the chloroform extracts, and, therefore, a third less stable substance may have been present.

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[12] This medium contained per liter 1.5 g. KH_2PO_4, 0.5 g. $MgSO_4 \cdot 7H_2O$, 50 g. dextrose, 2 g. neopeptone, 1500 mμ moles of thiamine, and a mixture of minor mineral elements as used in this laboratory.

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*CHROMOSOME SEGREGATION IN MAIZE TRANSLOCATIONS IN RELATION TO CROSSING OVER IN INTERSTITIAL SEGMENTS**

BY C. R. BURNHAM†

CALIFORNIA INSTITUTE OF TECHNOLOGY AND THE UNIVERSITY OF MINNESOTA‡

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In maize, the second meiotic division in the microsporocytes results in a quartet of four microspores in which it is possible to distinguish the two division planes. In normal material, the prominent feature of these spores, as revealed by acetocarmine smears, is the single nucleolus. In translocations involving chromosome 6 which carries a nucleolar organizer region, non-disjunction may result in spores with two nucleolar organizers (potentially two nucleoli), or with no organizer (nucleolar material remains scattered or diffuse) in the same quartet. By this method, McClintock[4] established the fact that the chromosomes which crossover in an interstitial segment (between the centromere and the translocation break) pass to opposite poles.

A further relation between chromosome segregation in such maize translocations and the frequency of crossing over in an interstitial segment has been reported in abstracts.[1, 2] This is a report of additional studies of

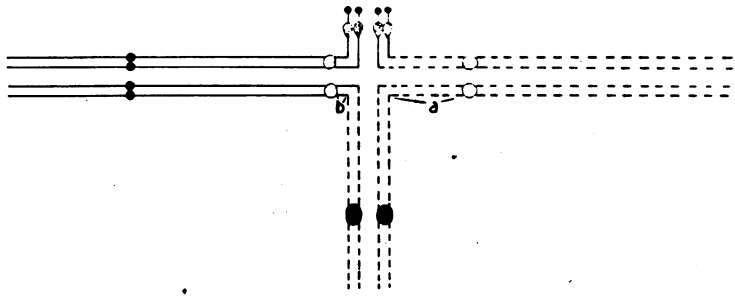

FIGURE 1

Pachytene diagram of a 5-6 translocation heterozygote with the break in chromosome 6 (solid line) in the short arm, that in 5 (dotted line) in the long arm, *b* and *a* being the interstitial segments in the respective chromosomes. The satellite and next to it the nucleolar organizer region (stippled) are shown at the end of the short arm of chromosome 6. The clear circles represent the centromeres, the dense ones the pycnotic knobs.

TABLE 1

SPORE QUARTET TYPES FROM A TRANSLOCATION HETEROZYGOTE WITH THE BREAK IN THE SHORT ARM OF CHROMOMOSOME 6

The figures 0, 1, 2 in the body of the table refer to the quartets containing no, one, and two spores with diffuse nucleolar material. The "no diffuse" may have four normal or four abortive spores, the latter indicated as (ab) in the table; the "two diffuse" have four abortive, and the "one diffuse" has two normal and two abortive, except a few cases where all four abort, indicated by (ab).

EVENTS IN INTERSTITIAL SEGMENTS	ORGANIZER MAKE-UP OF CHROMATID PAIR	SPORE QUARTET TYPES AFTER SEGREGATIONS OF ALTERNATE	ADJACENT 1	ADJACENT 2
No crossover in *a* or *b*	.. / .. / -- / --	0	2	0 (ab)
Single crossover in *a* or *b*	.. / .-* / -- / .-	1	1	0 (ab), 1 (ab)
Single crossovers in *a* and *b*	.- / .-* / .- / .-	0, 2, 1†	0, 2, 1†	0 (ab), 2, 1†
Double crossover in *a* or *b*				
2-strand	.. / .. / -- / --	0	2	0 (ab)
3-strand	.. / .- / -- / .-	1	1	0 (ab), 1 (ab)
4-strand	.. / -- / -- / ..	2	0	0 (ab)

* Crossover chromatids, see note at end of paper. . = organizer, - = its absence.

† Ratio of 1:1:2 in each. Here in the two-diffuse type the two diffuse spores are on opposite sides of the division I plane, and in the one-diffuse type the two abnormal spores are on the same side. In the other lines of the table, the two diffuse spores are on the same side and in the one-diffuse quartet the two abnormal ones are on opposite sides of the division I plane.

chromosome segregation in maize translocations involving chromosome 6, the detailed data to be published elsewhere. The ultimate objective is to determine why chromosome segregation is directed (i.e., largely alternate) in certain species and not in others.

