	4 B			K^*/K^S	499	446	63	52	9	14	127	136	0	1	2	3	0	0	0	0	1352
11				K^S/K^S ♂	1435	1343	539	675	43	47	409	436	8	6	53	66	4	1	0	1	5066
12				K^*/K^S ♂	1413	1369	452	504	40	38	542	556	3	6	64	56	4	1	0	0	5048

APPENDIX 16 Recombination values from test crosses of plants with different combinations of knobs and number of B-chromosomes.

	+ + + + ♀ yg 1 sh 2 bz 3 wx		Non crossovers	Crossovers in region							Total recombination			
				(1)	(2)	(3)	(12)	(13)	(23)	(123)	(1)	(2)	(3)	⁻
1	0 B	K^S/K^S	58.38	23.34	2.52	15.49	0.02	0.20	0.04	0.00	23.56	2.59	15.74	41.88
2		K^*/K^S	72.28	8.56	1.77	17.35	0.00	0.05	0.00	0.00	8.61	1.77	17.39	27.76
3	1 B	K^S/K^S	58.98	21.20	2.45	17.02	0.04	0.29	0.04	0.00	21.52	2.52	17.34	41.38
4		K^*/K^S	66.63	11.21	1.48	20.36	0.05	0.25	0.02	0.02	11.52	1.57	20.64	33.73
5	2 B	K^S/K^S	58.99	22.80	1.99	15.60	0.03	0.58	0.00	0.00	23.41	2.02	16.18	41.62
6		K^*/K^S	69.87	8.83	1.47	19.57	0.00	0.26	0.00	0.00	9.09	1.47	19.83	30.39
7	3 B	K^S/K^S	58.94	21.36	1.21	18.03	0.00	0.45	0.00	0.00	21.82	1.21	18.48	41.52
8		K^*/K^S	63.93	10.61	2.33	22.79	0.00	0.29	0.06	0.00	10.90	2.39	23.14	36.42
9	4 B	K^S/K^S	58.49	23.20	1.47	16.36	0.12	0.24	0.12	0.00	23.56	1.71	16.73	42.00
10		K^*/K^S	69.90	8.51	1.70	19.45	0.07	0.37	0.00	0.00	8.95	1.78	19.82	30.55
11		K^S/K^S ♂	54.84	23.96	1.78	16.68	0.27	2.35	0.10	0.02	26.61	2.17	19.15	47.93
12		K^*/K^S ♂	55.11	18.94	1.54	21.75	0.18	2.38	0.10	0.00	21.49	1.82	24.23	47.54

The recombination values were calculated from data in Appendix 15.

APPENDIX 17 Recombination data from test crosses of plants with different combinations of knobs and number of B-chromosomes.

	+ + + +			Gametic classes														
	yg sh bz wx		♀	(0) + + + +	(0) yg sh bz wx	(1) + sh bz wx	(1) yg + + +	(2) + + bz wx	(2) yg sh + +	(3) + + + wx	(3) yg sh bz +	(12) + sh + +	(12) yg + bz wx	(13) + sh bz +	(13) yg + + wx	(23) + + bz +	(23) yg sh + wx	(123) + sh + wx	(123) yg + bz +	Total
	1 2 3																		
1	0 B	K^S/K^S		998	963	410	397	48	26	239	275	0	0	5	6	0	4	0	0	3371
2		K^*/K^S		1379	1320	156	149	33	24	385	374	0	0	5	4	0	1	0	0	3830
3	1 B	K^S/K^S		865	824	315	319	33	27	233	246	1	0	10	6	0	0	0	0	2879
4		K^*/K^S		1302	1236	187	195	35	32	432	427	0	1	2	2	1	3	0	0	3855
5	2 B	K^S/K^S		1210	1188	516	495	44	47	354	303	1	0	15	8	0	0	0	0	4181
6		K^*/K^S		1351	1417	167	131	28	21	425	409	2	0	7	3	1	3	1	0	3966
7	3 B	K^S/K^S		544	555	201	183	22	15	161	138	0	1	6	8	0	0	0	0	1834
8		K^*/K^S		878	886	135	133	24	19	319	309	1	1	3	1	2	3	0	0	2714
9	4 B	K^S/K^S		1138	1208	483	424	30	40	296	309	1	1	10	5	2	1	1	0	3949
10		K^*/K^S		968	944	119	127	14	24	328	316	0	0	2	8	0	0	0	0	2850
11	5 B	K^S/K^S		278	281	106	112	8	8	84	80	0	0	7	5	0	2	0	0	971
12		K^*/K^S		301	311	58	62	9	5	106	108	0	0	1	1	1	3	0	0	966

