PREFERENTIAL SEGREGATION OF STRUCTURALLY MODIFIED CHROMOSOMES IN MAIZE[1]

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IN *Zea mays* there are two different types of chromosome 10, distinguishable not only on the basis of their morphology but also by the fact that one segregates preferentially at megasporogenesis and the other does not. The present investigation is concerned with the behavior of two modified chromosomes derived from an abnormal 10 which exhibits the phenomenon of preferential segregation.

LONGLEY (1937, 1938), working with strains of *Zea mays* from southwestern United States and Mexico, found an exceptional form of chromosome 10, known as the abnormal 10. According to RHOADES (1942, 1952), this chromosome differs from the normal 10 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 of chromatin consists of (1) a proximal euchromatic region, (2) a large heterochromatic segment, and (3) a distal euchromatic region. Figures 1–3 illustrate pachytene configurations of the knobbed and knobless homologues. It will be seen in Figures 1 and 2 that the abnormal 10 possesses three prominent chromomeres in the distal one sixth of the long arm which are not present in the normal strain (Figure 3). It should be also noticed that there is another less conspicuous chromomere near the base of the knob (Figure 2). The normal chromosome is shorter in length than the abnormal 10. In plants heterozygous for normal and abnormal 10, pairing of the normal strand ends in the region between the most distal of the three prominent chromomeres and the chromomere close to the knob.

In 1942 RHOADES discovered that this abnormal chromosome 10 segregated preferentially at megasporogenesis in plants heterozygous for a normal and an abnormal 10. He found, among the progeny of backcrosses in which the abnormal 10 carried the *r* locus (one unit from knob) and normal 10 the *R* locus, approximately 70 percent of the abnormal type (*r*) instead of the expected 50 percent. Evidence was also obtained by RHOADES (1952) that preferential segregation of the knobbed chromosome occurred only when the two homologues were heterozygous for the knob. In plants homozygous for the abnormal 10 and heterozygous for the marker gene *R*, he found random segregation of the knobbed chromosomes 10. From these tests RHOADES concluded that the causative factor or factors for preferential segregation are located either in the region of the

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dissimilar chromomeres or in the knob of the abnormal chromosome 10. Another possibility is that the euchromatic portion of the extra chromatin in abnormal 10 might be responsible for preferential segregation.

In this connection RHOADES' and VILKOMERSON's (1942) cytological studies of abnormal 10 are of importance. They found that abnormal 10 was responsible for the formation of neocentromeres (or secondary sites of centric activity) in chromosomes other than abnormal 10 when one of the two chromatids of a non-homologous dyad had a knob. More important still, the chromosome segments possessing neocentromeres move precociously to the poles. As a consequence, chromatids possessing neocentromeres reach the poles before those without. Neocentromeres occurred in plants both heterozygous and homozygous for the abnormal 10, but the extent of neocentric activity was less in heterozygous plants.

In 1952 RHOADES proposed the following scheme to account for the origin of neocentromeres: (1) in the presence of abnormal 10, the true centric regions yield a surplus of chromosomal fiber substance which is extruded from the centric regions of abnormal 10 and other non-homologous chromosomes and flows along the chromosomes until it comes in contact with a knob; and (2) in the presence of a knob this excess centric substance forms neocentromeres from which supernumerary fibers arise. According to RHOADES the knobs either stimulate neocentric activity or have a special affinity for this substance.

In view of the evidence that the abnormal chromosome 10 causes the formation of neocentromeres and concurrently produces the phenomenon of preferential segregation, it has been postulated by RHOADES (1952, 1955a) that the two are intimately related. He suggests that two events are essential for the occurrence of preferential segregation namely, crossing over and neocentric activity.

As a consequence of crossing over between the centromere and the knob in plants heterozygous for abnormal 10, heteromorphic dyads are formed, of which one chromatid is knobbed and the other is knobless. At anaphase I the knobbed

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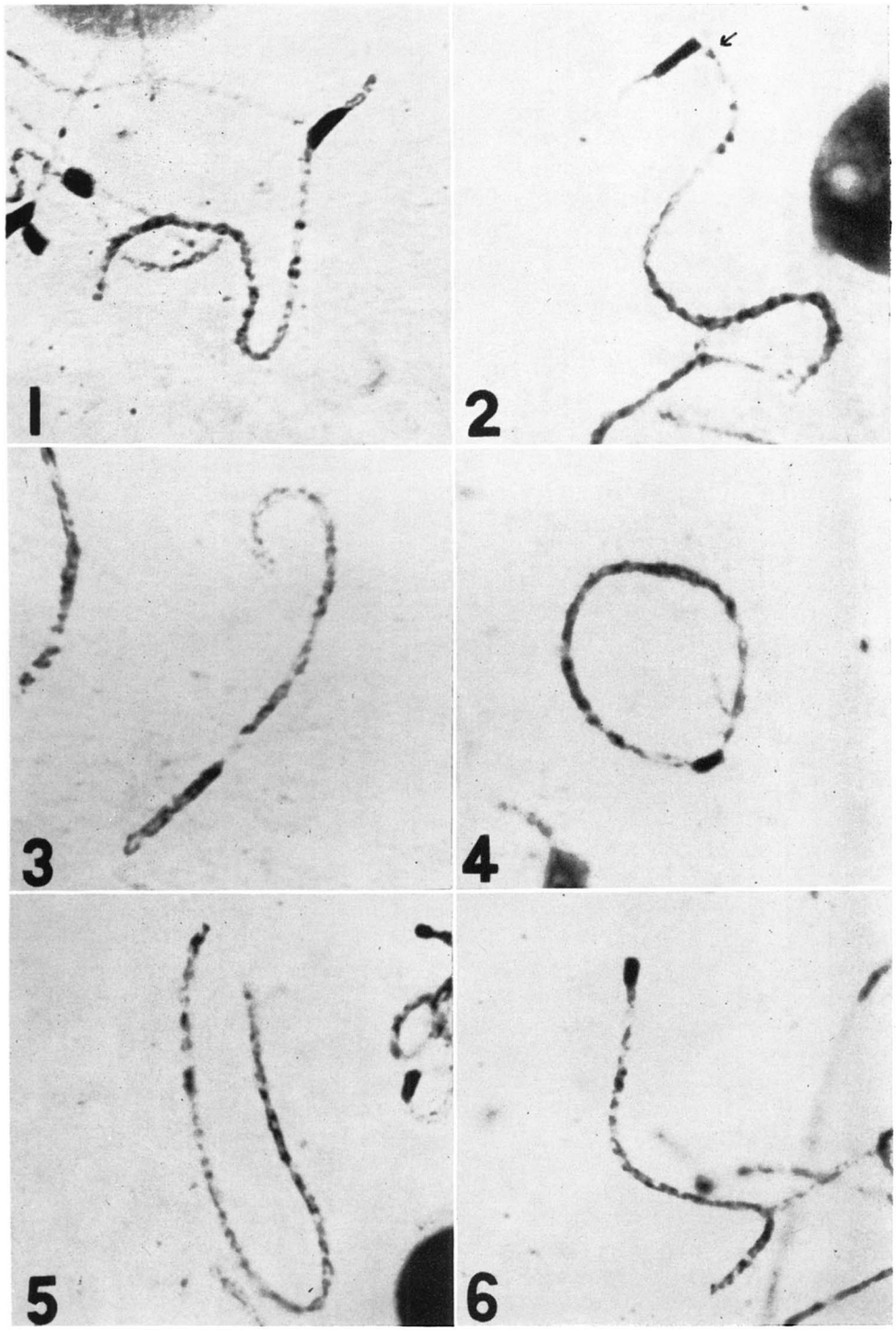
FIGURES 1–6.—Represent different forms of chromosome 10 at pachynema.

