CROSSING OVER AND DIPLOID EGG FORMATION IN THE ELONGATE MUTANT OF MAIZE[1]

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ABSTRACT

RHOADES (1941) found recombination in the proximal regions of chromosome 5 to be higher in male than in female flowers. Two explanations were proposed to account for the lower female values, namely: (1) there is a basic difference in rates of crossing over in mega- and microsporocytes, or (2) selective orientation of the chromosome 5 bivalent on the meiotic spindle leads to the preferential segregation of noncrossover chromatids to the basal megaspore. These alternatives have been tested by carrying out a half-tetrad analysis of the diploid eggs produced by plants homozygous for the recessive *elongate* (*el*) allele. The *A2–Bt* crossover values determined from the diploid eggs of elongate plants were much lower than those calculated from haploid sperm of both *El el* and *el el* plants. Since male and female flowers should have similar crossover values if the orientation hypothesis were correct, it was concluded that the amount of crossing over in the *A2–Bt* region of chromosome 5 is intrinsically higher in male than in female meiocytes. In the analysis of diploid eggs the use of the *Bt* locus, which marks the centric region of chromosome 5, provided information on the origin of diploid eggs. The genotypic constitution of 425 diploid eggs was ascertained. Of these, 20.4% were *Bt bt*. They could not be accounted for by failure of the second meiotic division or by replication during the interphase between the two meiotic divisions, but are expected if there is a single division with an equational separation of the centromere regions of chromosome 5. The *Bt Bt* and *bt bt* genotypes arise from a disjunctional separation. It is proposed that diploid eggs are produced by an abnormal meiosis in which there is one division with either disjunctional or equational separation. Disjunctional separation is more frequent but the ratio of the two types varies from ear to ear. Recombination in the *A2–Bt–Pr* region of chromosome 5 was found to be higher in the haploid gametes of elongate homozygotes than in *El El* and *El el* plants. On the other hand, crossing over was reduced in the *Sh–Bz* segment of chromosome 9 in elongate plants, but the adjacent *Bz–Wx* interval was unaffected.

DIFFERENT rates of recombination in male and female flowers have been reported for several chromosome regions in maize (reviewed by RHOADES 1941, 1955; and by PHILLIPS 1966, 1969b). The most intensively studied of these regions are those proximal to the centromere of chromosome 5. RHOADES (1941) found crossing over in the *A2–Bt*, *A2–Bm*, *Bt–Pr* and *Bm–Pr* intervals to be

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higher in male than in female meiocytes. Since these segments are adjacent to the centromere, he suggested that the differences in recombination might be confined to the proximal regions. Confirmation of this hypothesis was provided by PHILLIPS (1969b) who found the sex effect in the *Pr–Ys* segment of 5L but not in the more distal *Ys–V2* region.

Two explanations have been proposed to account for the higher recombination values in the staminate inflorescence. The first assumes a basic difference in the rates of crossing over in mega- and microsporocytes (RHOADES 1941). A second possibility is that selective orientation of the chromosome 5 bivalent on the meiotic spindle leads to the preferential segregation of noncrossover chromatids to the basal megaspore (RHOADES 1955). In a metaphase bivalent having one exchange or chiasma, the two chromatids participating in the cross lie nearest to the equatorial plate, while the other two chromatids face the spindle poles. There is strong evidence that the cytologically visible chiasma involves the crossover chromatids (PEACOCK 1968, 1970; reviews by RHOADES 1961; JOHN and LEWIS 1965; and WHITEHOUSE 1965). If the metaphase I orientation is retained at metaphase II in megasporogenesis, preferential recovery of noncrossover strands in the basal megaspore would result. Since all four spores arising from a microsporocyte produce viable gametes while the egg develops from the basal megaspore only, the observed amount of recombination would be less in the female gametes even though the frequency of crossing over was the same in male and female flowers.

It is possible to test these alternatives by making use of one of the properties of the elongate mutant, namely the production of unreduced eggs. Since the diploid eggs originate by omission of one of the meiotic divisions (RHOADES and DEMPSEY 1966b), half the products of meiosis can be recovered within individual cells. Thus, if the first hypothesis is correct, crossover values determined in unreduced eggs should be lower than those found in male flowers. On the other hand, if there is a selective orientation of the bivalent, the half-tetrad analysis should show recombination frequencies similar to those of the male gametes. The results of such a test are reported here. In addition, information concerning the mechanism of diploid egg formation in elongate homozygotes and an effect of the elongate gene on crossing over is presented.

