A CYTOGENETIC STUDY OF A CHROMOSOME FRAGMENT IN MAIZE¹

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Received March 6, 1936

INTRODUCTION

EACH of the ten groups of linked genes in maize has been associated with a specific member of the haploid set of ten chromosomes. The morphology of the ten chromosomes has been studied at pachytene by McCLINTOCK (1933) and each member found to differ from the others in such characters as length, position of the spindle fiber insertion region and presence of deeply staining knobs in definite positions, so that they can be recognized by their morphological characteristics.

The fifth longest chromosome of the haploid set of maize has a spindle fiber insertion region that is nearly median, dividing the chromosome into two arms. The ratio of the length of the two arms is 1.1:1.0. In certain strains the longer of the two arms, however, is differentiated at pachytene by the presence of a deeply staining knob that makes it easy to distinguish between the two nearly equal arms of the chromosome.

Plants which possess an extra chromosome V are said to be trisomic for this chromosome. These trisomic plants differ markedly in appearance from their disomic sibs and it is possible to classify a segregating progeny into trisomic and disomic types with considerable accuracy. For this reason plants trisomic for chromosome V have been successfully utilized in an attack on certain problems.

There are two secondary trisomes possible for each primary trisome since the extra chromosome in the secondary trisome is composed of two identical arms, that is, one of the arms of the chromosome is represented twice in the extra chromosome. One of the two secondary trisomes for chromosome V has been found (RHOADES 1933b). The extra chromosome consisted of two short arms of chromosome V. This secondary differed strikingly in appearance from both primary trisomes and disomes.

OCCURRENCE OF A FRAGMENT PLANT

An individual which was intermediate in its appearance between its trisomic and disomic sibs arose in the progeny of a chromosome V primary

¹ Contribution from the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Farm Crops Subsection, Iowa Agricultural Experiment Station, Ames, Iowa, cooperating.

² Journal Paper No. J331. Iowa Agricultural Experiment Station, Ames, Iowa.

trisome. Cytological examination of this exceptional plant showed that it contained a fragment of chromosome V. Studies at pachytene showed that this fragment consisted of the entire short arm of chromosome V with a terminal spindle fiber insertion region. That the break which produced the fragment occurred exactly at the spindle fiber region is indicated by the fact that in many clear pachytene figures no evidence of any chromatic material on one side of the insertion region was ever found. The nature of this fragment in its relation to a normal chromosome V is shown diagrammatically in figure 1.

FIGURE 1.—Diagrammatic representations of normal chromosome V showing relative length of the two arms, position of spindle fiber insertion region, location of knob and of fragment chromosome which consists of the entire short arm of chromosome V and which has a terminal spindle fiber insertion region. The insertion regions are designated by the stippled areas.

FIGURE 2.—Trivalent groups of the two normal and the fragment chromosomes at metaphase I showing the orientation of the fragment chromosome to be such that it disjoins to the same pole as one of the normal chromosomes.

As previously reported, maize plants trisomic for chromosome V differ markedly in their appearance from their disomic sibs. Especially noticeable are the thick broad leaves with relatively blunt tips, the stubby tassel and the reduced height of the trisomic plants. Plants carrying the short arm of chromosome V in excess are intermediate in appearance between primary trisomes and disomes.

CYTOGENETICS OF FRAGMENT CHROMOSOME

Cytological observations of microsporocytes at metaphase I showed that the fragment chromosome was associated with the two normal chromosomes V in approximately 53 percent of the cases to form a trivalent group. This frequency of trivalents is considerably lower than the 85–90 percent found in plants trisomic for the whole chromosome V. If assortment of the members of the trivalent group is completely random six gametic types are possible. If these six types are produced with equal frequencies the genetic backcross ratio from a plant carrying two dominant and one recessive alleles (AAa) would be $5A:1a$. (This ratio ignores the effect of chromatid crossing over on genetic ratios.) Random assortment

of the three homologous chromosomes occurs in primary trisomes but the occurrence of univalents and their failure to pass to either pole in anaphase results in a deviation of the genetic ratio from a 5:1 to about 3.35:1 (RHOADES 1933a) for genes in chromosome V.

Typical trivalent figures involving the fragment chromosome are shown in figure 2. The orientation on the metaphase plate of the trivalent group was such that the fragment would pass to the same pole as one of the normal chromosomes and was rarely of the type that would permit the two normal chromosomes to pass together to one pole while the fragment went to the other pole. Only four gametic types are usually formed. The theoretical gametic ratio for genes located in the short arm would be 3A:1a if the four gametic types are formed with equal frequencies and if the dominant allele is carried in the fragment chromosome and in one of the two normal chromosomes.

