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PREFERENTIAL SEGREGATION INVOLVING A KNOBLESS CHROMOSOME IN MAIZE[1]

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1. INTRODUCTION

Two kinds of chromosome 10 were found in a survey of maize strains collected throughout the world (*1, 2*). The infrequently occurring type designated abnormal 10 (K10), differs from the normal or common chromosome 10 (k10) in having an extra piece of chromatin attached to the end of the long arm. The proximal and distal portions of this piece are euchromatic with a large knob lying between the two euchromatic regions.

RHOADES (*7*) observed that K10 segregated preferentially at megasporogenesis in K10/k10 plants. Approximately 70%, instead of the expected 50%, of the ovules received the abnormal chromosome 10. Later, LONGLEY (*3*) demonstrated that knobbed chromosomes other than abnormal 10 segregate preferentially at megasporogenesis in the presence of abnormal 10. K10 does not induce preferential segregation in other chromosome pairs when both homologues are knobless or if both have knobs of identical size.

Abnormal 10 is also responsible for the formation of secondary sites of centric activity, or neocentromeres, during anaphase I and metaphase II. Chromosomal fibers arise not only from the knob of K10 but from all knobs. The neocentromeres move precociously in advance of the true centric regions to the poles (*11*). Since the K10 chromosome produces both the phenomena of preferential segregation and of neocentromere formation, RHOADES (*8*) postulated a mechanism by which the neocentromere accounted for preferential segregation.

RHOADES (*9*) and RHOADES and DEMPSEY (*10*) observed that the degree of preferential segregation depended on the frequency of crossing over. They concluded that the formation of heteromorphic dyads via crossing over between the centromere and the knob is an essential prerequisite for preferential segregation, and that it is the knobbed chromatid which is preferentially included in the basal megaspore at anaphase II. Neither preferential segregation or neocentric activity

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was found in the absence of K10. The two phenomena clearly stem from interaction between two heterochromatic organelles which differ genetically since one cannot be substituted for the other.

Since a new 9^B translocation carries the whole distal heterochromatic region of the B chromosome, a study was made to see if the B-heterochromatin translocated to the 9^B chromosome would interact with K10, as do knobs, to produce preferential segregation of genes on chromosome 9 and neocentromere formation.

2. MATERIALS AND METHODS

The *Yg2* was used not only because the alleles produce discrete phenotypes, which are easily classified at the seedling stage, but also because of its close physical proximity to the knob on 9S. Yellow-green plants of constitution 9^B (Def. for *Yg2*)/K^S *yg2* were pollinated by green plants (Yg2/Yg2) from a strain homozygous for knobs on nine chromosomes, including K10, and for a pair of knobless chromosomes 9(k9) with the *Yg2* allele (the so-called «high-knob» strain). The offspring from this cross were all green and all were heterozygous for K10; they included two different genotypes for chromosome 9-namely, 9^B/k9 *Yg* and K^S yg/k9 *Yg*. The two chromosome 9 compounds could be distinguished by genetic tests. When plants of 9^B/k9 *Yg2* constitution were used as the male parent in crosses with yellow-green testers (*yg2/yg2*), all of the offspring would be green because of the failure of 9^B pollen grains to function; whereas, K^S *yg2*/k9 *Yg2* plants would produce equal numbers of green and yellow-green seedlings in male test-crosses. The same plants were also used as female parents in crosses with *yg2* plants in order to test for preferential segregation of the 9^B chromosome with its long segment of B-heterochromatin, against the knobless k9 chromosome. The K^S/k9 plants served as controls.

Preferential segregation of the 9^B chromosome would be indicated if an excess of yellow-green over green seedlings occurred in the female test-cross. Since a modified chromosome such as 9^B may affect the viability of female gametophytes, a second control was needed, namely 9^B heterozygotes with normal chromosomes 10, for comparison of female transmission rates.