MATERIALS AND METHODS

Genetic stocks: The genes used as chromosome 5 markers were *A2*, *Bt* and *Pr*. *A2* is located at a position between 0.13 and 0.23 in the short arm (PHILLIPS 1969a); *Bt* is in the long arm and close to the centromere (RHOADES 1936), while the cytological position of *Pr* is approximately 0.4 in the same arm (PHILLIPS 1969a).

The recessive elongate allele (*el*) has a number of effects when homozygous. These include, among others, the production of unreduced eggs in varying proportions with haploid eggs, pollen and ovule abortion, and uncoiling of the chromonemata at both meiotic anaphases and telophases (RHOADES and DEMPSEY 1966b). It has not yet been placed in a linkage group.

When fertilized by haploid pollen, a diploid egg produces a triploid embryo. The resulting shriveled kernel has a low viability (RHOADES and DEMPSEY 1966b) and is difficult to score for marker genes. For the genetic analysis of unreduced gametes, elongate plants heterozygous for

a2 and *bt* were therefore pollinated with diploid pollen from tetraploid plants having appropriate recessive marker genes. Fertilization by *2n* pollen grains results in the development of plump kernels with *4n* embryos (RHOADES and DEMPSEY 1966b).

To derive the genotypes of the diploid eggs, individuals showing dominant phenotypes were used as females in a second testcross. From the segregation ratios of the specific gene, the F_1 genotype could be deduced, as illustrated in Table 1. In addition, different ratios of the four phenotypic classes in the testcross made it possible to distinguish *A2 Bt/a2 bt* and *A2 bt/a2 Bt* eggs. The second cross was unnecessary for testcross progeny showing only recessive phenotypes.

All material synthesized during this investigation was derived from stocks supplied by DR. M. M. RHOADES and MISS E. DEMPSEY of Indiana University, Bloomington, the Maize Genetics Stock Center in Urbana, Illinois, and DR. D. L. SHAVER of Cornnuts, Inc., Salinas, California.

Determination of recombination values: The control and experimental plants used in recombination studies were sibs. Recombination was tested in both male and female flowers. In chromosome 5 tests, recombination in the *Bt–Pr* region was calculated from the *A2* classes only, since *a2* kernels lack aleurone color and cannot be directly classified for *Pr* and *pr*. Except where otherwise specified, all recombination values for elongate female parents were derived from haploid gametes. The frequencies of crossover strands in unreduced gametes of elongate plants were obtained by determining the genotypes of diploid eggs according to the method outlined above.

In some testcrosses, it was suspected that the ratios of phenotypic classes were influenced by the presence of gametophyte factors. The discrepancies were not large enough to alter recombination frequencies significantly, and no corrections were made.

Statistical analyses: The segregation of alleles in testcrosses involving haploid gametes was tested for agreement with a 1:1 ratio by the chi-square method. Tetraploid segregations were not checked statistically except where there was doubt as to whether a pair of alleles showed a ratio characteristic of the 3.7:1 to 5:1 range or of the 1:1 to 13:15 range.

Within groups, the homogeneity of the recombination fractions for each region was determined by means of chi-square contingency tables, comparing the numbers of recombinant *vs.* nonrecombinant individuals. In the absence of significant heterogeneity, the data were pooled. Differences between groups were tested for significance using 2×2 chi-square contingency tables.

If one or both groups showed significant heterogeneity for recombination in a particular region, the following procedure was adopted. The recombination percentages for the individual families within each group were converted to angles, using the arcsin transformation. The values were not weighted because no correlation was observed between family size and recombination frequencies, and also because the family totals from which they were calculated were greater

TABLE 1

Determination of diploid egg genotypes from testcross progenies

Genotypes				
Diploid egg	Diploid pollen	First testcross progeny	Diploid pollen	Phenotypes of second testcross progeny
AA	x *aa* ⟶	*AAaa*	x *aa* ⟶	5*A*:1*a* to 3.7*A*:1*a**
Aa	x *aa* ⟶	*Aaaa*	x *aa* ⟶	1*A*:1*a* to 13*A*:15*a**
aa	x *aa* ⟶	*aaaa*		

* Depends on amount of crossing over between *A* and centromere (SWANSON 1957).

than 100 in nearly all cases. Where the variances calculated from the transformed data were homogeneous according to the F-test, means were compared using the formula

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

where s is an estimate of the standard deviation based on both samples jointly, $\bar{x}_1$ and $\bar{x}_2$ are the means and n_1 and n_2 the numbers of recombination values in the first and second groups, respectively.

If the variances differed significantly, the formula used was

$$t' = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

where s_1^2 and s_2^2 are the variances of the two groups. Values of t' larger than

$$\frac{s_1^2 t_1}{n_1} + \frac{s_2^2 t_2}{n_2} \Big/ \frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}$$

were judged to be significant, t_1 and t_2 being the values of Student's t for $n_1 - 1$ and $n_2 - 1$ degrees of freedom at the chosen level of significance. However, in view of the heterogeneity in the recombination frequencies, the results obtained with the t and t' tests were used with caution in the interpretation of data.