FIGURE 3.—Diagrammatic representation of the four gametic types produced by a fragment plant. The locus a_2 , which is included in the fragment, is used to mark the different chromosomes.

The rare occurrence of primary trisomes in the progeny from fragment plants substantiates this idea of a non-random segregation of the members of the trivalent group.³ Since the spindle fiber insertion region of the fragment chromosome is comparable in size with that of the two normal chromosomes, the failure of occurrence of random segregation indicates that the insertion region does not of itself control chromosome segregation. Such a conclusion has been reached on genetic grounds by DOBZHANSKY and STURTEVANT (1931) in their studies on *Drosophila* translocations. The four gametic types from a fragment plant are shown diagrammatically in figure 3.

³ Actually 1.5 percent of the progeny from a fragment plant, used as the female parent, are primary trisomes. The occurrence of these primary trisomes has a slight effect upon the genetic ratios but this has been disregarded to facilitate calculations,

When a trivalent group was present at metaphase, disjunction occurred and the three chromosomes were distributed between the two poles. If a bivalent and a univalent were present, however, the behavior of the univalent was variable. Often it never reached the metaphase plate and remained in the cytoplasm during the formation of the daughter nuclei. If it reached the metaphase plate it was often late in beginning its migration to one of the two poles and consequently was not included in the telophase nuclei. Occasionally the univalent apparently succeeded in reaching one of the poles and was, therefore, included in the nucleus.⁴ So far as observed the univalent never divided in the first meiotic division. If a univalent was present it was always the fragment chromosome.

Knowing that only four gametic types are formed, that trivalent groups are formed at metaphase in about 50 percent of the cells, we can calculate the gametic ratio for a gene located in the short arm of chromosome V if we assume that only those fragment chromosomes which were members of trivalent groups reach the pole. Based on the above the expected back-cross ratio from fragment plants of *AAa* constitution with *a* in one of the normal chromosomes is 5*A*:3*a* or 37.5 percent recessives.

Studies at pachytene showed that the fragment was often paired with the two normal chromosomes. The fragment was paired in approximately one-half of the microsporocytes. The difficulty in interpreting all figures makes this ratio of doubtful value but there was at least rough agreement between the frequency of trivalent complexes observed at pachytene and at metaphase.

That the insertion region is not the place where synapsis must be initiated is indicated by the following observations at pachytene. In those microsporocytes in which the fragment had paired with the short arms of the two normal chromosomes V the terminal insertion region of the fragment chromosome was usually some distance removed from the insertion regions of the normal chromosome. In most synaptic configurations it was some distance from the terminal insertion region of the fragment to the place where the fragment first paired with one of the normal chromosomes. Pairing between the three homologous arms was always between two of the three chromosomes, that is, one of the chromosomes was always unpaired at any given region. There was never any tendency for all three chromosomes to be associated at the same point. The fact that the insertion region of the fragment chromosome had no tendency to be associated

⁴ If the fragment chromosome was paired with the two normal chromosomes at metaphase it had a characteristic V-shaped appearance during anaphase I as it passed to one of the two poles. On the other hand if the fragment chromosome was unpaired at metaphase it had a ball-like or spherical shape at anaphase. In an occasional figure a spherical fragment chromosome accidentally lay so near one of the poles that it probably would be included in the telophase nucleus.

with the insertion regions of the normal chromosomes V indicates that the insertion regions in themselves have no specific attraction. In disomes at pachytene the insertion regions of two synapsed chromosomes lie side by side. This is probably caused by their being brought together by the synapsing of the homologous loci on either side and not because of any specific attraction to one another. It was not uncommonly observed that even though the three homologous arms were involved in a synaptic complex the terminal insertion region of the fragment was attached or stuck to the insertion regions of other pairs of chromosomes. This "stickiness" of insertion regions has often been observed in normal preparations and is not a characteristic feature of the insertion region of the fragment chromosome.

FIGURE 4.—Camera lucida sketch of synaptic relationships of the fragment chromosome and the two normal chromosomes V at pachytene. In this figure the insertion region of the fragment is in juxtaposition with the two insertion regions of the normal chromosomes. One of the normal chromosomes has a deeply staining knob in the long arm.

