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INFLUENCE OF A NEW 9^{B} TRANSLOCATION ON RECOMBINATION IN MAIZE (*Zea mays* L.)[1]

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1. INTRODUCTION

RHOADES (*3*) demonstrated that a difference in crossing over in male and female meiocytes was induced by a large terminal knob on the short arm of chromosome 9 (9 S) in maize. Recombination was studied in the *C-Wx* region of chromosome 9 in related plants of k9/k9, $K^{L}9/K^{S}9$ and $K^{L}9/K^{L}9$ constitutions. He showed that in knobless 9 homozygotes (k9/k9), crossover values are the same in male and female flowers. However, plants carrying a large knob (K^{L}) had higher crossing over in microsporocytes than in the megasporocytes. In K^{L}/K^{L} plants there was 33% more recombination in male than in female meiocytes, while with K^{L}/K^{S} plants there was about twice as much crossing over in the male gametes. These results demonstrate that the terminal knob in 9S is responsible for a sex difference in recombination.

In the course of his investigation, RHOADES re-analyzed data from CHANG and KIKUDOME (*1*) bearing on the distribution of crossovers in 9S in plants with varying knob and B chromosome constitutions. These data support his conclusion that the terminal knob on chromosome 9 causes a difference in crossover frequencies in male and female inflorescences. Recombination between *Bz* and *Wx* in the proximal segment of 9S and between *Yg2* and *Sh* in the distal segment was studied in male and female gametes from related plants homozygous for a small knob (K^{S}/K^{S}), and in heterozygotes for a large and a small knob (K^{L}/K^{S}). In K^{S}/K^{S} plants, crossing over was slightly higher in male than in female meiocytes in both the *Yg-Sh* and Bz-*Wx* regions. However, in K^{L}/K^{S} plants, crossing over in the *Yg-Sh* region of male flowers was more than twice the value in female flowers while the proximal *Bz*-Wx region showed a 32% increase in male gametes. Thus,

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the increase in recombination in the microsporocytes was more pronounced in the segment of 9S nearest the knob (the *Yg-Sh* region).

The data of RHOADES (*3*), as well as the reinterpreted data of CHANG and KIKUDOME (*1*), suggest that the sex difference in recombination becomes greater with increasing knob size; compounds with knobless chromosomes gave no sex difference, those with small knobs had a slight increase in the males gametes, while plants with large knobs showed much higher recombination in the male. The sex differential in recombination apparently involves a reduction in crossing over in female meiocytes rather than an increase in male.

Since a new 9^B translocated chromosome carries all of the large segments of distal heterochromatin present in the B chromosome, studies were undertaken to determine if the B heterochromatin behaves like a knob with regard to its ability to induce a sex difference on recombination following its translocation to a terminal position on 9S. In fact, a pronounced effect should be obtained because of the large quantity of heterochromatin in the 9^B chromosome.

2. MATERIALS AND METHODS

A translocated chromosome involving chromosome 9 and a B chromosome was used. The breakpoint in chromosome 9 was close to the tip of the short arm, the deficient fragment including only one or two chromomeres, while the break in the B chromosome was near the junction of the enchromatin and the proximal heterochromatin of the long arm, the translocated chromosome carrying all of the distal heterochromatic blocks of the B chromosome.

The marker genes on chromosome 9 and the phenotypes contrasting the dominant allele with the recessive mutant allele are as follows: Yg_2 — green seedling and plant; yg_2 — yellow-green seedling and plant. Bz — deep pigmentation in aleurone and plant; bz — pale or bronze pigment in aleurone and plant. Wx — I-KI gives blue staining starch in endosperm and pollen grains; wx — I-KI gives red staining starch in endosperm and pollen grains.

The 9^B chromosome was tested in three heterozygous compounds in which the normal chromosome 9 possessed knobs of different sizes: K^S — small knob; K^M — medium-size knob and K^L — large knob.

Plants of $9^B/K^S$ constitution were used as female and male parents in reciprocal crosses with *bz wx* tester plants and recombination between *bz* and *wx* was studied. The 9^B chromosome carried the dominant *Bz* and *Wx* alleles while the K^S chromosome had the recessive *bz wx* alleles. Since the 9^B chromosome is not transmitted in male test crosses, reciprocal classes will not be equal. The parental *bz wx* and recombinant *bz Wx* classes having a normal chromosome 9 (N9) will be more frequent than the *Bz Wx* and *Bz wx* classes. Although the latter two classes also possess the N9, they arise from infrequent events: the *Bz Wx* class, from crossing over between the *Bz* locus and the breakpoint in the 9^B chromosome; and, the *Bz wx* classes from double crossovers in the *Bz-Wx* region and the *Bz*-breakpoint intervals.

