LOCATION OF THE CENTROMERES ON THE LINKAGE MAPS OF MAIZE[1]

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IN RELATING genetic data to the morphology of the chromosomes in maize, the relation of gene loci to the position of the centromere, or region of spindle attachment, is of primary interest and importance. The relation of the genes to each other on the linkage maps is established directly from crossing over percentages, but the physical position of the genes in the chromosomes must necessarily be determined indirectly from both genetical and cytological data, since suitable techniques for the visual identification of individual genes are not yet available.

Chromosomal alterations that produce both genetical and visible cytological effects may be used to locate the position of the genes in the chromosomes. Of these, the most useful are the translocations, inversions and deletions that produce both phenotypic effects suitable for mapping with the techniques used for genes and visible disturbances of the normal synaptic relations by means of which their location in the chromosome can be determined. This correlation of loci on the genetic maps with specific regions of the chromosomes concurrently establishes the location of the genes with reference to the centromere and other differentiated regions of the chromosome.

The locations of the centromeres on the linkage maps were determined chiefly from translocations which were observed cytologically at the midprophase of the first meiotic mitosis. The cytological determinations were subject to minor errors of interpretation due to failure of pairing and non-homologous association of the chromosomes in the region of exchange of segments. A single case of duplication, that of a telocentric half-chromosome (RHOADES 1936, 1940) located the centromere of chromosome 5 with a high degree of precision. Deficiencies have been useful in correlating the genetic maps with the distal regions of the chromosomes, less so for the regions near the centromeres. However, the internal deficiencies and ring fragments studied by MCCLINTOCK (1938, 1941) delimited within narrow boundaries certain locations near the centromere of chromosome 5.

Translocations may be identified by the sterility they produce when in the heterozygous condition. Thus their position in the linkage maps can be established in much the same manner as are the positions of dominant genes. The

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sequence of translocations within a given region between two genes cannot be determined satisfactorily, and measurement of map distances are unreliable due to various amounts of suppression of crossing over. But the order of the genes and translocations on the linkage maps can be determined reliably and can be checked in the homozygous translocation.

Homozygous translocations are of especial value in mapping the centromeres. If it can be determined on which side of the centromere the translocation has occurred in the one chromosome, a simple test for linkage with known genes may tell which arm of the other chromosome is involved. As in the case of T2-3b, the map location of the translocation in chromosome 2 is to the right of *v4* well out on the long arm. It is near *ts4* in chromosome 3. In the homozygous translocation the linkage of *B* and *v4*, which are on opposite sides of the centromere in chromosome 2, with *ts4* places the break in chromosome 3 between *ts4* and the centromere; and the undisturbed linkage of *ts4* with *lg2*, which is approximately 30 units to the right of *ts4* places the break in the long arm of chromosome 3. In this manner much information relative to the location of genes and centromeres often can be obtained even in the absence of cytological observations. Moreover, cytological observations also may be checked in the same manner.

An effort has been made to present only such information as is needed to locate the centromeres within narrowest boundaries. The data were selected to show for each chromosome the nature of the evidence on which our conclusions are based, and to show the limits of our actual information.

Chromosome 1

Information on translocations in the short arm of chromosome 1 was summarized by ANDERSON in 1941. The gene *P* is about two-thirds of the distance out on the short arm. A minimum map distance from *P* to the centromere may be determined from T1-9a which is known to be located in the short arm. On the basis of 730 plants, the percentage of crossing over between *P* and T1-9a was found to be 21.2 ± 2.5. Thus the location of the centromere on the linkage map is 21.2 units or more to the right of *P*.

A number of translocations in the long arm of chromosome 1 give less than 5 percent of crossing over with brachytic. These are distributed from about L.2 to about L.6. The gene *br* is probably located in the neighborhood of L.3 or L.4. Only two of the translocations in the long arm are definitely placed to the left of *br*. T1-6a was reported by COOPER and BRINK (1931) and BRINK and COOPER (1932) to be in the long arm of chromosome 1 a short distance from the centromere. From their diagrams and figures a position of about L.2 is indicated, which is also in accord with other data. The map position, based on 75 plants, is given as 13.4 units to the left of *br*. T1-6b has been described by BURNHAM (1932). The locus in chromosome 1 is given as L.25. Very good linkage data involving 952 plants place the translocation to the left of *br* with 3.8 percent of crossing over. (Data by BURNHAM cited by EMERSON, BEADLE and FRASER 1935.)