In those sporocytes in which the fragment was present as a univalent at pachytene it was less contracted than the paired short arms of the two normal chromosomes V with which it is homologous. HUSKINS and HEARNE (1933) observed a similar attenuation in unpaired chromosomes. Non-homologous pairings (foldbacks) were of infrequent occurrence in the unpaired fragment. The equational split of the unpaired fragment chromosome into two chromatids was clearly evident at pachytene. Of considerably more interest was the observation that the terminal insertion

region of the unpaired fragment in some cells, where it was not stuck to other insertion regions, appeared to be split or divided at pachytene. The distal end of the insertion region was cleft to form a heart-shaped structure. As the diagrammatic sketch of the fragment chromosome in figure 5 illustrates there is a concentration of deeply staining chromatin near the insertion region while the more distal portions contain much less deeply staining material. A considerable amount of genetic data strongly suggests that the disjunction of the insertion regions is always reductional in the first meiotic division. DARLINGTON (1931) in his theory of meiosis accounts for this by assuming that the split of the insertion region occurs some time after the equational split of the chromosome into two chromatids. If the observation of the split nature of the terminal insertion region of the unpaired fragment is to be considered applicable to paired chromosomes, then the previous failure to observe the double nature of the insertion region at midprophase is probably due to its homogenous appearance and because its usual interstitial location is such as to conceal the split if it occurred. Only when the insertion region is favorably located, that is, terminally, could the split be evident. There is, of course, the possibility that the split condition of the insertion region of the unpaired fragment is not representative of conditions in paired chromosomes with internal insertion regions. Moreover, if the insertion does split at the same time as the chromosome there is no reason why the two daughter insertion regions cannot behave as "one effective insertion region" at metaphase and anaphase.

LEWITSKY (1931) and others hold that no chromosome has a strictly terminal insertion region. It is true that none of the normal complement of maize chromosomes (McCLINTOCK 1933) has a terminal insertion region but LEWITSKY'S statement does not hold for the fragment chromosomes described in this paper since conditions for cytological observations are exceptionally good at pachytene in maize and the fragment chromosome clearly had a terminal insertion region. The fragment chromosome was as stable as the regular complement of 20 chromosomes in its behavior throughout all the somatic mitoses and no irregularities were manifested.

LOCATION OF GENES IN LONG AND SHORT ARMS OF CHROMOSOME V

Since the fragment consists of the entire short arm of chromosome V it can be employed in determining which of the genes located in this chromosome are situated in the short arm and which are in the long arm. The A_2 locus will be used as an illustration of how this was accomplished. A plant carrying the fragment and homozygous for A_2 was pollinated by a plant homozygous for a_2 and for A_1CR , the other factors concerned with aleurone color. The F_1 fragment plants were then backcrossed with recessive a_2 pollen. If the A_2 locus is in the short arm of chromosome V a deviation from

a 1:1 backcross ratio will be had for $A_2:a_2$. The magnitude of the deviation will depend upon the frequency with which the fragment chromosome, which carried the dominant allele, is included in functioning female gametes. The fragment chromosome was paired with the two normal chromosomes at metaphase I in about 50 percent of the microsporocytes. If we assume that a similar condition holds for the megasporocytes and if we make the further assumption that the unpaired fragment chromosome does not succeed in reaching either of the two poles in anaphase I, and is consequently lost, the expected ratio of $A_2:a_2$ seeds in the backcross of fragment plants is 5 A_2 :3 a_2 seeds. Table I gives the ratio of $A_2:a_2$ seeds actually found and indicates that A_2 is in the short arm of chromosome V. Disomic sister plants gave 869 A_2 seeds to 890 a_2 seeds or a 1:1 ratio. The average of

FIGURE 5.—Diagrammatic sketch of an unpaired fragment chromosome at pachytene. The equational split into two chromatids has occurred and the heart-shaped appearance of the terminal insertion region suggests that it also is double at this stage. There is more deeply staining chromatin near the insertion region than in the more distal portions of the chromosome.

33.6 percent recessives suggests that either approximately 67 percent of the megasporocytes had a trivalent group or else that the unpaired fragment reached one or the other of the two poles in the first anaphase with a considerable frequency. A more useful and efficient test for the presence of any locus in the short arm is the occurrence of fragment plants which are homozygous for the recessive gene in the backcross progeny of a fragment plant carrying one recessive allele in a normal chromosome. If the locus under consideration is in the short arm, no fragment plants will occur, barring the rare occurrence of exceptional individuals through chromatid crossing over, which are homozygous for the recessive allele. This latter test was used for all the genes tested, as it required fewer plants and was of a more critical nature.