Tests were also made for preferential segregation in 9^B/k9 plants homozygous for K10. To this end, crosses were made with pollen from K10 *r*/K10 *r*;k9 *Yg wx*/k9 *Yg wx* plants of the «high knob» strain onto K10 *r*/k10 *R*;9^B *Wx*/k9 Yg^2 *wx* silks. The colorless (r), starchy kernels were selected to produce the desired individuals homozygous for K10 and of 9^B/k9 constitution. Sporocytes were collected to confirm the chromosomes 9 and 10 constitutions cytologically. Plants of K10/K10;9^B/k9 *Yg2* constitution were used as females in crosses with a *yg2* tester and the progeny kernels were germinated in a sandbench to score for green (k9) and yellow-green (9^B) seedlings.

3. RESULTS AND DISCUSSION

From 37 female test-crosses of 9^B/k9 *Yg2* heterozygote, 5,421 seedlings were obtained, of which 3,369 were green (carrying the normal knobless chromosome) and 2,052 were yellow-green (with the 9^B chromosome) (Table 1). Thus, in the presence of K10, the 9^B chromosome was recovered in the functional magaspore with an average frequency of 37.8% (2,052/5,421); whereas, the k9 was found in 62.1% (3,369/5,421) of the megaspores. One might suggest that haploid gametophytes possessing both 9^B and K10 chromosomes show reduced viability. If this were true, a transmission rate of about 38% for the 9^B chromosome would correspond

to an expected frequency of 12% ovule abortion. Although no counts were made of ovules on the ears, a distortion in regularity of kernel rows would have been noticeable with the predicted amount of abortion. No indication of missing ovules or irregularities was observed. Moreover, when K10 *r*/k10 *R* plants, some of which were 9^B/k9 heterozygotes, were crossed by *r* male parents, the expected 70:30 ratio of *r*:*R* kernels was found, indicating that the K10 chromosome was undergoing the usual preferential segregation and no loss of K10 in inviable gametophytes was occurring. Since it is known that 9^B chromosome in k10/k10 plants is transmitted in a nearly normal frequency (47.6%), the data indicate that K10 unexpectedly induced preferential segregation of the shorter knobless k9 chromosome over the physically longer 9^B chromosome with its large heterochromatic segment derived from the B chromosome.

In the K^S *yg/k9 Yg* crosses there was, as expected, preferential segregation for the small K^S knob which was recovered in 56% of the megaspores, and with k9 recovered in 44% (Table 1).

The results from the $9^{\bar{B}}$/k9; K10/k10 plants were not anticipated since the knobless chromosome was preferentially recovered. In all previous experiments with K10 it was the chromosome with the heterochromatic knob which segregated preferentially when opposed by a knobless homologous.

Observations by RHOADES (*8*) demonstrated that the distal heterochromatic segments of the intact B chromosome do not interact with K10 to form neocentromeres as do true knobs. Clearly, knobs and the distal heterochromatin of the B are not alike and constitute different species of heterochromatin. Indeed, a pure DNA probe extracted from knobs did not hybridize with B distal heterochromatin (*6*).

Since the above results were obtained with K10 in a heterozygous condition, tests were made for preferential segregation in 9^B/k9 plants homozygous for K10. The data from the crosses are given in Table 1.

TABLE 1. Summary of tests of female transmission of structurally modified chromosomes 9 in plants with and without K10

Female	Classification of seedlings				Total
	Green (Yg^2)		Yellow green (yg^2)		
	#	%	#	%	#
k10/k10; 9^B/k9 ($\underline{Yg}_2$)	824	52.4	748	47.6	1572
K10/k10; 9^B/k9 ($\underline{Yg}_2$)	3369	62.2	2052	37.8	5421
K10/k10; K^S 9($\underline{yg}_2$)/k9($\underline{Yg}_2$)	1507	44.0	1921	56.0	3428
K10/K10; 9^B/k9($\underline{Yg}_2$)	1011	61.3	610	38.7	1621
k10/k10; Df9(Df $\underline{Yg}_2$)/k9 ($\underline{Yg}_2$)	193	48.4	206	51.6	399
K10/k10; Df9(Df $\underline{Yg}_2$)/k9($\underline{Yg}_2$)	772	54.6	641	45.4	1413

In the K10 homozygotes, the 9^B chromosome was recovered in 38.7% (610/1,621) of the ovules and the shorter normal knobless 9 recovered in 61.3% (1,011/1,621). Thus, in 9^B/k9 plants, the knobless 9 is preferentially segregated with the same frequency in K10/k10 and K10/K10 plants. RHOADES and DEMPSEY (*10*) also found a similar degree of preferential segregation of a knobbed chromosome in heterozygous and homozygous K10 plants.