FIGURE 1 is the uncommon type of chromosome 10, known as abnormal 10. This figure, which was published in RHOADES (1952), is homozygous for abnormal 10. Note the three prominent chromomeres in the distal one sixth of the long arm, the large knob, and the euchromatic strand distal to the knob. FIGURE 2 shows a normal knobless 10 paired with the abnormal 10. The last chromomere (indicated by an arrow) is included in both of the altered chromosomes which are shown below. FIGURE 3 is the common type of chromosome 10, known as normal or knobless 10. Note that the three prominent chromomeres of the abnormal 10, as well as the knob and distal euchromatic thread, are not found in the knobless strain. This figure was obtained from RHOADES (1954). FIGURE 4 shows a ring chromosome for abnormal 10. This ring lacks a small portion of the short arm, approximately one half of the knob, and the entire strand distal to the knob. FIGURES 5 and 6 represent two modified abnormal chromosomes 10 which originated from the ring chromosome. FIGURE 5 is homozygous knob$^{\circ}$ (K°). This chromosome possesses the three characteristic chromomeres of the abnormal 10 but is deficient for the entire knob and distal euchromatic strand. Note that the next to last chromomere of the long arm is the one shown by an arrow in FIGURE 2. FIGURE 6 is homozygous knob^{s} with the three prominent chromomeres of abnormal 10. This chromosome lacks a portion of the knob and the strand distal to the knob.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chromatid of the dyad moves to the pole more rapidly than the knobless one since the knobbed chromatid possesses neocentromeres. As a result the knobbed chromatid lies nearer to the pole than does the knobless one. This orientation is maintained until the second metaphase stage of megasporogenesis where the two spindles are arranged in tandem. At anaphase II the knobbed monads pass to the terminal poles while the knobless monads migrate to the inner poles. In maize only the basal cell survives to form the functional megaspore. Consequently, the knobbed chromosome is expected to be recovered more often than its knobless homologue, since it moves precociously to the terminal poles. On this hypothesis, therefore, preferential segregation takes place only when crossing over occurs and if neocentromeres are present.

Before discussing the evidence that supports the foregoing hypothesis of preferential segregation, it is necessary to consider one other aspect of abnormal 10. The work of LONGLEY (1945) demonstrated that knobbed chromosomes other than abnormal 10 segregated preferentially at megasporogenesis when the plants were heterozygous for abnormal 10. It was shown that non random segregation occurred only if the non-homologous chromosome was heteromorphic, as was the case for abnormal 10. He analzed preferential segregation of chromosomes 6 and 9. RHOADES and DEMPSEY (1957) reported that non random segregation occurred in plants heterozygous for knobbed chromosomes 3 and possibly 8. In other experiments RHOADES (1955b) and RHOADES and DEMPSEY (1957) showed that preferential segregation of knobbed chromosome 9 marked with the *wd* character not only took place in plants heterozygous for abnormal 10 but also in homozygous plants.

In 1942 RHOADES reported that the extent of preferential segregation depended on the map position of the locus. Subsequent experiments by RHOADES and DEMPSEY (1957) have confirmed and extended the data. They found that loci more distant from the centromere and closer to the knob underwent a higher degree of preferential segregation than did loci less favorably situated.

More recently RHOADES (1958) presented supporting evidence for the relation of crossing over to preferential segregation. This experiment was performed by using a structural abnormality in the short arm of chromosome 9 which was known to cause a great reduction in the normal amount of crossing over. On his hypothesis that preferential segregation occurs only when crossing over takes place, it follows that any event reducing the normal frequency of crossing over would also reduce the degree of preferential segregation. This proved to be true in tests of plants heterozygous for abnormal 10 with the structurally modified chromosome 9 marked with the *yg* locus, which is situated near the terminal knob on 9S, and the knobless homologue marked with the *Yg* allele. The control data showed 65.2 percent preferential segregation of the knobbed chromosome, while the data of modified-9/knob-9 compound exhibited only 54.7 percent preferential segregation of the knobbed chromosome. These data show beyond doubt that preferential segregation is greatly affected by the amount of crossing over.

Recently KIKUDOME (1958), who studied the effects of heterozygous abnormal 10 on preferential segregation of chromosome 9, found that the degree of preferential segregation was greatly influenced by the size of the knob on chromosome 9.

Source of modified chromosomes 10

It was reported in a previous study (EMMERLING 1955) that a large ring chromosome involving nearly the whole of abnormal 10 was produced in the progeny of plants exposed to an X-ray dosage of 400r units (Figure 4). As is shown schematically in Figure 16A, the initial break points must have occurred close to the distal end of the short arm and within the knob, so that the ring chromosome included approximately one half of the knob and the three prominent chromomeres of the long arm of abnormal 10. As a result, the ring chromosome is deficient for the distal part of the knob, the euchromatic segment distal to the knob, and the tip of the short arm. With the exception of the latter deficiency, the segment lacking in the 10L region of the ring does not represent a true deficiency for this is extra chromatin not present in a normal knobless 10. A number of ring/rod heterozygotes were examined at pachynema in order to determine the extent of the deficiency in the region of the "short arm" of the ring. A deficiency was not found. In each figure the homologous rod chromosome was paired in its entire length with the ring chromosome. However, it must be assumed that the ring chromosome possesses a small deficiency at the distal end of 10S, since two broken ends are necessary to produce a ring-shaped chromosome.

In the backcrossed progeny of plants heterozygous for the ring chromosome carrying R^r (colored seed and plant) and the normal rod homologue carrying r^g (colorless seed and plant), a number of exceptional plants occurred which exhibited stable plant color. Ordinarily, plants of ring-R^r/rod-r^g constitution show variegated plant color, due to the unstable behavior of a ring chromosome. Cytological analysis of these exceptional plants revealed that the ring chromosomes had been converted to stable rods.

SCHWARTZ (1953), who encountered a similar situation in a ring-6/rod heterozygote, suggested that the ring to rod conversion arose as a consequence of crossing over in the heterozygote. If the dicentric chromatids produced by crossing over broke at anaphase, the broken ends would heal to form stable rods. A number of Drosophila workers (MORGAN 1933; STURTEVANT and BEADLE 1936; NOVITSKI 1951) have obtained similar results following crossing over in ring/rod heterozygotes.

Such a scheme could account for the origin of the stable rod chromosomes reported herein. Figure 16B shows only one of the four possible types of crossovers that would result in the production of a dicentrid chromatid at the anaphase I stage. Chromosomal bridges may also arise from both types of 3-strand and from 4-strand double exchanges. As shown in Figure 16B, a single crossover either in the long or short arms gives rise to a single chromatid bridge at anaphase I which is expected to break at telophase I (MCCLINTOCK 1938). The position of the

break may occur at any point between the two centromeres of the dicentric chromosome, producing both deficient and duplicated derivatives. McCLINTOCK (1941) has shown that the broken ends of the two newly formed chromosomes heal in the embryo to form stable rods, while in the endosperm they continue the bridge-breakage-fusion cycle.