Linkage group 5 contains 23 genes. The order of the following genes in the genetic map is correct, $\frac{a_2 \text{ } bm_1 \text{ } bv \text{ } pr \text{ } ys \text{ } v_2}{0 \text{ } 6 \text{ } 12 \text{ } 31 \text{ } 40 \text{ } 72}$, according to the data summarized by EMERSON et al (1935). The exact location of the other genes in the linkage map is uncertain. Eight of the 23 genes in this chromosome were tested against the fragment. The V_2 , Ys_1 , Pr , V_{12} , V_3 and Bt_1 loci were

found to be in the long arm of chromosome V while only A_2 and Bm_1 were situated in the short arm. Of especial interest is the placing of Bm_1 and Bt_1 on opposite sides of the spindle fiber insertion region as these two loci show little crossing over (BURNHAM and RHOADES in EMERSON et al 1935). A_2 is only some 6-7 crossover units from Bt_1 , which is in the long arm, and it occupies, at present, the leftmost position of all the factors in the genetic map of chromosome V. If the magnitude of crossing over per unit of physical length is the same in the two arms of the chromosome, and we have no reason to believe otherwise, the length of the genetic map for

TABLE I

$$\text{Ratio of } A_2:a_2 \text{ seed from backcross of } \frac{A_2}{A_2} \times \frac{a_2}{a_2}$$

PEDIGREE OF FRAGMENT PLANT	A_2 SEEDS	a_2 SEEDS	PERCENT RECESSIVES
2524 6	187	87	31.8
2524 14	166	66	28.4
2525 9	206	118	36.4
2525 6	228	130	36.3
2525 3	59	25	29.8
2524 25	174	77	30.7
2525 2	117	66	36.1
2525 8	92	44	32.4
2524 29	97	47	32.6
3117 2	195	111	36.3
Total	1521	771	Aver. % = 33.6

chromosome V is due for a considerable extension as new genes are located in the short arm to the left of A_2 . Three-point backcross data for Bm_1-Pr-V_2 are available. Bm_1 is so close to the insertion region that we can consider the amount of crossing over between Bm_1-Pr essentially equal to that between the spindle fiber and Pr . Let us calculate, as best we can, the approximate length of the genetic map for the long arm of chromosome V. The three-point backcross data give the following recombination values: $Bm_1-Pr = 22.3$ percent, $Pr-V_2 = 43.4$ percent. According to HALDANE'S (1919) table for estimating the map distance from recombination percentages the map distance for the Bm_1-Pr interval is 24.5 units and that for the $Pr-V_2$ interval is 60.8 units. The sum 85.3 units gives an estimate of the map distance from the insertion region to the V_2 locus but we do not know how far removed V_2 is from the end of the long arm. Since the two arms of chromosome V are nearly equal it appears that the length of the genetic map for this chromosome will exceed 170 map units.

In the course of the studies with the fragment several plants were obtained which carried either recessive a_2 or bm_1 in the two normal chromosomes V and the dominant allele in the fragment chromosome. Considering the bm_1 locus these plants were of the following constitution: $bm_1Pr/bm_1pr/Bm_1$ and for the a_2 locus had $a_2pr/a_2pr/A_2$ constitution. When a plant carrying the bm_1 gene was used in reciprocal crosses with a recessive individual the following results were obtained:

From the above data where the fragment was used as the pollen parent it is evident that even though the fragment plant was Bm_1 in phenotypic appearance it bred true for bm_1 excepting those cases where fragment pollen functioned or where a crossover had occurred which transferred the Bm_1 allele to a normal chromosome.

The following calculations can be made from the data in table 2. (1) The percentage of functioning eggs carrying the fragment chromosome was 33.

TABLE 2.

FRAGMENT PLANT AS FEMALE	
2N PROGENY	2N+FRAGMENT PROGENY
<i>Pr bm₁</i> —62	<i>Pr Bm₁</i> —31
<i>Pr Bm₁</i> —1	<i>pr Bm₁</i> —35
<i>pr bm₁</i> —64	<i>wh Bm₁</i> —19
<i>wh. bm₁</i> —45	
FRAGMENT PLANT AS MALE	
2N PROGENY	2N+FRAGMENT PROGENY
<i>Pr bm₁</i> —146	<i>Pr Bm₁</i> —5
<i>Pr Bm₁</i> —1	<i>pr Bm₁</i> —1
<i>pr bm₁</i> —109	<i>wh Bm₁</i> —2
<i>pr Bm₁</i> —1	
<i>wh. bm₁</i> —123	

The above crosses were segregating for colored and colorless aleurone. The colorless (wh.) class was included because of the bm_1 data.