APPENDIX 18 Recombination values from test crosses of plants with different combinations of knobs and number of B-chromosomes.

+ + + + yg sh bz wx 1 2 3		♀	Non crossovers	Crossovers in region							Total recombination			
				(1)	(2)	(3)	(12)	(13)	(23)	(123)	(1)	(2)	(3)	Σ
1	0 B	K^S/K^S	58.17	23.94	2.19	15.25	0.00	0.33	0.12	0.00	24.27	2.31	15.69	42.27
2		K^*/K^S	70.47	7.96	1.49	19.82	0.00	0.24	0.03	0.00	8.20	1.51	20.08	29.79
3	1 B	K^S/K^S	58.67	22.02	2.08	16.64	0.04	0.56	0.00	0.00	22.61	2.12	17.19	41.93
4		K^*/K^S	65.84	9.91	1.74	22.28	0.03	0.10	0.10	0.00	10.04	1.87	22.49	34.40
5	2 B	K^S/K^S	57.36	24.18	2.18	15.71	0.02	0.55	0.00	0.00	24.75	2.20	16.26	43.22
6		K^*/K^S	69.79	7.51	1.24	21.03	0.05	0.25	0.10	0.03	7.84	1.41	21.41	30.66
7	3 B	K^S/K^S	59.92	20.94	2.02	16.30	0.06	0.76	0.00	0.00	21.76	2.07	17.07	40.89
8		K^*/K^S	65.00	9.88	1.58	23.14	0.07	0.15	0.18	0.00	10.10	1.84	23.47	35.41
9	4 B	K^S/K^S	59.41	22.97	1.77	15.32	0.05	0.38	0.08	0.03	23.42	1.93	15.80	41.15
10		K^*/K^S	67.09	8.63	1.33	22.60	0.00	0.35	0.00	0.00	8.98	1.33	22.95	33.26
11	5 B	K^S/K^S	57.57	22.45	1.65	16.89	0.00	1.24	0.21	0.00	23.69	1.85	18.33	43.87
12		K^*/K^S	63.35	12.42	1.45	22.15	0.00	0.21	0.41	0.00	12.63	1.86	22.77	37.27

The recombination values were calculated from data in Appendix 17.

APPENDIX 19 Recombination data from test crosses of plants with different combinations of knobs and number of B-chromosomes.