Since neocentromere formation is believed to be responsible for preferential segregation of knobbed chromosomes (*8, 10*) cytological studies were made on pollen mother cells from K10/K10; 9^B/k9 plants to see if neocentromeres were formed by the 9^B chromosome in the meiotic mitoses. The fact that all of the chromosomes, except chromosome 9, were knobbed facilitated the recognition of the 9^B and 9 chromosomes at anaphase I and metaphase II. As expected, neocentromeres were formed in as many as nine chromosomes but not by all ten. Since the only knobless chromosome was chromosome 9 it could be assumed that the 9^B/k9 pair did not form neocentromeres. This was verified observationally. In some meiotic metaphase and anaphase figures it was possible to identify the 9^B and k9 chromosomes by the differential staining of the B heterochromatic segment on the 9^B chromosome and by the unequal length of the two chromatids in crossover dyads. Neocentromeres were never observed coming from this pair.

Inasmuch as the 9^B chromosome did not form neocentromeres, it should not be, and was not, preferentially recovered in the basal megaspore. Unanticipated was the finding that in K10 plants, preferential segregation did occur but it involved the knobless k9 chromosome. The deficiency of the 9^B chromosome cannot be attributed to inviability of some 9^B gametophytes since it is recovered in a 1:1 ratio in k10/k10 plants. Instead of the B-heterochromatin promoting preferential recovery of the 9^B chromosome it had the opposite effect. The ability of K10 to induce preferential recovery for the knobs of A chromosomes and against the 9^B chromosome with its segment of B-heterochromatin may add a new dimension to its genetic potential.

The mechanism responsible for the preferential recovery of k9 chromosome at the expense of 9^B is unknown. The K10 chromosome plays an essential role in decreasing the recovery of the 9^B chromosome but what can be said about the role of the B-heterochromatin? If it interacted with K10, as do the heterochromatic knobs of the A set, preferencial segregation should have occurred for the 9^B chromosome. Instead, the normal chromosome 9 was recovered preferentially. At issue is the question whether the B-heterochromatin was actively or passively involved in the negative transmission of 9^B. Seemingly, this matter could be resolved if it were possible to remove the segment of B-heterochromatin from 9^B and then test for preferential segregation. Unfortunately, this is not easily accomplished.

However, an approximation of the above condition was available in a strain with a normal knobless 9(k9) and a deficient 9(Df9), from plant 309-3, which lacks a terminal segment of 9S of the same extent as that in 9^B, but is not capped by a piece of heterologous chromatin. The deficient 9 had a terminal deletion of three or four chromomeres, including the *Yg2* but not the *C* locus. The female transmission of the deficient chromosome was normal in k10/k10 plants of Df9 *C*/k9 *c* constitution. In a population from four testcrossed ears, there were 332 *C* kernels and 320 *c*. Since about two percent of crossing over occurs between the *C* locus and the breakpoint in the deficient chromosome, the test was repeated with Df9/k9 *Yg2* compounds to follow more directly the transmission of the deficient chromosome. Three testcrossed ears gave 206 yellow-green seedlings with the deficient 9, and 193 green with the normal 9 (Table 1). Thus the deficient chromosome, from plant 309-3, is normally transmitted in the ovules of k10/k10 plants. Similar

tests with K10/k10 plants indicated a reduced female transmission of the Df9 chromosome since only 45.4% of the offspring were yellow-green (Table 1).

If the heterochromatic segment of 9^B actively supresses transmission of the translocated chromosome in K10/k10 female parents, one might predict that the Df9/k9;K10/k10 compounds, possessing the deficiency but not the heterochromatic segment, would show normal transmission. Instead, transmission was reduced in K10/k10 plants, although to a lesser extent than with 9^B/k9 heterozygotes.

An important difference between 9^B/k9 and Df9/k9 bivalents is that in the former the 9^B chromosome is longer than the k9, while in Df9/k9 individuals the Df9 is shorter than the k9 chromosome. Heteromorphic dyads will be generated by crossing over in both kinds of plants. If preferential segregation from heteromorphic dyads were simply a matter of a size difference between the two chromatids, the prediction is that in 9^B/k9 plants the k9 would be preferentially recovered, because it is the shorter chromosome.