The results differ, depending on whether the break in chromosome 6 is in the long arm or in the short arm between the centromere and the nucleolar organizer. The theoretical results for a translocation with the break in 6 in the short arm will be considered first, followed by the experimental data.

The pachytene configuration in a translocation heterozygote with one break in the short arm of 6 between the centromere and the nucleolar organizer is shown in Fig. 1. Alternate or adjacent chromosomes in this complex may pass to the same pole, the latter being of two types—adjacent 1, in which homologous centromeres pass to opposite poles at division I, and adjacent 2 in which they pass to the same pole. When no crossing over has occurred in the interstitial segment, adjacent 1 brings about non-disjunction of the translocated pieces including the nucleolar organizers; adjacent 2 brings about non-disjunction of the non-translocated pieces. Unless stated otherwise, the crossovers referred to in this paper are the cytological ones occurring in interstitial segments. Crossovers in other segments do not change the quartet types or the pollen abortion.

The theoretically expected spore quartet types with and without crossing over in the interstitial segments are listed in table 1. The second column of this table shows the nucleolar organizer constitution of the chromatid pairs (sister centromeres) after crossing over, i.e., before orientation. Reference to this column should facilitate determining the quartet type resulting from each kind of segregation. With no crossing-over, alternate as well as adjacent 2 segregation results in a "no diffuse" spore quartet, the former with four normal or functional spores, the latter with four abortive ones, the two being indistinguishable cytologically, while adjacent 1 segregation results in the "two-diffuse" type in which the four spores are expected to be abortive (two spores have potentially two nucleoli, two have diffuse nucleolar material). A single crossover or a 3-strand double followed either by alternate or by adjacent 1 segregation results in the "one diffuse," also termed a "crossover-type" quartet, expected to have two functional (one nucleolus each) and two abortive (one with potentially two nucleoli, one with diffuse nucleolar material) spores. Adjacent 2 segregation following these crossovers is not expected since the evidence indicates that chromosomes that cross over pass to opposite poles. If it did occur, a "no-diffuse" and a "one-diffuse" type of spore quartet would be expected, but the two abnormal spores in the latter are on the same side of the first division plane. The absence of this class in a translocation with only one interstitial segment furnished the evidence that chromosomes that

cross over pass to opposite poles.[4] Simultaneous crossing over in both interstitial segments gives rise to the three quartet types following each of the three kinds of segregation (table 1). Consequently, to determine segregation types by this method it is necessary to use translocations in which these simultaneous crossovers are infrequent, i.e., at least one segment genetically short, or if both are long, to have a genetic measure of the amount of simultaneous crossing over.

The three available translocations in which one break was in the short arm of chromosome 6, were: T2-6a (2L.4?, 6S?), T6-10b (6S.5, 10L.58), and T5-6c (5L.89, 6S0.0). In T5-6c the break in 6 was adjacent to the centromere, so that there is no interstitial segment in chromosome 6, while in the other two translocations it is not long enough to give much crossing

TABLE 2

SUMMARY OF SPORE QUARTET AND POLLEN STERILITY DATA AND FREQUENCIES OF CHROMOSOME SEGREGATION TYPES IN PLANTS HETEROZYGOUS FOR TRANSLOCATIONS HAVING ONE BREAK IN THE SHORT ARM OF CHROMOSOME 6; THE FIRST THREE WITH SHORT INTERSTITIAL SEGMENTS, THE LAST WITH ONE VERY LONG.

	QUARTETS				POLLEN ABORTION %			CHROMOSOME SEGREGATION IN NON CROSSOVER		
		%			OBSERVED					
TRANSLOCATION HETEROZYGOTE	TOTAL	NO DIFFUSE	TWO DIFFUSE	ONE DIFFUSE	TOTAL	PARTLY FILLED	PREDICTED	QUARTETS ALTERNATE	ADJACENT 1	ADJACENT 2
T5-6c In5a / + In5a	7189	61.7	27.8	10.6	50.5	13.1	33.0	49.4	31.0	19.6
T2-6a / +	2361	73.6	25.3	1.1	51.6	..	25.7	48.4	26.2	25.5
T6-10b / +	2374	76.6	18.1	5.3	42.9	..	20.7	58.0	22.4	19.7
T5-6c / +	4229	20.3	17.9	62.8	48.1	22.1	49.3	..*	..*	0.0