Recombination percentages in italics in the tables of results represent the pooled values for the families concerned but signify that heterogeneity was present and significance was tested by calculating t or t'.

RESULTS AND DISCUSSION

Recombination in male and female flowers: To test the two hypotheses proposed to explain the difference in male and female recombination values for chromosome 5, crossing over was studied in elongate plants and their normal sibs. *El el* and *el el* plants heterozygous for the markers *A2* and *Bt* were testcrossed as males and as females to *a2 bt* testers. The genotypes of the original diploid eggs were deduced from the progeny phenotypes as described in the section on MATERIALS AND METHODS.

Table 2 shows the frequencies of phenotypic classes among tetraploid offspring from the first testcross of four F_1 elongate plants. The second testcross data, from

TABLE 2

Progenies from the testcross of A2 Bt/a2 bt, *el el* ♀ *(family No. 453)* × *tetraploid* a2 bt ♂

Phenotypic class	F_1 plant no.			
	453–77	453–96	453–103	453–138
A Bt	68	121	71	88
a bt	37	35	39	25
A bt	11	19	14	14
a Bt	1	4	5	7
Total progeny	117	179	129	134

The data are from plump kernels, i.e. unreduced eggs.

which the genotypes of 425 diploid eggs were determined, are summarized in Table 3. Corrections were made to these frequencies (Table 4) to allow for incomplete germination of certain classes of kernels among the first testcross progeny and for misclassification of the *A2* phenotype in a few individuals. Crossover values from the individual F_1 plants, calculated from both the corrected and uncorrected genotypic analyses of diploid eggs, are summarized in Table 5, together with data from haploid sperm of the same plants and from haploid eggs and sperm of *El el* sibs.

When plants of *El el* constitution heterozygous for *A2* and *Bt* were reciprocally testcrossed, the 10.1% of recombination found in the female gametes was significantly lower than the 20.1% in the male (Table 5). According to the hypothesis that no basic difference in recombination values exists between the two sexes and that the observed discrepancy comes from a preferential segregation of the noncrossover chomatids to the basal megaspore, the percent of recombinant strands found in the diploid or unreduced eggs of *el el* plants should be similar to that in the haploid male gametes of *El el* sibs. In both *El el* microsporocytes and elongate diploid eggs, a representative sample of the meiotic products is recovered and these should include the crossover strands presumed to be preferentially lost in the *El el* megaspores. Thus, recombination should be higher in *el el* diploid eggs than in the haploid eggs of *El el* sibs and should approach the value observed in the haploid sperm of *El el* individuals.

TABLE 3

Uncorrected frequencies of diploid eggs of various genotypes produced by elongate plants of family No. 453

Diploid egg genotype	F_1 plant no.				Total	Percentage
	453–77	453–96	453–103	453–138		
$\frac{A\ Bt}{A\ Bt}$	30	34	29	19	112	26.4
$\frac{a\ bt}{a\ bt}$	37	35	39	25	136	32.0
$\frac{A\ Bt}{a\ bt}$	10	30	13	19	72	16.9
$\frac{A\ Bt}{A\ bt}$	1	1	1	4	7	1.6
$\frac{a\ Bt}{a\ bt}$	0	2	1	3	6	1.4
$\frac{A\ Bt}{a\ Bt}$	5	8	11	24	48	11.3
$\frac{A\ bt}{a\ bt}$	6	15	10	11	42	9.9
$\frac{A\ bt}{a\ Bt}$	1	0	0	1	2	0.5
Total	90	125	104	106	425	100.0

TABLE 4

Corrected frequencies of diploid eggs of various genotypes produced by elongate plants of family No. 453

Diploid egg genotype	F_1 plant no.				Total	Percentage
	453–77	453–96	453–103	453–138		
$\frac{A\ Bt}{A\ Bt}$	43.5	54.1	38.1	23.6	159.3	29.4
$\frac{a\ bt}{a\ bt}$	37.0	35.0	39.3	25.0	136.3	25.1
$\frac{A\ Bt}{a\ bt}$	14.2	47.8	17.1	24.6	103.7	19.1
$\frac{A\ Bt}{A\ bt}$	1.4	1.6	1.3	4.8	9.1	1.7
$\frac{a\ Bt}{a\ bt}$	0.0	4.0	4.0	4.0	12.0	2.2
$\frac{A\ Bt}{a\ Bt}$	7.1	12.7	14.5	28.5	62.8	11.6
$\frac{A\ bt}{a\ bt}$	11.0	19.0	12.7	14.0	56.7	10.4
$\frac{A\ bt}{a\ Bt}$	1.4	0.0	0.0	1.2	2.6	0.5
Total	115.6	174.2	127.0	125.7	542.5	100.0