Both duplicated and deficient rod chromosomes arose from the ring-10 heterozygote, of which only two will be considered in this paper. One of the modified chromosomes originated from breakage of the anaphase bridge at position 1 (Figure 16B). This chromosome which will be referred to as knob° (or K°) includes all that is present in the long arm of an abnormal 10 except for the knob and the distal euchromatic segment (Figure 16C, Figure 5). That is, the K° chromosome possesses the three adjacent chromomeres characteristic of an abnormal 10 and the strand distal to this which terminates at the base of the knob. Since the K° chromosome lacks the knob of abnormal 10, it could be confused with a normal knobless chromosome 10 in homozygous K° plants if it were not for the presence of the three prominent chromomeres (see Figures 3 and 5 for comparison). The K° chromosome is apparently free from a gross aberration either in the distal region of the short arm or in the region proximal to the last deeply stained chromomere of the long arm. Several pachytene figures of K° paired with its normal knobless homologue or with an unchanged abnormal 10 failed to reveal either a deficiency or duplication.

A second type of modified abnormal 10 originated from breakage of the anaphase bridge at position 2 (Figure 16B). This chromosome which is designated knob^{s} (or K^{s}) possesses the region of the dissimilar chromomeres and approximately one half of the heterochromatic substance (Figure 16D and Figure 6). It differs from an unchanged abnormal 10 by the absence of part of the knob and the chromatin distal to the knob, and from the K° chromosome by the presence of about one half of the knob. The paired regions of normal 10 and K^{s} chromosomes showed no indication of deficiency or duplication. This is important to note since the genetic data from tests of K^{s} with various types of homologues are not in agreement with the cytological findings. The evidence for this will be discussed later.

Knob symbols

A summary of the knob symbols that will be used for chromosome 10 in the first section of this paper is as follows:

k 10— a normal knobless chromosome (Figure 3).

K 10— an unchanged abnormal 10 (Figures 1 and 2).

K°10— a modified abnormal chromosome which is deficient for the entire knob and distal euchromatic strand but possesses the characteristic chromomeres of an abnormal 10 (Figure 5).

K^{s}10— a modified abnormal chromosome which lacks part of the knob and the distal euchromatic strand (Figure 6).

In the second section the following symbols with the chromosome number will

be used to describe the type of knob at the distal end of the short arm of chromosome 9.

k 9— a normal knobless chromosome.

k (*wd*)9—a knobless 9 associated with a minute deficiency at the distal end of the short arm.

K 9— knobbed chromosome used by RHOADES (1955b) and LONGLEY (1945).

K^L 9— a large knob employed in the present experiment (Figure 15).

Viability test of K° *and* K^8

The transmission of the modified chromosomes through the pollen was tested by crosses of the type *r k/r k* × *R* modified-*K/r k*, in which the seeds heterozygous for the modified knobs were colored (*Rr*) while their knobless sibs were colorless (*rr*). The *R* locus is located approximately one to two crossover units proximal to the knob (RHOADES 1942). In the absence of reduced male transmission, the ears should show equal frequencies of colored and colorless seeds.

Ordinarily a test of this type provides an extremely sensitive screen to detect the slightest chromosomal abnormality. However, in the case of plants heterozygous for the modified chromosomes, the data are complicated by the fact that an unchanged abnormal 10 exhibits reduced transmission through the male gametes. In tests of type *r k/r k* × *R K/r k*, RHOADES (1942) recovered only 42 percent of abnormal 10 where 50 percent was expected. RHOADES (1942) correlates the lowered transmission of the abnormal 10 through the pollen with the extra segment of the abnormal 10, postulating hyperploidy.

In the present experiment the compound *R K/r k*, including an unchanged abnormal 10, yielded 1125 *R r* (*K*) and 1419 *r r* (*k*), or 44.2 percent *K*. Two different plants of the same stock were used as male parents. The data from the transmission tests of K^8 come from two different male stocks. One culture, in which four different pollen parents of *R* K^8*/r k* constitution were tested, produced 1043 *R r* (K^8) and 1570 *r r* (*k*), or 39.9 percent K^8. The second culture, in which five different male parents were tested, gave 1685 *R r* (K^8) and 3105 *r r* (*k*), or 35.2 percent of K^8. The average frequency of K^8 transmission is 36.9 percent. In male transmission tests of K°, two *R K°/r k* pollen parents of one stock gave 1955 *R r* and 2018 *r r*, or 49.2 percent K°. In another stock, four *R K°/r k* plants yielded 1056 *R r* and 1084 *r r*, or 49.4 percent K°.

Although these results show a decrease in the rate of male transmission of K^8 as compared with an unchanged abnormal 10, they do not justify the conclusion that the K^8 chromosomes carries a factor reducing transmission until more comparable tests are made. The reason for this is that the above data come from two unrelated stocks. There is considerable evidence against the possibility of a segmental deficiency in the K^8 chromosome. If this chromosome possesses a gross cytological aberration, it might be expected that the pollen would exhibit a considerable amount of sterility. However, plants of K^8 *k* constitution showed only 8 to 10 percent abnormal pollen which is about what RHOADES (1952) found in

plants homozygous for abnormal 10. Furthermore, there is no evidence of ovule sterility in ears heterozygous for K^s. In addition, the fact that the K^s chromosome is viable in the homozygote also argues against a gross deficiency. Consequently while it is impossible at this time to disprove the existence of an aberration associated with K^s, there is ample evidence to show that K^s has all the chromatin possessed by a normal 10. However, in view of the genetic data to be presented in later sections, it is important to bear in mind the lowered transmission rate of the K^s chromosome through the pollen.

With the K° chromosome, the tests showed approximately normal transmission through the male gametes. These data are of interest since it has been demonstrated by RHOADES (1942) that an unchanged abnormal 10 with the extra segment exhibited lowered male transmission. Presumably the loss of only the knob and distal chromatin restored viability through the pollen.

Additional evidence suggests that the K° chromosome is of normal constitution. The pollen examinations showed that abortion was not higher than in plants homozygous for normal 10; and the cytological observations failed to reveal any abnormality. Also, ears from both heterozygous and homozygous K° plants did not exhibit an appreciable amount of ovule sterility.

Effect of K^s *and* K° *chromosomes on crossing over*

In view of the hypothesis that preferential segregation takes place only when crossing over occurs (RHOADES 1952), it is essential to test the effects of the two modified chromosomes on crossing over. It is expected on the basis of RHOADES' experiment (1958) involving a structurally modified chromosome 9 that the degree of preferential segregation would be reduced if either of the modified chromosomes (K° or K^s) is associated with an abnormality that reduces the normal amount of crossing over in chromosome 10.