				Gametic classes														
+ + + +			♂	(0)	(0)	(1)	(1)	(2)	(2)	(3)	(3)	(12)	(12)	(13)	(13)	(23)	(23)	(123)	(123)	
yg sh bz wx				+	yg	+	yg	+	yg	+	yg	+	yg	+	yg	+	yg	+	yg	
1 2 3				+	sh	sh	+	+	sh	+	sh	sh	+	sh	+	+	sh	sh	+	Total
				+	bz	bz	+	bz	+	+	bz	+	bz	bz	+	bz	+	+	bz	
				+	wx	wx	+	wx	+	wx	+	+	wx	+	wx	+	wx	wx	+	
1.		K^s/K^s		745	651	252	325	25	29	172	219	1	2	40	14	2	2	0	0	2479
2.	0 B	K^*/K^s		948	873	310	300	46	40	349	418	0	4	37	39	4	1	0	0	3369
3.		K^s/K^s		705	601	216	300	19	20	193	234	0	1	26	27	0	2	0	0	2344
4.	1 B	K^*/K^s		739	550	170	206	24	16	241	300	0	2	19	18	0	0	0	0	2285
5.		K^s/K^s		752	658	261	347	13	21	168	221	2	0	25	26	0	0	0	0	2494
6.	2 B	K^*/K^s		990	764	185	224	21	24	293	405	4	5	35	21	0	2	0	0	2973
7.		K^s/K^s		784	693	254	320	18	23	145	236	1	1	21	34	0	0	0	0	2530
8.	3 B	K^*/K^s		900	666	215	263	30	20	304	351	2	1	33	27	2	0	0	0	2814
9.		K^s/K^s		1115	1042	370	478	24	33	247	358	3	4	35	24	1	0	0	0	3734
10.	4 B	K^*/K^s		606	517	167	161	25	17	212	225	1	1	19	15	2	2	0	0	1970
11.		K^s/K^s		414	365	148	179	9	13	105	138	1	1	14	20	1	0	0	1	1409
12.	5 B	K^*/K^s		406	346	102	97	11	10	146	144	0	0	8	6	2	0	0	0	1278

APPENDIX 20 Recombination values from test crosses of plants with different combinations of knobs and number of B-chromosomes.

+ + + + ♂ yg 1 sh 2 bz 3 wx			Non crossovers	Crossovers in region							Total recombination			
				(1)	(2)	(3)	(12)	(13)	(23)	(123)	(1)	(2)	(3)	
1	0 B	K^s/K^s	56.31	23.28	2.18	15.77	0.12	2.18	0.16	0.00	25.58	2.46	18.11	46.15
2		K^*/K^s	54.05	18.11	2.55	22.77	0.12	2.26	0.15	0.00	20.48	2.82	25.17	48.47
3	1 B	K^s/K^s	55.72	22.01	1.66	18.22	0.04	2.26	0.09	0.00	23.32	1.79	20.56	46.67
4		K^*/K^s	56.41	16.46	1.75	23.68	0.09	1.62	0.00	0.00	18.16	1.84	25.30	45.30
5	2 B	K^s/K^s	56.54	24.38	1.36	15.60	0.08	2.05	0.00	0.00	26.50	1.44	17.64	45.59
6		K^*/K^s	58.99	13.76	1.51	23.48	0.30	1.88	0.07	0.00	15.94	1.88	25.43	43.26
7	3 B	K^s/K^s	58.38	22.69	1.62	15.06	0.08	2.17	0.00	0.00	24.94	1.70	17.23	43.87
8		K^*/K^s	55.65	16.99	1.78	23.28	0.11	2.13	0.07	0.00	19.23	1.96	25.48	46.66
9	4 B	K^s/K^s	57.77	22.71	1.53	16.20	0.19	1.58	0.03	0.00	24.48	1.74	17.81	44.03
10		K^*/K^s	57.01	16.65	2.13	22.18	0.10	1.73	0.20	0.00	18.48	2.44	24.11	45.03
11	5 B	K^s/K^s	55.29	23.21	1.56	17.25	0.14	2.41	0.07	0.07	25.83	1.84	19.80	47.48
12		K^*/K^s	58.84	15.57	1.64	22.69	0.00	1.10	0.16	0.00	16.67	1.80	23.94	42.41

The recombination values were calculated from data in Appendix 19.

VITA

Chia-cheng Chang was born [redacted] in Tamsui Taiwan. In 1962 he received the BS degree from the Taiwan Provincial Chung-Hsing University with a major in Agronomy. From 1962 to 1963 he served in the Chinese Army as a Second Leutenant. He was a research assistant in the Department of Agronomy, Chung-Hsing University from 1963 to 1965. Since 1966 he has been a graduate assistant in Genetics at the University of Missouri. He was married to Li-hwa Wu in 1965. They have two daughters, Li-chee and Helen.

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