Another consequence of the "orientation" hypothesis is that no difference should be found in crossing over in the male and female gametes of elongate plants. The female values are based on diploid eggs, while the male values come from haploid gametes. It might be argued that comparisons should not be made between two populations originating by different meiotic mechanisms. However, RHOADES and DEMPSEY (1966b) found no difference in *Lg–A* (chromosome 3) or *Sh–Wx* (chromosome 9) crossover frequencies in the haploid and diploid eggs from the same ear. Thus, a comparison of recombination in diploid eggs and haploid sperm of the same plant should also be legitimate.

Table 5 shows that neither of the above expectations of the orientation hypothesis is fulfilled. Recombination in elongate megasporocytes, as measured by the number of recombinant strands in diploid eggs, is not significantly higher than that found in haploid eggs from *El el* megasporocytes and is much less than the value observed in *El el* microsporocytes. Moreover, the amount of recombination in diploid eggs from *el el* plants was much lower than the value obtained from haploid male gametes of the same individuals. (It should be noted that recombination in the haploid gametes of elongate microsporocytes is higher than in those of *El el* sibs. The possible influence of the elongate allele on crossing over is discussed in a later section.)

The significant differences between male and female recombination values in both *El el* and *el el* plants eliminate the hypothesis involving selective orienta-

TABLE 5

A2-Bt *recombination in diploid eggs and haploid sperm of* el el *plants and in haploid eggs and sperm of* El el *sibs*

El el			*el el*			
Plant no.	No. of progeny strands	Percent recombination	Plant no.	No. of progeny strands	Percent recombination	
					Uncorrected	Corrected
F_1 used as female parents						
453–91	387	13.2	453–77	180	7.8	9.6
453–113	185	7.0	453–96	250	10.4	10.7
453–123	315	12.7	453–103	208	11.1	12.8
453–132	581	5.9	453–138	212	20.8	21.4
453–140	284	8.1				
453–142	389	14.1				
Pooled value	2141	*10.1***		850	*12.6***	*13.4***
F_1 used as male parents						
453–91	109	15.6	453–77	71	18.3	
453–113	361	15.2	453–88	484	32.0	
453–123	352	24.4	453–96	267	28.5	
453–132	531	17.3	453–121	270	35.9	
453–140	434	23.5				
453–142	334	22.2				
Pooled value	2121	*20.1*		1092	*31.2*	

Based on data from family No. 453. Recombination in diploid eggs expressed as percentage of crossover chromatids among total chromatids analyzed.

** Significantly lower at the 1% level, using a one-tailed test, than the corresponding male value.

tion. It may be concluded that the amount of recombination in the *A2–Bt* region of chromosome 5 is intrinsically higher in male than in female meiocytes. The factors responsible for the effect of sex on crossing over in chromosome 5 are obscure. It has been suggested (RHOADES 1941) that differences in the tissues surrounding the male and female sporocytes may be involved or that the degree of pycnosis in the proximal segments of the chromosome may vary in the two kinds of sporocytes.

Where the sex effect has been found in maize, the male flowers usually have the higher recombination values (PHILLIPS 1969b) and the regions concerned are generally adjacent to or near a centromere. The only segment which consistently shows lower crossing over in male than in female sporocytes is the *Y-Su2* interval of chromosome *6* (PHILLIPS 1969b, 1971). Other regions tested either exhibit similar recombination in mega- and microsporocytes or have given inconsistent results (reviewed by RHOADES 1941, 1955; and by PHILLIPS 1966, 1969b). The total amount of crossing over may be greater in the male than in the female flowers, or the increases reported for certain regions could be compensated for by decrease elsewhere in the genome. A distinction between these alternatives is not

possible at present because a limited portion of the genome has been tested and the data for distal regions in particular are scanty.

Mechanism of diploid egg formation: RHOADES and DEMPSEY (1966b) discussed possible mechanisms by which the unreduced eggs of elongate plants could arise. Following a genetic study of the segregation of various marker genes in diploid gametes, they were able to rule out somatic doubling of the genome in sporogenous cells and doubling in the gametophytic generation. The three remaining alternatives were: (1) the suppression of the first meiotic division followed by a normal second division, (2) a normal first division with omission of the second and (3) a normal first division with chromosomal replication occurring during interphase, followed by the second meiotic division. From their analysis of the diploid eggs from elongate plants heterozygous for *sh wx* on chromosome *9* and *lg a* on chromosome *3*, it was concluded that the evidence did not support hypothesis (1) but was consistent with either hypotheses (2) or (3), which have similar expectations in progeny tests.