The effects of the K^s and K° chromosomes on crossing over can be measured in the *R-K* (1–2 units) and *G-R* (14 units) regions of chromosome 10 and in regions to the left of *g*. At the present time data are available only from the *G-R* segment (Table 1). With the K^s chromosome, the amount of recombination was 10.3 percent which was lower than the expected frequency of crossing over in this region. Similarly with the K° chromosome, there was a reduction from 14 percent to 11.5 percent. Two possibilities may be mentioned as tentative explanations of the lowered frequency of recombination. The first is that the K^s and K° chromosomes are associated with an abnormality, causing a reduction in the normal amount of crossing over. As mentioned before, the cytological observations showed no evidence of a gross aberration. Alternatively, these differences could be explained as being due to variation since crossing over in the *G-R* region was found to vary markedly in normal stocks. With normal chromosome 10 (*k k*), the frequency ranged from 11 percent to 24 percent (EMERSON, BEADLE and FRASER 1935) while in stocks with heterozygous abnormal 10 (*K k*), 14 percent to 19.2 percent was found (RHOADES 1942 and 1952). RHOADES (1942) stated that the presence of the additional segment in abnormal 10 caused no decrease in crossing over in the *g-R* region.

TABLE 1

Effect of K^S *and* K^o *on crossing over in the* G–R *region in female gametes*

Cross		Noncrossovers		Crossovers		Crossover
g R^r K^S/G r^g k × *g r^g k*/same		*g R^r K^S*	*G r^g k*	*G R^r K^S*	*g r^g k*	Percent
56:353–14		35	35	2	2	...
–10		87	82	7	11	...
–3		65	63	8	9	...
–9		107	114	15	9	...
–1		100	70	12	13	...
–13		117	135	13	15	...
–12		62	67	7	6	...
–6		66	48	7	8	...
Total	1397	639	614	71	73	10.3
G R^r K^o/g r^g k × *g r^g k*/same		*G R^r K^o*	*g r^g k*	*g R^r K^o*	*G r^g k*	
79:870–8		107	103	12	12	...
–6		67	75	15	13	...
–10		65	55	7	6	...
–1		98	94	9	15	...
–5		81	76	6	12	...
–2		112	94	17	12	...
–4		112	100	14	11	...
Total	1400	642	597	80	81	11.5

Consequently, it is not possible to conclude with certainty, without further crossover data from sib comparisons of abnormal and modified chromosomes 10, that the K^S and K° chromosomes cause a reduction in crossing over.

A. Effect of modified chromosomes on preferential segregation for the R *allele*

1. *Tests of* K^S *and* K^o *chromosomes with a knobless 10:* The effect of the modified chromosomes on preferential segregation was determined in backcrossed ears produced by pollinating female plants of *R* modified-*K*/*r k* constitution by *r k*/*r k*. The relative frequency of colored and colorless seeds was used to measure the degree of preferential segregation of the altered chromosomes. The results are presented in Table 2.

TABLE 2

The behavior of K^S *and* K^o *when tested with a normal knobless chromosome 10 in crosses of* ♀♀ R K (*modified*)/r k × ♂♂ r k/r k

Knob type	Parental culture	Total population	Numbers of Colored *R K*	Colorless *r k*	Percent *K*	χ^2 (50:50)	p
R K (unchanged)/*r k*	77:1661C	3,397	2,384	1,013	70.2	...	.....
R K^S/r k	77:1661B–62C	14,967	7,433	7,534	49.7	...	.....
	79:1051B	25,147	12,420	12,727	49.4	...	.....
Total		40,114	19,853	20,261	49.5	0.96	0.50–0.20
R K^o/r k	77:1664A–D	10,278	5,432	4,846	52.7	33.42	<0.01

K^s k *Test*: Plants of *R* K^s/r *k* type gave approximately 49.5 percent of K^s in separate experiments, conducted during two growing seasons. This percentage does not differ significantly from 50 percent (p = 0.5–0.2). Control data from a closely related stock had exhibited 70 2 percent of abnormal 10.

The apparent absence of preferential segregation in K^s *k* heterozygotes cannot be satisfied by a simple hypothesis, since the evidence presented here are inconsistent with data to be given in later sections. However, if these data are considered apart from other tests involving K^s, two alternative mechanisms could account for the finding of normal backcross ratios in plants heterozygous for K^s.

One interpretation is that the effect is due to an unknown factor which is causing a reduction of K^s transmission through the female gametes. It has been previously demonstrated that there was a probable reduction of K^s transmission through the pollen. If such be the case in the female, then the ears should exhibit approximately 20 percent of ovule sterility. A number of ears were examined for ovule abortion but no appreciable amount was found. However, a small amount of sterility might not be detected unless numerous ovule counts were made.

A second explanation of the normal 1:1 backcross ratios from K^s *k* plants is that crossing over was suppressed in chromosome 10. As mentioned before RHOADES (1958) reported that preferential segregation was lowered when crossing over was also reduced. If for some reason the presence of the K^s chromosome inhibited crossing over throughout the entire arm of 10L the ratio of K^s:*k* should be 1:1 instead of 0.7:0.3. From the evidence available at present there is a possibility that crossing over is decreased in the *G-R* region from 14 to 10.3 percent but there is not the complete suppression which would be necessary in order to account for the observed 1 K^s: 1 *k* ratios. Crossover data from the *R-K* segment have not yet been obtained. Consequently, the interpretation of suppressed crossing over lacks sufficient support from the data on hand but cannot be excluded.

The interpretation that a factor for preferential segregation is missing from the K^s chromosome is immediately excluded on the basis of the data from plants heterozygous for K°. It follows that if the preferential segregation factor were present in either the distal one half of the knob or in the euchromatin distal to the knob, then the K° chromosome without these segments should not segregate preferentially. The results show, however, that K° segregates preferentially.

Since the genetic data exhibit no evidence of preferential segregation of K^s in the heterozygote, it was of interest to examine these plants cytologically in order to determine whether neocentromeres were formed. The expectation here is that the neocentric activity would be absent since preferential segregation did not occur. Before describing the cytological observations, a word of caution must be added. The absence of preferential segregation in plants of K^s *k* constitution does not necessarily mean that the K^s chromosome behaves normally in other chromosomal combinations. It will be shown that K^s behaves similar to *K*10 in plants of K^s *K* constitution. Consequently, the cytological evidence from K^s *k* plants should be interpreted with this in mind.