The validity of this hypothesis was first established in *Drosophila* (*4, 5, 12*). Following the formation of heteromorphic dyads by crossing over between dissimilar homologues, the shorter chromatid of the two was preferentially included in the egg nucleus. The degree of preferential segregation depended upon the frequency of the crossovers which produced heteromorphic dyads. No specific genetic factors were involved; preferential segregation was simply a matter of physical disparity in size between the two chromatids and for this reason it is said to have a mechanical basis. It is known as the chromosomal type of meiotic drive.

As is shown in Table 1, the shorter k9 chromosome in 9^B/k9; K10/k10 plants had a transmission percentage of 62.2 in female test crosses.

There is an apparent similarity between the 9^B/k9 case in maize and those in *Drosophila* where the homologues are of unequal length. In both organisms, it is the shorter chromosome of the pair that is preferentially recovered from heteromorphic dyads. They differ in that no known genetic factors affect chromosomal meiotic drive in *Drosophila* while the K10 chromosome is indispensable in maize since preferential segregation occurs in K10 plants but not in k10. Unquestioned is the essentiality of K10 in preferential segregation in 9^B/k9 plants; less certain is the role, if any, played by the B-heterochromatin on the 9^B chromosome. It could be argued that chromosomal meiotic drive found in *Drosophila* is also operating in the 9^B/k9 situation in maize. The random segregation found in k10/k10; 9^B/k9 plants and the preferential segregation occurring in K10 plants could reflect different frequencies of crossing over needed to produce heteromorphic dyads. It has been well-established that K10 enhances recombination especially in proximal regions such as the Wx-centromere interval and heteromorphic dyads should be more frequent in K10 compounds. Thus, the 62% recovery of the normal chromosome 9 in 9^B/k9 female testcrosses could be based, not on neocentromere formation, but on the size differential in heteromorphic dyads, whose frequency was increased by K10 chromosome.

Although the data from 9^B/k9 compounds is in agreement with the hypothesis of chromosomal meiotic drive, the frequencies obtained with Df9/k9 plants are in conflict. Since the Df9 is the shorter of the two homologues, it should be recovered more frequently. Instead, in K10/k10 individuals it was found in only 45.4% of the ovules, while in k10/k10 plants the frequency was slightly over 50%. It is true that the difference in length between the Df9 and k9 chromosomes is not great, consisting of the distal three or four chromomeres, and the difference in length between the two at metaphase when they are tightly coiled and contracted might be relatively slight. Nevertheless, it cannot be denied that the longer of the two chromoso-

mes was recovered preferentially. It would be desirable to test a longer deficiency against a normal knobless chromosome to see whether an increase in size differential affects the degree of preferential segregation. An attempt to make this test gave inconclusive data because of the abortion of ovules containing the deficient chromosome.

A number of arguments can be marshalled in opposition to the explanation involving chromosomal meiotic drive. Although no data are available on the enhacement of crossing over by K10 in the proximal region of 9^B/k9 compounds, the amount of crossing over in k10/k10;9^B/k9 plants (more than 10% for the *Bz-Wx* interval) is sufficient to give some degree of preferential segregation if chromosomal meiotic drive is operating. In the absence of K10, no preferential segregation was observed. A similar argument holds for Df9/k9 plants where more than 30% recombination occurs between the *Wx* locus and the breakpoint. Heteromorphic dyads should be frequent and chromosomal meiotic drive should produce an excess of Df9 ovules. Instead, segregation in k10 plants was normal. Moreover, as mentioned above, the data from Df9/k9 individuals with K10 are not compatible with the hypothesis of chromosomal meiotic drive. A careful consideration of all aspects of the 9^B and Df9 data makes the hypothesis of chromosomal meiotic drive suspect and it seems unlikely that a simple difference in size can account for preferencial segregation.