When no exchanges occur between a locus and its centromere, the diploid eggs from a heterozygous plant would be expected to show 0% or 50% homozygosity for the recessive allele, depending on whether the first or second meiotic divisions, respectively, are omitted. With 100% single exchanges, the corresponding values are 25% and 0%. On the basis of studies with a number of loci on chromosomes *2*, *3* and *9*, RHOADES and DEMPSEY (1966b) concluded that the percentages of homozygosity were best explained by omission of the second meiotic division. Deviations from 50% homozygosity were attributed to crossing over between the gene and its centromere, and gene–centromere distances were derived for the various marker genes. The *Wx*–centromere value of 11.5% recombination was based on a frequency of 38.5% homozygosis of *wx* in the diploid eggs. This was higher than previous estimates obtained in translocation studies but crossing over is known to be reduced in regions close to the breakpoints in translocation heterozygotes. RHOADES and DEMPSEY did not consider the possibility that more than one mechanism was responsible for the origin of diploid gametes.

The chromosome 5 testcrosses described in the previous section provide information on the manner in which unreduced eggs are formed. *Bt* is in the long arm close to the centromere and is separated from the locus of *Bm* in the short arm by only one unit on the standard linkage map. It is thus a convenient centromere marker and can be used to follow the behavior of chromosome 5 during meiosis.

The genotypic constitution of diploid eggs is given in Tables 3 and 4. The frequency of *bt* kernels from the first testcross (Table 2) was 34.7%. The corrected data in Table 4, where the complete genotypes of the diploid eggs were determined, give 41% *Bt Bt*, 35.5% *bt bt* and 23.5% *Bt bt* eggs. The high frequencies of the homozygous *Bt Bt* and *bt bt* classes are at variance with the hypothesis postulating suppression of the first meiotic division. However, difficulties also arise if the results are interpreted on the basis of second division failure. On this hypothesis, the 23.5% *Bt bt* eggs would be ascribed to exchanges between *Bt* and the centromere. For short regions the frequency of recombination between a locus

and the centromere is 50 minus the percentage of the homozygous recessive class (RHOADES and DEMPSEY 1966b). Thus, a map distance of 50.0 − 35.5 = 14.5 units (or 8.1 for the uncorrected data) would be obtained between *Bt* and the centromere. The close linkage of *Bt* with *Bm* in the short arm makes this highly improbable. Moreover, most or all of the exchanges between *A2* and *Bt* would have occurred between the *Bt* locus and the centromere, which is very unlikely.

Secondly, there are four genotypes which are heterozygous for *Bt* (Tables 3 and 4). With omission of the second division, two of these types, namely, *A2 Bt/a2 bt* and *A2 bt/a2 Bt*, must involve double exchanges in the *A2*–centromere and centromere–*Bt* regions. In view of the small amount of recombination between *A2* and *Bt*, double exchanges should be rare, and yet nearly one-fifth (19.1%) of the diploid eggs are *A2 Bt/a2 bt*. This genotype could be derived from two-strand and one-half of the three-strand double crossovers, while the *A2 bt/a2 Bt* eggs could come from four-strand and one-half of the three-strand doubles in the same regions. Since the *A2 bt/a2 Bt* class represents only 0.5% of the diploid eggs, there would have to be a very great excess of two-strand double exchanges. The high frequency of eggs heterozygous for *Bt* cannot be accounted for by omission of the second meiotic division.

The possibility that eggs classified as *A2 Bt/a2 bt* were not disomic but were in fact *A2 Bt/*— — monosomics in which an *A2 Bt* chromatid had been lost during meiosis must be considered. The resulting *A2 Bt/a2 bt/a2 bt* plants would be aneuploid instead of full tetraploids and these would give the 1:1 segregation ratios for both pairs of alleles on which the genotype determinations were based. That aneuploids are produced by elongate plants was demonstrated by RHOADES and DEMPSEY (1966b). After pollination with haploid pollen, they determined chromosome numbers in root tips of triploid individuals arising from shriveled kernels. They found 82% to be euploids with 30 chromosomes, 11% hypoploids with 25 to 29 chromosomes and 7% hyperploids with 31 to 33 chromosomes. It is unlikely that the eggs classified as *A2 Bt/a2 bt* in family No. 453 arose through the loss of an *A2 Bt* chromatid. Among 21 progeny of plant No. 453–138 that were examined cytologically and used in the second testcross, only two had less than 40 chromosomes and the deficient chromosome could have been any one of the 10 chromosomes of the complement.