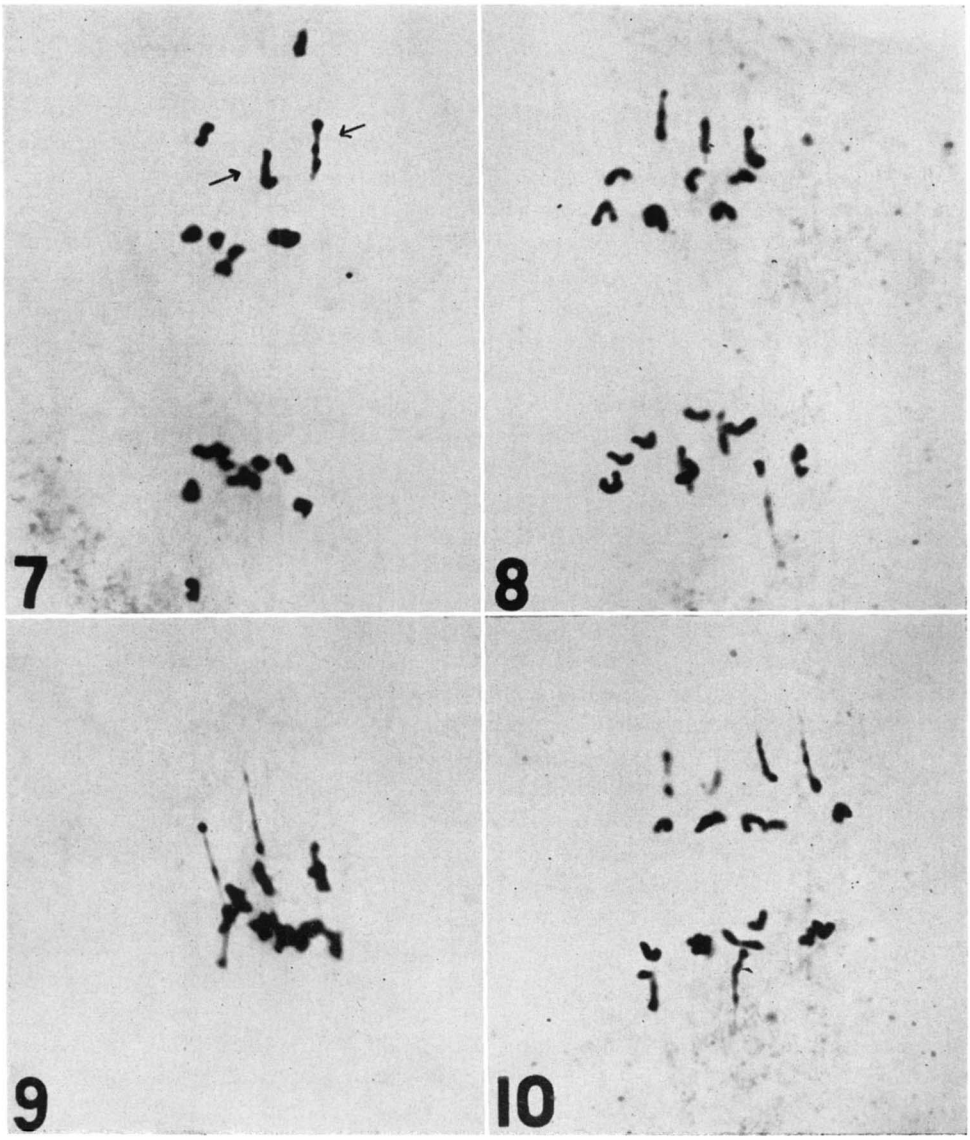
Examination of microsporocytes established that neocentromeres were formed in plants of *R* K^s/*R* K^s constitution. Typical anaphase II configurations are illustrated in Figures 7 and 8. In Figure 7 it will be noted that the arms of two of the monads in the upper group are moving poleward in advance of the true centric regions. Figure 8 shows neocentric regions in three monads migrating to the upper pole and in three monads moving to the lower pole.

From these observations there appears to be a reduction in the degree of neocentric activity of K^s when compared with an unchanged abnormal 10 (see RHOADES 1952 for figures of neocentric activity in plants with abnormal 10). This difference may be due to variation introduced by the stocks, or to an effect of the K^s chromosome. There is ample evidence to prove that the extent of neocentric activity varies markedly in stocks heterozygous and homozygous for abnormal 10. When a random sample of six plants carrying heterozygous abnormal 10 was examined, only two of the six plants had neocentromeres, while the other four plants which showed genetic evidence of preferential segregation were without neocentromeres. In view of this variation it will be necessary to compare the degree of neocentric activity in sib plants of K^s *k*, *K k*, and *k k* types before any conclusion can be drawn in regard to the apparent reduction observed in K^s.

Consequently, from the limited cytological information available on the neocentric activity of the K^s chromosome, we can be reasonably certain of only one conclusion: some degree of neocentric activity occurs in the absence of preferential segregation. This evidence does not disprove RHOADES' hypothesis on the role of neocentromeres in preferential segregation, but it is inconsistent with it. It is conceivable that a certain amount of neocentric activity is required before the chromosomes will segregate preferentially. If such be the case, the cytological results reported here would be without much significance unless the apparent reduction of neocentric activity proves to be invalid.

K° k *Test:* When plants of *R K°/r k* type were used as the female parent in backcrosses, the percentage of *K°* transmission was 52.7 (Table 2). This differs significantly from the normal 50 percent expected on basis of random segregation of the two types of chromosome 10, and also from the 70 percent predicted on the basis of preferential segregation of the knobbed chromosome. Thus the result shows that the *K°* chromosome is able to segregate preferentially, although at a much reduced rate.

It is obvious that the data reported here are inconsistent with the data of K^s, as shown in the previous section. In the case of K^s *k* plants, in which the modified chromosome possesses not only the three characteristic chromomeres but also part of the knob, the two types of chromosomes segregated wholly at random. In the *K° k* heterozygotes, in which the entire knob 10 is missing, the *K°* chromosome segregated preferentially in 52.7 percent of the functional megaspores. Why should *K°* segregate preferentially if K^s does not, for the latter has more of the original abnormal 10 than the *K°*? This immediately raises the question as to whether or not the data from the K^s *k* heterozygote represent an actual lack of



FIGURES 7–10.—Show neocentric activity in plants homozygous for the modified chromosome 10. FIGURE 7 shows an anaphase II cell in a plant homozygous for K^8. The two monads which exhibit neocentric regions are indicated by arrows. FIGURE 8 is another anaphase II cell in a plant homozygous for K^8. In this cell, six monads possess neocentromeres of which three are migrating to the upper pole and three to the lower pole. FIGURE 9 is metaphase II in a plant homozygous for knob°. The first dyad from the left has formed neocentromeres in each arm; also one other monad shows a neocentric region. FIGURE 10 is early anaphase II in a plant homozygous for knob°. Neocentric regions are exhibited by four monads directed to the upper pole and by two to the lower pole.

preferential segregation on the part of the K^s chromosome, or if some other mechanism is involved which is masking the true behavior of K^s. Unfortunately, this cannot be answered from the data available at the present time.

The results from plants of K° k constitution are of interest for two other reasons: (1) the formation of neocentromeres occurred in the absence of the heterochromatic knob; and (2) a slight degree of preferential segregation also occurred in the absence of the knob and distal euchromatic segment. These two points will be discussed in the following paragraphs.

Cytological examination showed that neo-centromeres were formed in plants of $R\ K^\circ/R\ K^\circ$ type. Figure 9 illustrates a typical metaphase II stage in which two dyads possess neocentric regions. Figure 10 shows an early anaphase II stage in which four monads exhibit neocentric activity. The fact that neocentromeres are formed in the absence of the large heterochromatic segment is of interest, since it was suspected that the knob plays an important role in their production (RHOADES 1952). The observations of K° plants do not necessarily indicate that the knob is without function in the formation of neocentromeres. It will be seen in the photomicrographs of K° that the degree of neocentric activity, as indicated by the number of dyads with neocentric regions and the extent of attenuation of the chromosomal arms, is not as great as it appears in plants with abnormal 10 (see RHOADES 1952) but is similar to what was found with K^s. It is conceivable that the absence of the knob in the K° chromosome, while not completely inhibiting the expression of neocentric activity, has the effect of diminishing it. That is, the presence of only the three prominent chromomeres of the K° chromosome may not be sufficient for the maximum expression of activity.