These data show merely that *br* is between one-quarter and one-half the distance out on the long arm. The map position of the centromere must be somewhere between the locus of T1-9a, 21.2 units to the right of *P* and the locus of T1-6a, 13 units to the left of *br*. This is a very long region. If crossing over were equally distributed over this portion of the chromosome, we might expect the location of the centromere to be about midway between *P* and *br*.

Chromosome 2

The map location of the centromere can be rather closely delimited by a number of translocations in the interval between *ts* and *v*4. Several of these will be considered. T2-9b is located cytologically at 2S.1 and 9L.2. Linkage tests give the order definitely as *B-ts-T-v*4. Crossing over between the nearest genes was:

ts-T = 33/662 = 5.0
T-*v*4 = 121/1542 = 7.8

Since the break in chromosome 9 is known to be in the long arm (ANDERSON 1938), the *wx* gene must be carried in the 9^2 chromosome. Tests of linkage relations in the homozygous translocations were used to verify the location of the break in chromosome 2. These tests gave the following results, showing that the break is between *ts* and *v*4.

B-*ts* = 86/319 = 26.3
ts-*v*4 = 108/195 = 55.4, or independence
wx-*B* = 159/721 = 22.1
wx-v4 = 2571/5056 = 50.9

The linkage of *wx* with *B* and its independence of *v*4 establishes the break in the short arm of chromosome 2 between *B* and the centromere. The linkage of *B* and *ts* shows that the break is to the right of *ts*, and the independence of *ts* and *v*4 locates the break between those genes. Thus the centromere in chromosome 2 is at least five units to the right of *ts*.

T2-5a was studied by RHOADES (1933) and described cytologically as in the long arm of chromosome 2 near the centromere. Linkage tests give the order as *B*-T-*v*4 with 7.3 percent of crossing over between T and *v*4.

T2-10a is located at L.2, with the break in chromosome 10 well out on the long arm, two or three cross-over units to the left of *g*. The order on chromosome 2 is *ts*-T-*v*4, and the data on crossing over are as follows:

ts-T = 52/384 = 13.5
T-*v*4 = 74/1145 = 6.5

Linkage data in the homozygous translocation are as follows:

B-ts = 50/282 = 17.7
B-g = 42/205 = 20.6

Since *g* is distal to the break in chromosome 10, the *B-ts* section of chromosome 2 must include the centromere—that is, the translocation must be in the long arm of chromosome 2.

These data may be summarized as follows:

T2-9b	*ts*-5.0-T-7.8-*v4*	Short arm
T2-5a	*ts*-T-7.3-*v4*	Long arm
T2-10a	*ts*-13.5-T-6.5-*v4*	Long arm

The centromere must be five or more crossover units to the right of *ts* and 7.3 or more units to the left of *v4*. Since there is usually some suppression of crossing over in the heterozygous translocation, the total map distance of the *ts-v4* interval is uncertain. The normal value is probably about 20 units. The centromere is probably a little closer to *ts* than to *v4*.

Chromosome 3

The summary of translocations involving chromosome 3 published by ANDERSON and BRINK (1940) places the centromere in the general neighborhood of *ts4*. Since then, additional data on T2-3b has indicated that *ts4* is in the long arm of chromosome 3. This translocation shows about 4 percent of crossing over with *v4*. The order is probably *B-sk-v4*-T. Linkage tests in homozygous T2-3b stocks give the following crossover values:

B-sk	$=38/399=9.5$
B-v4	$=128/289=44.3$
B-ts4	$=496/1171=42.4$
ts4-lg2	$=27/135=20.0$
v4-ts4	$=10/59=17.0$

These data all agree in placing the translocation beyond *v4*, consequently in the long arm of chromosome 2. The linkage of *ts4* with *B* and *v4* in the homozygous translocation places the break in chromosome 3 between the centromere and *ts4* and shows that it is the long arm that is involved. From this it may be concluded that the centromere is to the left of *ts4*—that is, between *d1* and *ts4*.