It is therefore proposed that diploid eggs are produced by an abnormal meiosis in which there is a single division with either disjunctional or equational separation. The first meiotic division is suppressed in some cells and the second division omitted in other meiocytes of the same ear or, alternatively, some chromosomes undergo a disjunctional separation of centric regions and other chromosomes an equational separation in the same cell. This proposal is supported by the close correlation between the observed distribution of *A2* genotypes within the *Bt Bt*, *bt bt*, and *Bt bt* classes and that expected on the basis of a known amount of recombination between *A2* and *Bt*. For example, if there is no crossing over between *Bt* and the centromere and if the *Bt bt* eggs come from first division elimination or an equational separation, the proportion expected to be homozygous at the *A2* locus is 13.4% on the basis of recombination between *A2* and *Bt* (corrected data,

Table 5) and 12.6% on the basis of the uncorrected crossover value. These percentages are in fair agreement with the frequencies of 16.6% and 14.9% derived from the corrected and uncorrected arrays of diploid eggs (Tables 4 and 3) respectively.

Similarly, if the *Bt Bt* and *bt bt* eggs arise through the omission of the second meiotic division or from a reductional separation, the expected proportions of homozygotes (*A2 A2* + *a2 a2*) are 73.2% and 74.8%, depending on whether the corrected or uncorrected recombination values are used. The actual frequencies are in close agreement, namely 71.2% for the corrected and 73.4% for the uncorrected data. The small discrepancies can be attributed to a low frequency of crossing over between *Bt* and the centromere, and to sampling errors.

TABLE 6

Effect of homozygous elongate on crossing over in the A2-Bt *and* Bt-Pr *regions of chromosome 5*

Based on data from family No. 713.

El el plants			*el el* plants		
	Percent recombination			Percent recombination	
No. of progeny	*A–Bt*	*Bt–Pr*	No. of progeny	*A–Bt*	*Bt–Pr*
F_1 used as female parents					
522	1.3	18.5	207†	7.7†	40.3†
488	2.7	19.8	202	8.4	37.9
546	3.3	30.6	336	10.1	30.1
458	3.5	15.7	114	10.5	30.6
459	4.8	14.7	100	11.0	37.8
464	5.0	25.4	137	13.1	27.1
427	5.4	35.5	279	13.3	32.2
422	6.2	24.8			
439	6.4	22.5			
481	6.7	32.8			
339	7.7	20.5			
Pooled value	*4.6*	*23.8*		10.5**	33.6**
F_1 used as male parents					
240	2.5	32.4	290	10.7	28.2
344	3.8	30.7	328	13.7	33.8
288	4.2	22.3	387	14.7	31.9
341	5.0	31.4	326	15.3	37.1
418	5.5	27.4	328	16.8	34.2
250	6.8	33.6	418	17.9	36.1
396	7.1	23.4	364	20.6	29.7
Pooled value	5.1	28.4		*15.9***	33.1**

** Significantly different, at the 1% level, from the *El el* value for the same sex.
† Pooled values for 3 ears.

The percentages of *A2 Bt/a2 bt* diploid eggs ranged from 12.3 to 27.4 in the four tested elongate plants (Table 4). Evidently the frequencies of reductional and equational separation are subject to considerable plant-to-plant variation. This is not surprising since the other manifestations of the *el* gene are variable in expression. In a system where two mechanisms are responsible for the production of diploid eggs, gene-to-centromere distances can be determined. However, in order to distinguish between the two types of separation, it is necessary to follow a centromere marker such as *bt*. Furthermore, by simultaneously using centromere markers on two or more nonhomologous chromosomes, it should be possible to determine whether all the chromosomes of the complement undergo the same type of separation in any individual meiocyte or not.

Modification of crossing over by the elongate mutant: It was previously noted that crossing over in family No. 453 was higher in the microsporocytes of elongate

TABLE 7

Effect of homozygous elongate on crossing over in the A2-Bt *and* Bt-Pr *regions of chromosome 5*

Based on data from family No. 717.

El el plants			*el el* plants		
	Percent recombination			Percent recombination	
No. of progeny	*A–Bt*	*Bt–Pr*	No. of progeny	*A–Bt*	*Bt–Pr*
F_1 used as female parents					
353	2.8	25.4	147	4.8	36.0
428	3.3	22.3	129	5.4	29.7
472	3.6	22.0	157	7.0	18.7
542	4.2	20.9	165†	8.5†	33.8†
456	4.2	27.6	134	10.4	32.8
334	4.2	21.4	143	10.5	37.9
464	4.3	27.6			
323	4.6	30.4			
483	4.8	24.3			
Pooled value	4.0	24.6		7.8**	31.4**
F_1 used as male parents					
437	3.2	24.6	319	11.3	27.7
274	4.0	20.8	208	12.5	27.9
457	4.2	26.0	318	13.2	40.8
270	6.3	26.5	337	13.6	33.8
248	7.3	22.8	396	14.4	38.6
299	7.4	15.8	429	15.4	40.3
326	7.4	24.7	362	17.1	39.8
435	8.3	26.4	234	20.9	39.8
Pooled value	5.9	23.8		14.8**	36.6**