Crossing over was also analyzed in the *Bz-Wx* region of $9^B/K^M$ and $9^B/K^L$ plants following reciprocal crosses with *bz wx* testers. In both kinds of crosses, the 9^B carried the dominant *Bz Wx* alleles while K^M and K^L chromosomes had the recessive *bz wx* alleles.

3. RESULTS AND DISCUSSION

It is apparent from the data in Table 1 that the distal B heterochromatin do-

es not give a sex difference in recombination. Although female gametes displayed a higher recombination value than did males, the difference was not statistically significant. Thus, the capacity of the K9 knob to induce a sex difference in recombination does not depend simply on its physical location but must also require a specific composition. That different kinds of heterochromatin exist and, more specifically, that knobs and the distal B heterochromatin are not alike in composition was discovered by PEACOCK *et alii* (*2*). Using a simple purified DNA probe they observed *in situ* hybridization with all knobs on the A chromosomes, with the heterochromatic block of K10, and with the knob-like region of the B chromosome adjacent to the centromere. The probe did not hybridize with the distal heterochromatin of the B, with the centric heterochromatin of the *A* set or with the nucleolus organizer region. The findings of PEACOCK *et alii* and the data from the present paper with the 9^B translocation strongly suggest that differences in behavior of knobs and the distal B heterochromatin should be attributed to their differing DNA composition.

RHOADES (*3*) pointed out that the data of CHANG and KIKUDOME (*1*) also reveal an interaction between the K9 knob and B chromosomes. K^L9/K^S9 plants with B's had a smaller sex difference in crossing over than did those with no B's. Since $9^B/K^S$ plants showed slightly more crossing over in female gametes than in male, it is possible that the presence of B chromatin counteracted the usual knob effect.

To determine whether the 9^B chromosome suppresses the inductive effect of K9 knob, crossing over was analyzed in the *Bz and Wz* region of 9^B *Bz Wx*/K^M *bz wx*

TABLE 1 - Effect of the 9^B chromosome on the induction of a sex difference in Bz-Wx recombination when opposed by normal chromosomes with knobs of different sizes (*)

Constitution	Sex	Number of kernels					% crossing over between Bz and Wx
		Bz Wx	Bz wx	bz Wx	bz wx	Total	
$9^B/K^S$	Female	1680	175	195	1717	3767	9.8%
	Male	108	13	408	4380	4909	8.6%
$9^B/K^M$	Female	1099	149	159	1264	2671	11.5%
	Male	58	8	179	1178	1423	13.1%
$9^B/K^L$	Female	555	45	57	567	1224	8.3%
	Male	41	4	138	1061	1244	11.4%

* For $9^B/K^S$ crossover data from 21 pairs of reciprocal test crosses; for $9^B/K^M$ data from ten pairs; for $9^B/K^L$ nine pairs were analyzed.

and 9^B *Bz Wx*/K^L *bz wx* plants following reciprocal crosses with *bz wx* testers. The results are presented in Table 1.

In $9^B/K^M$ plants, crossing over in the male meiocytes (13.1%) was higher than in the female (11.5%); however, the difference was not statistically significant. On the other hand, the 38% increase in male (11.4%) over female (8.3%) recombination values in $9^B/K^L$ plants was highly significant. In the presence of the 9^B chromosome, increasing knob size was accompanied by an increase in sex differences in recombination, with male values exceeding female values.

The question as to whether 9^B exerts a suppressive effect on knob activity remains to be answered. However, the 38% increase in male recombination in $9^B/K^L$ compounds compares favorably with the 32% increase found in K^L/K^S compounds of the CHANG and KIKUDOME (*1*) study and no suppression by 9^B is indicated.

Since the sex difference in recombination was greater in regions adjacent to the knob than in more distant regions (RHOADES, *3*), the percentage of crossing over in a segment closer to the terminal knob from *Bz* to the breakpoint in the 9^B translocation was tested in plants of $9^B/K^M$ constitution. The K^M chromosome had the *Yg2* and *bz* alleles, while the 9^B was deficient for the *Yg2* locus and carried the dominant *Bz* allele. $9^B/K^M$ plants were reciprocally test crossed with *yg2 bz*. Because of the deficiency in the 9^B chromosome, *Yg-Bz* recombination actually occurs in the *Bz*-breakpoint region. The results are presented in Table 2.