A noteworthy feature of the preferential segregation experiments is that in both the 9^B/k9 and Df9/k9 compounds, it is the normal knobless k9 chromosome that is preferentially recovered despite the fact that k9 is the shorter chromosome in the 9^B/k9 class and the longer in the Df9/k9 plants. It is possible that megaspores with the normal chromosome 9 substitute for those carrying a deficient or translocated chromosome when K10 is present in the plant, i.e., there is competition between the linear set of four megaspores in the development of the embryo sac. Normally, it is the basal megaspore which functions while the remaining three abort. It may be that a certain percentage of k9 megaspore develop into the functional female gametophyte even though they are not situated at the base of the linear set of four. This is the well-known Renner effect found in *Oenothera* (cited by RHOADES, 7). This hypothesis could be, but has not been, tested by cytological examination of megasporogenesis. If it can be demonstrated to occur in the present instance, the K10 chromosome would acquire another function, namely induction of megaspore competition.

Another hypothesis is that K10 induces a delayed replication of the B-heterochromatin in embryo mother cells which leads to the formation of a bridge at anaphase I following an exhange in 9S. If this orientation of the 9^B chromatids were maintained in the dyads of second meiotic metaphase, they would be oriented towards the two inner poles while the two knobless chromatids would be directed to the two terminal megaspores, the basal one developing into the embryo sac. However, no such bridges were observed in the pollen mother cells of 9^B/k9 plants carrying K10. Of course, it is possible that meiosis is dissimilar in the male and female inflorescences. There is no evidence supporting this hypothesis but it has not been disproven.

The preferencial segregation during megasporogenesis described by RHOADES (7) involves the interaction of abnormal chromosome 10 and heterochromatic knobs. The mechanism proposed to explain this phenomenon requires the formation of neocentromeres at the knob regions in response to K10 activity. For the 9^B case, however, there is no interaction involving knobs and no indication of neocentromere formation. If this case proves to be a deviant type of preferential segregation, a modification of the mechanism to permit occasional occur-

rence of the phenomenon in the absence of knobs and neocentromeres will be necessary. This could be a new, secondary, and alternative mechanism of preferential segregation induced by K10. However, before a revision of the mode of preferential segregation is attempted, all possible alternative explanations must be rigorously tested.

4. SUMMARY

A translocated chromosome (9^B) arose in which all of the distal heterochromatin of the B chromosome was attached to the short arm of chromosome 9 in maize. Test for preferential segregation of 9^B during megasporogenesis in plants heterozygous for a normal knobless chromosome 9 (k9) and the translocated 9^B and carrying the abnormal chromosome 10 (K10) gave unexpected results. The k9 was favored over the 9^B chromosome, the translocation being recovered in only about 38% of the ovules. The preferential segregation of the knobless chromosome was not associated with neocentromere formation.

Thus, the distal heterochromatin of the B chromosome following transposition does not acquire the property of the heterochromatic knobs in associating with K10 to be recovered preferentially, but remains passive.

The mechanism of preferential segregation of the knobless intact chromosome 9 remains to be elucidated.

5. RESUMO

(SEGREGAÇÃO PREFERENCIAL, ENVOLVENDO UM CROMOSSOMO SEM «KNOB», EM MILHO)

Uma nova translocação 9^B em milho foi utilizada para verificar se a heterocromatina distal do cromossomo B, translocada para o braço curto do cromossomo 9 (9S), se comportaria como os knobs heterocromáticos, com relação à capacidade de segregar preferencialmente durante megasporogênese na presença do cromossomo 10 anormal (K10).

Os resultados obtidos eram totalmente inesperados. Plantas de constituição 9^B/k9, homozigotas ou heterozigotas para K10, produziram resultados semelhantes. O cromossomo normal sem knob (k9) segregou preferencialmente, sendo recuperado em 62% dos óvulos, enquanto o cromossomo translocado 9^B foi recuperado em apenas 38% Conseqüentemente, a heterocromatina distal do cromossomo B translocada para o cromossomo 9 não adquiriu a propriedade dos knobs heterocromáticos de associar-se com o K10 e segregar preferencialmente. Logo, os knobs e a heterocromatina distal do cromossomo B constituem tipos distintos de heterocromatina. A segregação preferencial do cromossomo sem knob não foi associada com a formação de neocentrômero.

O mecanismo de segregação preferencial do cromossomo 9 intacto, sem knob, ainda não está elucidado.

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