** Significantly different, at the 1% level, from the *El el* value for the same sex.
† Pooled values for 4 ears.

plants than in those of *El el* sibs (Table 5). Additional information on crossover enhancement in *el* homozygotes is provided by the results from several three-point testcrosses involving chromosomes 5 and *9*.

Tables 6 and 7 contain data from reciprocal testcrosses of *El el* and *el el* plants (in families No. 713 and 717) to *a2 bt pr* stocks. Since both elongate and normal plants were pollinated with haploid pollen, all recombination values are derived from haploid gametes. In both families the *A2–Bt* and *Bt–Pr* regions of elongate plants show higher rates of crossing over in the female sporocytes than do the same regions in their normal sibs; this is in contrast to the previously studied family (Table 5) where the difference between the values for diploid eggs of elongate plants and haploid eggs of *El el* sibs was not significant. In the latter case, recombination was slightly higher in the unreduced eggs than in the controls, and the lack of significance may have been due to low numbers of plants and heterogeneity of the crossover values. Recombination is markedly higher than normal in the male flowers of elongate plants. The extent of the crossover increase above the frequency found in *El el* sibs is variable; in family No. 713, recombination is more than trebled in the *A2–Bt* region (Table 6), while in family 453 (Table 5) there is only a 50% increase. In some families (No. 717 and 453) a more striking increase was found in the male gametes of elongate plants; in others (No. 713) the percentage increase was greater in the female gametes. In both sexes enhancement of recombination in the *A2–Bt* segment exceeds that found in the *Bt–Pr* interval; the shorter *A2–Bt* region containing the higher proportion of proximal chromatin is apparently the more sensitive of the two segments.

Since comparisons were made between *el el* and *El el* genotypes, it could be argued that crossing over is reduced in the elongate heterozygotes as was true of

TABLE 8

Effect of elongate on crossing over in the A2–Bt *region of chromosome* 5

Plants of three F_1 families having the genotype *A2 Bt/a2 bt* and segregating for *El El, El el* and *el el* were used as male parents in testcrosses to *a2 bt* testers

Family no.	*El El*		*El el*		*el el*	
	No. of progeny	Percent recombination	No. of progeny	Percent recombination	No. of progeny	Percent recombination
408	581	7.2	396	13.4	270	39.6
			305	17.0		
			256	21.9		
418	371	7.8	323	18.3	327	29.1
	278	13.7	317	13.6	169	31.4
			235	18.7	128	33.6
			401	23.7	135	31.1
			129	17.8		
452	420	4.8	256	7.8	258	22.5
			431	11.1	571	27.3
					255	38.0

plants heterozygous for the recessive asynaptic mutant (NEL 1970), rather than increased in the homozygotes. A limited amount of information on *A2–Bt* crossing over in the microsporocytes of *El El*, *El el* and *el el* sibs is available from three families related to family 453 (Table 8). The *el el* recombination values are well above those of the heterozygous and the homozygous normal plants; there is no evidence that crossing over in the *El el* class is decreased. On the contrary, there is a distinct possibility that the frequency of recombination is higher in the *El el* group than in the *El El* plants. However, more data are needed before a real difference between these two classes can be established. In any event, recombination between *A2* and *Bt* is increased in elongate homozygotes, as compared to both *El el* and *El El* sibs.

Does the *el* allele itself enhance crossing over or is a linked locus responsible for the increases? If a linked factor enhances recombination, crossing over between the enhancer and *el* in the *El el* parents of the F_1 families should result in some overlapping of the *A2–Bt* values in the *El el* and *el el* sibs. An examination of the values in male flowers, which show the most striking differences (Tables 5, 6, 7 and 8), reveals only one case where recombination in an elongate plant is lower than the highest value in *El el* sibs, *viz.* plant No. 453–77 in Table 5. Since only one plant in a total of 58 *El el* and *el el* plants testcrossed qualifies as a possible crossover between *el* and the enhancer, the map distance between the two loci would be 1/58 or approximately 1.7 units. Therefore, the *el* allele itself or a very closely linked locus is responsible for the enhancement of crossing over in chromosome 5. Since the elongate mutant is known to affect meiosis in several different ways, it would not be surprising if it also influenced the process of crossing over.