TABLE 2 - Recombination in the Bz-breakpoint region in male versus female gametes of $9^B/K^M$ heterozygotes (*)

$9^B/K^M$ used as	Number of kernels					% crossing over Bz-breakpoint
	yg Bz	Yg Bz	g bz	Yg bz	Total	
Female	999	35	24	1180	2238	2.6%
Male	0	66	0	1355	1421	4.6%

* Crossover data from eleven pairs of reciprocal crosses.

A highly significant sex difference in crossing over in the distal *Bz*-breakpoint region was found; recombination was 77% higher in the male than in the female test crosses. The percentage of *Bz-Wx* recombination in $9^B/K^M$ compounds (see Table 1) was only 14% higher in male gametes than in females. These data are in good agreement with the conclusion reached by RHOADES (*3*) that the crossover differential is more pronounced in regions close to the knob. Of course, heterofertilization would increase the frequency of recombination in gametes for the distal region, since one trait is expressed in the endosperm (*Bz*) and the other in the sporophyte (*Yg*). The frequency of heterofertilization was not determined in the present studies so the reality of the increased sex difference in the distal segment has not been unequivocally demonstrated.

That the frequency of crossing over in the *Bz*-breakpoint region in male gametes is influenced by the knob constitution in the chromosome 9 bivalente was

shown by the following experiment. 9^B *Bz*/K^S *bz* and 9^B *Bz*/K^L *bz* plants were used as male parents in crosses with *bz* testers and the percentage of *Bz* kernels (representing *Bz*-breakpoint recombinants) determined. In $9^B/K^S$ male parents, the percentage of *Bz* kernels was 2.4%, whereas it was 3.6% in $9^B/K^L$ compounds. Thus, a 50% increase in crossing over between *Bz* locus and the breakpoint in the 9^B chromosome was produced with a change in knob size from K^S to K^L. It should be mentioned, however, that these two knobs originated in stocks with different genetic backgrounds; since crossover values are known to vary in different stocks, the possibility cannot be excluded that the increased recombination in the K^L heterozygotes was due to modifier genes.

In conclusion, the distal heterochromatin of the B, when translocated to chromosome 9, fails to induce a sex differential in crossing over in male versus female gametes, nor does it suppress the positive activity of the K9 knob.

The distal segment of the B is completely neutral in the ability to influence relative amounts of crossing over in microsporocytes and megasporocytes and it differs from the heterochromatic knob of chromosome 9 in this regard. Consequently, the sex differential in recombination caused by the K9 knob is a property of its specific composition and does not depend simply on position or heterochromatic appearance.

4. SUMMARY

A translocated chromosome involving chromosome 9 and a B chromosome was used. The breakpoint in chromosome 9 was close to the tip, the deficient fragment including only one or two chromomeres, while the break in the B chromosome was near the junction of the euchromatin and the proximal heterochromatin of the long arm. Since the translocated chromosome (9^B) carries all of the distal heterochromatic blocks of the B chromosome, it was possible to test whether the foreign heterochromatin acquired the genetic property associated with the terminal knob of 9S, namely the ability to produce a sex difference in crossing over in 9S. The results indicate that knobs and the distal heterochromatin of the B constitute distinct kinds of heterochromatin and that transposition of the latter to an A chromosome does not transform it into a knob-like entity. The B-heterochromatin not only failed to cause a sex difference in crossing over, it also showed no tendency to suppress or counteract the knob effect.

5. RESUMO

(INFLUÊNCIA DE UMA NOVA TRANSLOCAÇÃO 9^B SOBRE A PERCENTAGEM DE RECOMBINAÇÃO EM MILHO (*Zea mays* L.))

Uma nova translocação 9^B em milho foi utilizada para testar se a heterocromatina distal do cromossomo B, translocada para o braço curto do cromossomo 9 (9S), se comportaria como os knobs heterocromáticos, induzindo diferencial entre os sexos com relação à porcentagem de recombinação entre genes em 9S.

Os resultados mostraram que a heterocromatina do cromossomo B não somente não causou diferença em recombinação entre os sexos, como também não mostrou tendência para causar supressão da atividade positiva do knob. Portanto, os knobs e a heterocromatina distal do cromossomo B constituem tipos distintos de heterocromatina. Conseqüentemente, o diferencial em recombinação entre os sexos, causado pelos knobs, é propriedade da composição específica deles e não depende simplesmente da posição no cromossomo ou da aparência heterocromática.

6. LITERATURE CITED

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