Chromosome 4

A number of translocations in the proximal regions of both arms of chromosome 4 adjacent to the centromere all show close linkage with *su*, usually accompanied by much suppression of crossing over. These data indicate that the centromere is in the general region of the *su* locus.

Translocation 4-5*d* has been placed by linkage in chromosome 4 between *su* and *Tu;* in chromosome 5 between *bm* and *pr*. The frequency of crossing over between the translocation and *su* was 14/493 or 2.8 percent; between *bm* and the translocation it was 11/439 or 2.5 percent. In the homozygous translocation, the genes *Ts5* and *su* were linked, and these genes were also linked to both *bm* and *a2* from chromosome 5. RHOADES (1936) has shown that the centromere of chromosome 5 is in the very short region between *bt* and *bm*, a short region of about 1 unit. This is much less than the observed crossover value of 2.5 between *bm* and the translocation. Therefore, the location of the translocation must be in the long arm of chromosome 5, with *bm* remaining

with the centromere. The linkage of *Ts5* and *su* with *bm* in the homozygous translocation must involve a break in the short arm of chromosome 4 between *su* and the centromere. Thus the map position of the centromere of chromosome 4 must be at least 2.8 units to the right of *su*—that is, between *su* and *Tu*.

Translocation 2-4c is very near the centromere in the short arm of chromosome 4, and far out on the long arm of chromosome 2. The linkage relations are as follows:

su-9.1-T-30.8-*Tu*
v4-19.9-T-29.3-*Ch*

Linkage tests with the homozygous translocation confirm the order in chromosome 4 and give the further data:

su-v4 = 401/1057 = 37.9
su-Ch = 247/525 = 47.0 (independence)
Tu-Ch = 193/429 = 44.9

The linkage of *su* with *v4* places the translocation between *su* and the centromere and indicates a position for the centromere 9 units or more to the right of *su*. This confirms the conclusion drawn from translocation 4-5d.

Chromosome 5

The position of the centromere in relation to the known genes of chromosome 5 was determined accurately by RHOADES in 1936, with the aid of a fragment of chromosome 5, which apparently consisted of the centromere and the entire short arm of the chromosome. In the metaphase of the first meiotic division in the microsporocytes the fragment formed a trivalent with the two normal number 5 chromosomes in approximately half of the cells; in the remainder of the cells it was present as a univalent that was rarely included in either daughter nucleus. From the known cytological behavior of the fragment the expected backcross ratio from fragment plants of the constitution *AAa* with *a* in one of the normal chromosomes was calculated to be 5A:3a or 37.5 percent of recessives. This ratio differs sufficiently from the ordinary 1:1 backcross ratio of disomic inheritance so that, with the aid of the fragment chromosome, genes located in the short arm could be distinguished from those located in the long arm of chromosome 5.

Another test employed by RHOADES to identify the genes in the short arm was the occurrence of fragment-carrying plants homozygous for the recessive gene in the backcross progenies of fragment plants carrying a recessive allele in one of the normal number 5 chromosomes. If the locus under consideration was in the short arm, none of the fragment-carrying plants would be homozygous for the recessive allele, barring rare exceptions resulting from chromatid crossing over.

Utilizing these tests, it was found that the *a2* and *bm* loci were in the short arm and *bt*, *pr*, *ys*, *v2*, and *v12* were in the long arm of chromosome 5.

MCCLINTOCK (1938, 1941) studied deficiencies in the region about *bm* and

also placed *bm* in the short arm close to the centromere. The available data on translocations are in agreement with the findings of RHOADES and MCCLINTOCK.

The centromere is located in the very short region between *bm* and *bt*.