As regards crossing over in other portions of the genome, RHOADES and DEMPSEY (1966b) found no difference between elongate and standard values in the distal *Lg–A* and *Sh–Wx* regions of chromosomes *3* and *9*, respectively. This finding is confirmed by a study of recombination in the *Sh–Bz–Wx* region in haploid gametes of *el el* and *El el* plants. Table 9 shows that crossing over between *Bz* and *Wx* is slightly higher in elongate plants than in *El el* sibs, but not significantly so. Recombination in the adjacent *Sh–Bz* region is significantly reduced

TABLE 9

Effect of homozygous elongate on crossing over in the Sh–Bz *and* Bz–Wx *regions of chromosome* 9

Genotype	No. of plants	No. of progeny	Percent recombination	
			Sh–Bz	*Bz–Wx*
F_1 used as female parents				
El el	10	4968	2.3	17.4
el el	19	2165	1.4*	*17.8*
F_1 used as male parents				
El el	9	3914	2.4	*24.6*
el el	10	3472	1.6*	*26.7*

* Significantly different, at the 5% level, from the *El el* value for the same sex.

in the *el el* plants but the total recombination in both segments is similar in *el el* and *El el* individuals. In this experiment, the crossover values for the *Bz–Wx* segment are higher in male flowers than in female flowers. Thus, the effect of elongate on recombination is not uniform in all parts of the genome.

Other genetic factors which influence recombination in maize are B chromosomes (HANSON 1965, 1969; AYONOADU and REES 1968; RHOADES 1968; CHANG and KIKUDOME 1971, 1974; WARD 1972, 1973; NEL 1973) and abnormal chromosome *10* (KIKUDOME 1959; RHOADES and DEMPSEY 1966a; ROBERTSON 1968; NEL 1973). Crossover rates were also altered in plants homozygous (RHOADES 1947; RHOADES and DEMPSEY 1949; DEMPSEY, unpublished) and heterozygous NEL 1970) for the recessive asynaptic mutant. It is of special interest that the regions most strongly affected in plants with structurally normal homologs were the proximal segments of chromosome 5. The same regions also show large differences in recombination in the male and female flowers (RHOADES 1941; PHILLIPS 1969b and present experiments). The proximal regions of chromosome 5 were more responsive to the influence of B chromosomes and abnormal chromosome *10* than was the proximal *Wx–Gl15* segment of chromosome *9* or the more distal regions of *9S* (NEL 1973). The greater sensitivity of chromosome 5 was also evident in the asynaptic and elongate experiments, where crossing over in the *Gl6–Lg–A* and *Bz–Wx* regions, respectively, was relatively unaffected, while significant changes were observed in the *A2–Bt* and *Bt–Pr* intervals.

Crossing over in the proximal regions of chromosome 5, particularly the *A2–Bt* segment, is variable. The standard map distance for this interval is 7 units (NEUFFER, JONES and ZUBER 1968). However, the values obtained in specific tests vary widely, even among apparently "normal" strains. For example, 1.6% recombination was found in megasporocytes of some *A2 Bt/a2 bt* plants (PHILLIPS 1966), while RHOADES (1941) reported 26.3% recombination in the microsporocytes of a high crossover line. The effect of sex is also subject to fluctuation; in some strains male and female flowers may show essentially the same amount of crossing over (*El el* plants in Table 7, and PHILLIPS 1969b) while in others recombination in male meiocytes may be as much as three times that in female meiocytes (NEL 1970). It is therefore not surprising that heterogeneity in crossover values occurs within groups in most experiments. The high degree of responsiveness makes chromosome 5 particularly useful in detecting and assaying factors which affect crossing over.

While the centromere region of chromosome 5 is known to be more subject to the influence of abnormal *10*, B chromosomes and sex differences than the proximal segment of chromosome *9* (NEL 1973), comparisons with the proximal regions of other chromosomes have not been made. A unique behavior of the proximal chromatin of chromosome 5 is suggested by the studies of PETERSON (1955) on nonhomologous centromere associations at pachynema in the inbred line KYS. She found that the centromere region of chromosome 5 was more frequently involved in nonhomologous associations than were the centromeres of the other chromosomes.

It is patent from these investigations that no generalizations can be safely

drawn regarding the effects on recombination of B chromosomes, of abnormal chromosome *10* and of the *as* and *el* genes. Not only do different chromosomes vary in their response to these factors but, within a single chromosome, markedly different reactions are found in proximal as compared to distal segments. The mechanism by which recombination occurs is probably the same in all eukaryotes but the pattern of recombinational events may be different, even among the chromosomes of a single species. The maize chromosomes tested have dissimilar recombinational patterns and all ten may have unique systems which developed in the course of evolution.

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