- (2) The frequency of crossing over between the fragment chromosome and one of the normal chromosomes in the Bm_1 -insertion region was .5 percent.
 (3) Two percent of the functioning pollen from the fragment plant was hyperploid for the fragment chromosome. Incidentally the transference of the Bm_1 gene from the fragment chromosome to one of the normal chromo-

somes could be used as cytological proof of genetic crossing over if such evidence were needed after the conclusive work of STERN (1931) and CREIGHTON and MCCLINTOCK (1931). Obviously a more critical proof would be the transference of the recessive bm_2 allele from a normal chromosome to the fragment chromosome. Such crossovers have occurred in other progenies.

More extensive data from reciprocal crosses similar to the above were obtained for the A_2 locus. The percentage of fragment eggs was 33. The percentage of functioning fragment pollen was 1.2 and the amount of crossing over, based on a thousand individuals, between the fragment chromosome and the normal chromosomes in the A_2 -insertion region interval was six percent. JENKIN'S (1934) data places A_2 and Bt_1 7 units apart. Therefore the map distance from A_2 to the insertion region is not more than 7 units. The frequency of crossing over between the fragment and the normal chromosome in the A_2 -insertion region interval was six percent. It would seem then that the frequency of crossing over in this interval was the same in disomes as in fragment plants. But the fragment is associated with the normal chromosome in approximately 50 percent of the microsporocytes. Therefore the cross over value of six units should be doubled. This indicates that crossing over for this interval, when the fragment is associated with the two normal chromosomes, occurs considerably more frequently in fragment plants than in diploids.

The results obtained from this study of the fragment chromosome agree with previously known facts concerning the order of genes in this chromosome. MCCLINTOCK (1932) placed the Bm_1 locus in the short arm near the insertion region from her work with ring-shaped fragments. Moreover, cytogenetic studies with a reciprocal translocation (RHOADES 1933c) indicated that Bt_1 and Bm_1 were both near the insertion region of chromosome V. STADLER (1935) obtained a deficiency for the V_3 locus. This deficiency occurred in the long arm of the chromosome. In addition to substantiating the above, the fragment studied places the two closely linked genes Bt_1-Bm_1 (1-2 units apart) on opposite sides of the insertion region. Moreover it definitely places six loci in the long arm and two in the short arm of chromosome V.

When plants of $bm_1Pr/Bm_1pr/Bm_1$ constitution were used as the pollen parent in a backcross the following data were obtained: 515 $Pr\ bm_1$:568 $pr\ Bm_1$:383 $Pr\ bm_1$:386 $pr\ bm_1$. These data give a recombination value of 41.5 percent for the $Pr-Bm_1$ interval. Diploid sister plants had a cross-over value of 22.3 percent for the same region. This large and highly significant increase in the amount of crossing over in the long arm of chromosome V in fragment plants is inexplicable, as somewhat comparable data from *Drosophila* showed no increase in crossing over in that arm which

was not represented in the duplicating fragment (RHOADES 1931). Unfortunately only the *Pr*-insertion region interval was involved in the present studies. More data on this problem will be obtained later.

SUMMARY

1. A plant which was intermediate in appearance between the trisomic and disomic individuals occurred in the progeny of a plant trisomic for chromosome V. This exceptional plant carried in addition to the normal complement of 20 chromosomes a fragment of chromosome V. The fragment chromosome consisted of the entire short arm of chromosome V with a terminal spindle fiber insertion region.

2. The cytological behavior of this fragment chromosome was studied at pachytene and metaphase in microsporocytes. The fragment chromosome was associated with the two normal chromosomes in approximately one-half the cells. Segregation of the three members of the trivalent groups was not random, but in such a manner that the fragment chromosome usually accompanied one of the normals when it passed to a pole.

3. The spindle fiber insertion region of the fragment chromosome was observed to be split or double in those cells where it was lying free in the nucleus as a univalent. The equational split into two chromatids was clearly evident.

4. The V_2 *Ys* *Pr* V_{12} V_3 and Bt_1 loci were located in the long arm of chromosome V. The Bm_1 and A_2 loci were located in the short arm of the chromosome.

5. Pollen hyperploid for the fragment chromosome occasionally functioned in competition with haploid pollen.

6. Crossing over in certain regions was apparently more frequent in fragment plants than in diploids.

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