Still another interpretation, that of variation, can be considered as applicable to the discussion on the reduced neocentric activity in the K° chromosome. This possibility has been mentioned previously in connection with the apparent reduction of neocentric activity in the K^s chromosome. To test this possibility it would be desirable to examine a number of K° stocks of diverse background in order to determine whether any one stock exhibits more neocentric activity than another. It would also be essential to make sib comparisons of the degree of neocentric activity in K° and in abnormal 10 plants.

Until these tests have been completed, both alternative interpretations—actual reduction and variation—are equally compatible with the present cytological observations. It can be concluded, however, that some degree of neocentric activity occurs in the absence of the knob. Whether the segment bearing the three deeply staining chromomeres carries an agent responsible for the production of neocentromeres is unknown. Preliminary examinations of an abnormal chromosome 10 which lacks this segment but has the normal knob and the strand distal to the knob, showed no neocentric activity in 390 metaphase II and 41 anaphase II cells. However, cytological observations of one sib plant carrying a heterozygous abnormal 10 without the deficiency also exhibited no neocentric activity in 344 metaphase II cells. In view of the absence of neocentromeres in a sib plant it

becomes less certain that the lack of neocentromeres in plants carrying a deficiency for the three chromomeres is of any significance.

Another observation of interest in the K° k study was the fact that the K° chromosome segregated preferentially in 52.7 percent of the functional megaspores. These data could be interpreted to mean that the heterochromatic segment and possibly the euchromatic strand distal to the knob, which are missing in the K° chromosome, have a pronounced effect on the degree of preferential segregation. Without this additional chromatin the maximum amount of preferential segregation may not be manifested. Another interpretation is that the K° chromosome is associated with an aberration which is reducing its viability. This is rather unlikely for the reason that male transmission tests and pollen examination provide no such evidence. Also, no appreciable amount of ovule sterility was found on K° k ears. There is also the possibility that crossing over was reduced in the *G-R* region, but this could be explained on the basis of variation. Thus it seems probable that the knob and the distal euchromatic strand play an important role in the manifestation of preferential segregation.

2. *Tests of* K^{S} *and* K° *chromosomes with abnormal 10:* In experiments designed to test the behavior of the modified chromosomes 10 with an unchanged abnormal 10, plants of R^{r} modified-K/R^{r} modified-K type were crossed with a stock homozygous for abnormal 10 and R^{st}, a stippled R allele. These F_1 plants of R^{r} modified-K/R^{st} abnormal-K constitution were used as the female parent and were backcrossed to a pollen parent homozygous for r^{g} k. In crosses of this type, the fully colored seeds identified the modified knobbed chromosome while the stippled seeds identified the abnormal 10. Where the classification of seed color was questionable, the type of plant color (which is closely linked with seed color) was used to identify the two chromosomes; the R allele of the modified knob was linked with colored plant, while the R^{st} allele of the abnormal knob was without plant color.

Control Test: One type of control cross, in which the abnormal 10 carried R^{st} and the normal knobless 10 carried r, gave 70.2 percent preferential segregation of the knobbed chromosome (Table 3). Data from a second type of control cross,

TABLE 3

The behavior of K^{S} *and* K° *when tested with an unchanged abnormal chromosome 10 in crosses of* R^{r} K (*modified*)/R^{st} K (*unchanged*) × r k/r k

Knob type	Parental culture	Total population	Numbers of Unchanged K (R^{st} K)	Knob-less (r k)	Percent unchanged K	χ^2 (50:50)	p	χ^2 (70:30)	p
R^{st} K(unchanged)/r k	57:144	8,297	5,825	2,472	70.2	....	...	....	...
			Unchanged K (R^{st} K)	Modified K (R^{r} K^{*})					
R^{st} K(unchanged)/R^{r} K^{S}	57:142	8,845	4,798	4,047	54.3	63.76<0.01		....	...
R^{st} K(unchanged)/R^{r} K^{o}	57:143	10,284	6,659	3,625	64.8	895.10<0.01		134.92<0.01	

* K represents modified knob chromosomes 10.

in which the same knobbed R^{st} chromosome was tested with another abnormal 10, are not available at this time. RHOADES (1952), who tested a compound of *R K/r K* constitution found normal 1:1 ratios for the *R* locus. As mentioned before, he concluded that preferential segregation of chromosome 10 does not occur in the homozygote (*K K*).

K^s K *Test:* A photomicrograph of a K^s chromosome paired with an abnormal 10 is shown in Figure 11. This configuration reveals the three homozygous chro-

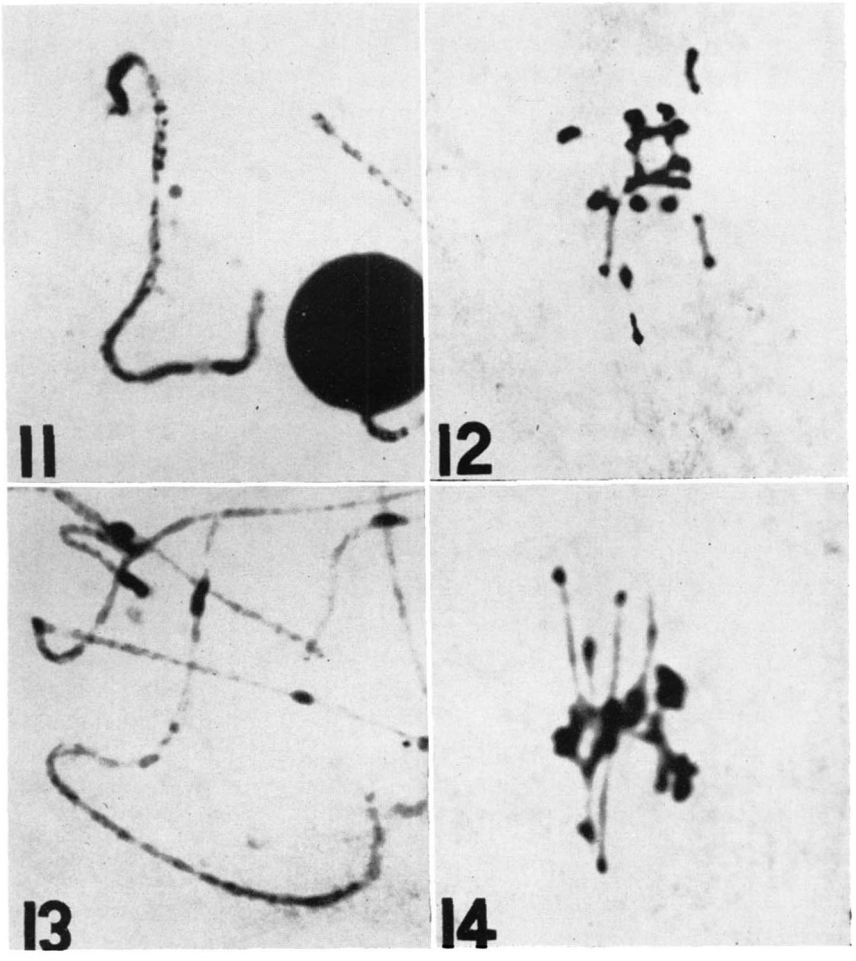


FIGURE 11.—Shows abnormal 10 paired with knob^s. Note that synapsis is normal throughout the entire length of the chromosomes.

FIGURE 12.—Early anaphase II in a plant with an abnormal 10 and knob^s chromosome. Neocentric regions are exhibited by two to three monads going to the lower pole and one to the upper pole.