Chromosome 6

Translocation 6-9b, about one-fourth of the way out on the long arm, is to the right of *Y* but very closely linked to it. Five other translocations are recorded cytologically at about L.2 or L.25. These are T 1-6c, 2-6c, 4-6a, 4-6b, and 4-6c. All are closely linked with *Y* and definitely to the left of *Pl*. When heterozygous, all show a reduction of crossing over between *Y* and *Pl* to 5 percent or less. Proven crossovers with *Y* have not yet been obtained for study. With so much suppression of crossing over, little can be inferred as to the location of the *Y* locus with reference to the centromere. Translocations in the satellite or nucleolar region have given less than 20 percent of crossing over with *Y*. Data on translocations between the centromere and the nucleolar region are too meager to give any satisfactory evidence as to the position of the centromere.

The map location of the centromere may be very near *Y*, or to the left of *Y*, probably not more than five or ten units.

Chromosome 7

Two translocations, T1-7b and T2-7b, place the genes *ra*, *gl*, and *ij* on the long arm. Translocation 1-7b is far out on the long arm of chromosome 1 and about one-fifth of the way out on the long arm of chromosome 7. The map position of the break in chromosome 1 is a little to the right of *br;* in chromosome 7 it is a trifle less than one unit to the left of *ra*. This is confirmed by data on the homozygous translocation showing *ra* linked to *P* and *br* and independent of *bm2*. The translocation is therefore to the left of *ra* and between it and the centromere.

Translocation 2-7b is about one-fourth of the distance out on the long arm of chromosome 7 and is in about the same relative position on the long arm of chromosome 2. Linkage tests place it near *ra*, with slightly over one percent of crossing over. Linkage tests in the homozygous translocation show linkage of *ra* and *gl*, which indicates that the translocation is to the left of *ra*. This is also confirmed by the linkage of *B* and *ra* (*B-ra* = 167/462 = 36.1). Since *B* is in the short arm of chromosome 2 and thus in the 2^7 chromosome, *ra* must be in the translocated portion of the long arm of chromosome 7.

Three translocations in the short arm of chromosome 7 also have been tested for linkage with *ra*. These together with the two described above have given the following data:

T1-7d S.4	5/231 = 2.2
T2-7c S.1+	24/376 = 6.4
T5-7 S.1	14/153 = 9.2
T1-7b L.2	5/546 = 0.9
T2-7b L.2+	4/364 = 1.1

The centromere is to the left of *ra*, probably only a few units. Its relation to *v5* is unknown.

Chromosome 8

The only genes known to be located in chromosome 8 are in the distal region of the long arm. From the data of ANDERSON (1939), the location of the centromere must be 30 units or more to the left of *ms8*.

Chromosome 9

Information on chromosome 9 has been summarized by ANDERSON (1938). Translocation 5-9a is located in the short arm near the centromere and is to the right of *wx* with about 2 percent of crossing over (BURNHAM 1934a). This places the centromere at least two units to the right of *wx*. Translocation 3-9a, in the long arm gave only 3.6 percent of crossing over with *wx*, indicating that the centromere is probably not far beyond the minimum of two units. The gene *v* has not been located definitely but is believed to be in the long arm not far from the centromere (BEADLE 1932; BURNHAM 1934b). Its map position is 12 units from *wx*.

Chromosome 10

The only chromosome 10 genes which have been adequately used in tests with translocations are *g* and *R*. Both are located in the distal part of the long arm. Translocations to the left of L.3 have given from 9 to 23 percent of crossing over with *g*. Probably different amounts of suppression are involved.

Crossing over T-*g*

8-10a	S.6	104/613 = 17.0
8-10c	S.4	122/535 = 22.8
9-10b	L.1−	12/135 = 8.8
6-10a	L.1	33/342 = 9.6
3-10a	L.1+	74/471 = 15.7
1-10a	L.3	21/137 = 15.3

The centromere must be placed at least 15 units to the left of *g*. Its relation to such genes as *nl*, *li*, and *sp2*, previously mapped to the left of *g* by EMERSON et al. (1935) or by RHOADES and RHOADES (1939), cannot be determined from present data. The gene *Rp*, mapped at 43 units to the left of *g* by RHOADES and RHOADES (1939), is placed in the distal one-fourth of the short arm by the earlier studies of V. H. RHOADES (1935).