FIGURE 13.—Represents abnormal 10 paired with knob°. This figure shows that no gross abnormality is present in the knob° chromosome since pairing is normal.

FIGURE 14.—A metaphase II cell from a plant with an abnormal 10 and knob° chromosome.

momeres of the long arm, as well as the absence of a structural modification in the K^s chromosome for pairing is normal in its entire length. The strand distal to the knob appears double rather than single in this particular figure. The reason for this is that in maize the two chromatids comprising each chromosome can often be seen in unpaired regions.

The results from tests of the K^s K compound are given in Table 3. The percentage of abnormal K transmission was 54.3 which was wholly unexpected on the basis of previous evidence. It will be recalled that in the K^s k test, the K^s chromosome behaved similar to a normal knobless 10 for it did not segregate preferentially. Accordingly, if the K^s chromosome is without effect on preferential segregation in the heterozygote, it might be expected that it would show no effect when tested with an abnormal 10. If such be the case, then the expected frequency of abnormal 10 would be 70 percent, since K^s K should act similarly to K k. On the other hand, if for some unknown reason both knobs of the K^s K compound were equally effective, then the expected frequency would be 50 percent, as shown by RHOADES (1952). The data in Table 3 show that the ratio of $K10$:K^s is not greatly different from a normal 1:1 ratio. This definitely does not support the previous suggestion that the K^s chromosome is without the ability to segregate preferentially. The slight excess of abnormal 10 above 50 percent could be ascribed to either megaspore competition between the two types of knobbed chromosomes, or to a reduction in viability of the K^s chromosome.

Cytological examinations of K K^s plants exhibited neocentric activity at the metaphase II and anaphase II stages. Figure 12 illustrates an early anaphase II cell in which only a few of the monads exhibit neocentromeres. In this compound it might be expected that the neocentric activity would be greater since an abnormal 10 is present. However, the configurations of this particular stock displayed reduced neocentric activity, similar to that which was found for K^s k and K° k.

K° K *Test*: A pachytene configuration of a K° chromosome paired with abnormal 10 is shown in Figure 13. This photomicrograph shows that no gross cytological aberration is present in the K° chromosome for pairing is normal. It will be also noticed that the three chromomeres of the long arm are homozygous, and that the chromosomes appear double up to the knob which is in agreement with Figure 5. The most distal chromomere in Figure 13 corresponds to the second most distal chromomere in Figure 5. The last chromomere in Figure 5 is apparently either the last chromomere adjacent to the knob, which is not visible in Figure 13, or a small fraction of the knob.

The genetic results in Table 3 show that 64.8 percent of the backcross progeny of K° K plants possess the abnormal 10. As explained before in connection with the data from K^s K heterozygotes, the expected frequency of abnormal 10 in the presence of two equally effective knobs is 50. In cases where one knob is less effective than the other, the percentage of K is predicted to lie between 50 and 70. Inspection of the data of K° K compounds demonstrate that while K° is included

less often in the functional megaspores than K, it is not wholly without some capacity to compete with abnormal 10. If the K° chromosome were without any effect, then approximately 70 percent of the functional megaspores should include abnormal 10 instead of the 64.8 percent which was observed. It could be argued that the difference between 64.8 and 70 percent is not real since sib comparisons were not made. However, the data from the K° k test are against this view. It was shown in Table 2, that when K° was tested with a normal chromosome, 52.7 percent of the functional megaspores included K°. Consequently, the K° chromosome should also exhibit a slight degree of preferential segregation in the presence of an abnormal 10, which it did. For this reason the results from K° K are not unexpected and are consistent with the previous data of K° k.

The extent of neocentric activity observed in K° K plants (Figure 14) was much the same as found in K^s k, K° k, and K^s K plants. Here again the degree of neocentric activity appears reduced as compared with homozygous abnormal 10 (see RHOADES 1952).

3. *Test of* K° *with* K^s: The results from crosses of type $R\ K^\circ/r\ K^s \times r\ k/r\ k$, in which the two modified chromosomes were tested against each other, are presented in Table 4. The data show that the K° chromosome was included in approximately 51.9 percent of the functional megaspores, a value which deviates significantly from 50 percent. Thus the K^s chromosome is less effective than the K° chromosome.

B. Effect of modified chromosomes 10 on preferential segregation of chromosome 9

The effect of the K^s and K° chromosomes on preferential segregation of heteromorphic chromosome 9 bivalents was studied by using RHOADES' method (1955b). Plants which were heterozygous for modified chromosomes 10 and for knobbed chromosome 9 were backcrossed as the female parent by a stock homozygous for knobless chromosomes 9 and 10. The degree of preferential segregation for the knobbed chromosome 9 was measured by using the *wd* character which is a minute deficiency for the tip of the short arm of chromosome 9 (MCCLINTOCK 1944). The advantage of using this deficient chromosome as a marker is that it is always associated with the knobless chromosome 9, and it has no effect on viability. The knobbed chromosome 9 used in these tests carried the marker *Wd* and a large terminal knob (K^L) on the end of the short arm (Figure 15).

TABLE 4

The results from crossing females heterozygous for K° K^s *to males homozygous for knobless 10*
(♀♀ R K°/r K^s × ♂♂ r k/r k)

Total population	Numbers of Colored (*R K°*)	Numbers of Colorless (*r K^s*)	Percent *K°*	χ^2	p
12,153	6,308	5,845	51.9	17.62	<0.01

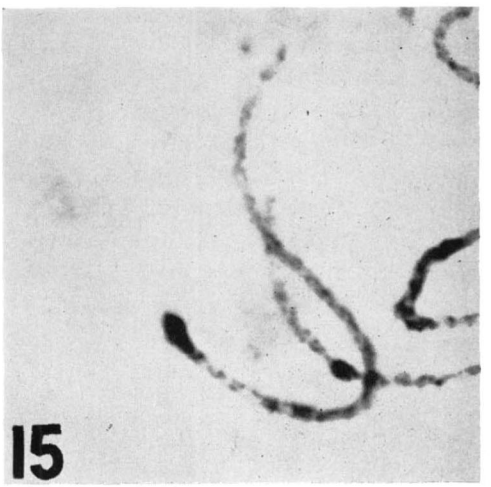


FIGURE 15.—A chromosome 9 with a large terminal knob on the short arm.