LINKAGE MAPS

For graphic representation, linkage maps of the ten chromosomes showing the location of the centromeres are presented in figure 1. Only the better known genes, or the genes used in the present studies are listed. Genes whose order in relation to the centromere is unknown or in much doubt are omitted. Untested genes near the centromere whose location is uncertain are enclosed in parentheses.

These maps bring up to date and replace similar maps published earlier by RANDOLPH (1941).

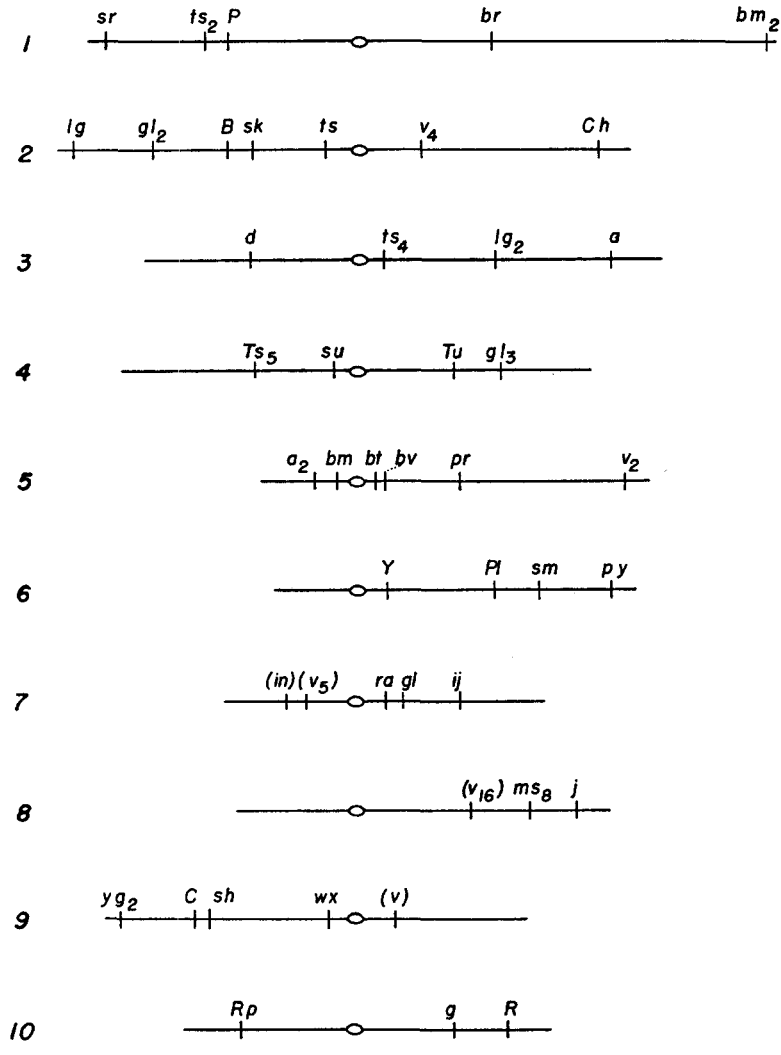

FIGURE 1.—Approximate locations of the contromeres on the linkage maps of selected genes in maize.

SUMMARY

The evidence relating to the locations of the centromeres on the linkage maps of maize has been presented, and, so far as can be determined from the available data, these locations are as follows:

Chromosome 1. Midway between *P* and *br*.

Chromosome 2. Between *ts* and *v4* but closer to *ts*.

Chromosome 3. A short distance to the left of *ts4*.

Chromosome 4. A few units to the right of *su*.

Chromosome 5. Between *bm* and *bt*.

Chromosome 6. Near, or to the left of *Y*.

Chromosome 7. A few units to the left of *ra*.

Chromosome 8. Thirty or more units to the left of *ms8* and *j*. No genes known in the region.

Chromosome 9. A few units to the right of *wx*, probably between *wx* and *v*.

Chromosome 10. Fifteen or more units to the left of *g*.

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