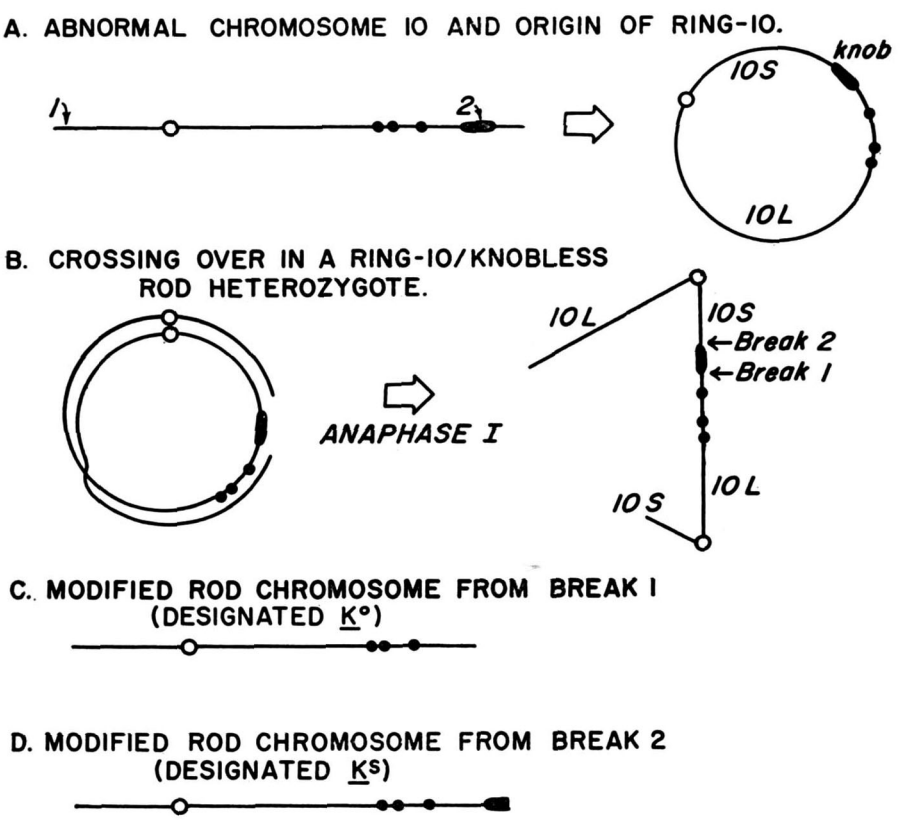


FIGURE 16.—The origin of two modified abnormal chromosomes 10 resulting from crossing over in a ring-10/rod heterozygote. The ring chromosome includes the three prominent chromomeres of an unchanged abnormal 10 and approximately one half of the original knob.

Test of K^S: In the test with the K^S chromosome, the cross was of the following type: $K^S10/k10$, K^L9 $Wd/k9$ $wd \times k10/k10$, $k9$ $wd/k9$ wd. The data are summarized in Table 5. These results show that the knobbed chromosome 9 carrying *Wd* was included in 53.5 percent of the functional megaspores, a value which deviates significantly from 50 percent at the one percent level. Thus K^S 10 has some effect on preferential segregation of K^L 9. Had the K^S chromosome been without any effect on chromosome 9, the percentage of *Wd* should have been 50, for a heterozygous knobbed chromosome 9 does not segregate preferentially in the absence of abnormal 10 (RHOADES 1955b). If, on the other hand, the K^S chromosome were wholly efficacious, then approximately 68 percent of the functional megaspores would include the K^L 9. This percentage is based on the 1958 data of KIKUDOME who used the same sized knob on chromosome 9 as was employed in the tests of the K^S chromosome.

Test of K°: The data obtained from tests of K° on preferential segregation of the knobbed chromosome 9 are presented in Table 5. The cross is the same as that described in the previous section. The result shows that 53.2 percent of the functional megaspores received the K^L 9 chromosome. This percentage deviates significantly from 50 at the one percent level.

The effect of K° on K^L 9 was low, as might be expected, since the other tests involving K° also showed a low degree of preferential segregation. It will be recalled that in the heterozygote (K° k) only 52.7 percent of K° was found instead of the usual 70 percent. Similarly, in the case of the K° K analysis, 35.2 percent of K° was recovered rather than the normal 50 percent. Therefore, the data presented here are consistent with the previous findings.

From these observations it is possible to conclude that the K° chromosome has some effect on the segregation of the knobbed chromosome 9. This is of interest since previous workers (LONGLEY 1945; RHOADES 1955b; RHOADES and DEMPSEY 1957; KIKUDOME 1958) have shown that preferential segregation of other knobbed chromosomes occurred only in the presence of abnormal 10 with the large heterochromatic segment. The K° chromosome which lacks this knob is still capable of influencing the behavior of chromosome 9. However, the effect is greatly reduced, a fact which could be attributed to the absence of the heterochromatic segment.

TABLE 5

The effect of K^S *10 and* K$^\circ$ *10 on the behavior of the knobbed chromosome 9 in crosses of* ♀♀ *modified* K *10*/k *10*, K^L *9*/k *9* × ♂♂ k *10*/k *10*, k *9*/k*9*

Knob type	Parental culture	Total population	*Wd* K^L 9	*wd* *k* 9	Percent *Wd*	χ^2 50:50	p
K^S 10/*k* 10, *Wd* K^L 9/*wd* *k* 9*	57:158	4,172	2,233	1,939	53.5	20.72	<0.01
K° 10/*k* 10, *Wd* K^L 9/*wd* *k* 9	57:159	5,337	2,840	2,497	53.2	22.04	<0.01

* Knobbed chromosome 9 was marked for *Wx* in 2338 of the 4,172 gametes tested. The K^L 9 chromosome showed approximately 52 percent *Wx*.

TABLE 6

The summarized results of the modified chromosomes tested with a normal knobless chromosome 10, an abnormal 10, and a knobbed chromosome 9

	Female transmission			Male transmission
	Knobless 10	Abnormal 10	Knob^{L} 9	Knobless 10
Abnormal 10	70.2	50.0*	68.0†	44.2
Knob^{S} 10	49.5	54.3	53.5	36.9
Knob$^{\circ}$ 10	52.7	64.8	53.2	49.3

K^S/K°—51.9 percent, K°.
G–R recombination in K° $k = 10.3$ percent.
G–R recombination in K^S $k = 11.5$ percent.

* Data from RHOADES (1952).
† Data from KIKUDOME (1958).

SUMMARIZED RESULTS

The summarized results of the modified chromosomes tested with a normal knobless chromosome 10, an abnormal 10, and a knobbed chromosome 9 are shown in Table 6. In the first three columns the percentage of female transmission (or preferential segregation) are recorded. Column 1 shows the results from tests of the various knobbed chromosomes 10 with a normal knobless chromosome. Column 2 gives the percentages of abnormal 10 resulting from trials with the modified chromosomes. In column 3 the effect of the modified chromosomes on a knobbed chromosome 9 are recorded in terms of the frequency of knobbed chromosome 9 segregation. The last column refers to the percentages of the various knobs resulting from male transmission tests. Immediately below the table the frequency of K° in K° K^S heterozygotes is given. The percentages of recombination in the *G-R* region in plants of K° k and K^S k constitution are also shown below the table.

SUMMARY

(1) An analysis was made of the effects of two structurally modified chromosomes 10 on preferential segregation of chromosomes 9 and 10.

(2) The knob$^\circ$ chromosome, an altered abnormal 10 without the heterochromatic strand, has a reduced effect on the segregation of chromosomes 9 and 10. This suggests that the knob and the distal euchromatic thread play an important role in the manifestation of preferential segregation. The knob$^\circ$ chromosome also shows an apparent reduction in the degree of neocentric activity, a fact which could be ascribed to the absence of the heterochromatic segment.

(3) The knob^S chromosome, an altered abnormal 10 lacking about one half of the heterochromatic segment and distal euchromatic strand, shows normal 1:1 ratios when tested with a knobless 10 but forms neocentromeres. In tests with an abnormal 10 and a knobbed chromosome 9, knob^S has some effect. At the present time it is difficult to formulate a single hypothesis that would account for the contradictory results from the K^S chromosome.

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