* These classes cannot be determined.

over since this arm of 6 is very short. The break in T2-6a is in the short arm but the exact point was not determined. The interstitial segment in the other chromosome of these translocations differed in length. An inversion in chromosome 5 (In5a-S0.0-L.67) served to shift the position of the centromere in chromosome 5. By crossing a stock homozygous for T5-6c and In5a with one homozygous only for In5a, plants were produced which were homozygous for In5a and heterozygous for T5-6c. In these plants, due to the shift in centromere position, the interstitial segment in chromosome 5 is short (a length equal to 0.22 of the long arm). In those heterozygous for T5-6c and not carrying the inversion that segment is long (0.89 of the long arm). In both T5-6c/+ stocks the relative lengths of the two axes and the lengths of the translocated pieces remain the same.

The frequencies of the different spore quartet types were determined

cytologically (in all but the early studies, without reference to division planes) and from them the expected degree of pollen abortion was calculated. The amount by which the observed pollen abortion exceeded the calculated was used as a measure of the frequency of adjacent 2 segregation. The data for each translocation are based on at least two plants and two slides for each, each slide made from one anther from different florets. The counts on the pollen to determine the percentages of abortion were made on the same plants. The spore quartet and pollen abortion data and the calculated segregation frequencies for these translocations are summarized in table 2. Comparison shows the first three stocks have 20 to 26% of adjacent 2 segregation while the last one, T5-6c/+, has none. In the latter, the observed pollen abortion was actually higher than that predicted from the cytological count of the spore quartets. In the first three, the percentages of crossover-type quartets were low, 10.6, 5.3 and 1.1, respectively, while in the latter it was high, 62.8%. This indicates a close relation between the length of the interstitial segment, in terms of cytological crossing-over, and chromosome segregation, i.e., low crossover is accompanied by frequent adjacent 2, high crossover is accompanied by little or no adjacent 2 segregation. In the stock with 62.8% of crossover-type quartets, there was probably a considerable amount of double crossing-over and some higher multiples. If as much as 15% doubles occurred, practically all the tetrads would have had one or more crossovers in the interstitial segment, using Weinstein's[6,7] and Sax's[5] method of calculation. The absence of adjacent 2 segregation is then expected since chromosomes that cross over pass to opposite poles. Adjacent 2 segregation could occur only in those with no such crossovers. A comparison of the results for the two T5-6c/+ stocks is especially convincing since in these the lengths of the translocated pieces remained the same, the difference being in the length of the interstitial segment. The differences in segregation, 20% of adjacent 2 in one, none in the other, were achieved in spite of very unequal axes lengths and no change in the lengths of axes or of the translocated pieces. In an earlier report[1] the differences were supposed to be the result of a centromere effect. It now seems probable that they are the result of the relation between crossing-over in the interstitial segment and disjunction, centromere position being incidental in determining the length of that segment, but still important in that it may initiate the separation of the crossover chromosomes. Although probable in corn, there is no proof in this study that alternate and adjacent 1 segregations following these crossovers are equally frequent, hence the frequencies of alternate and adjacent 1 segregations are not calculated for T5-6c/+ in table 2.

In those translocations with a low frequency of crossover-type quartets, the two-diffuse class represents the frequency of adjacent 1 segregation

since double crossovers probably were lacking. The frequency of quartets from alternate segregation is the difference between the total of the no-diffuse class and those from adjacent 2. From the segregation frequencies calculated on this basis, for the first three stocks in table 2 it will be noted that alternate segregation is roughly 50%, adjacent 1 and 2 segregations making up the other 50%. In other words, adjacent 2 segregation is probably at the expense of adjacent 1.

Translocations with one break in the long arm of chromosome 6 were also studied. In these, only two quartet types can be recognized cytologically, no-diffuse and two-diffuse, the latter following adjacent 2 segregation with or without crossing-over in the interstitial segments. Here when there is little crossing-over, the difference between observed and predicted pollen abortion measures the frequency of adjacent 1 segregation. Segregation of three chromosomes of the ring to one pole and one to the other, resulting in $n + 1$ and $n - 1$ spores, also are expected (part of them) to result in the two-diffuse spore quartet, offering some difficulty. Usually its total frequency is not expected to be higher than about 5%. For ten such translocations with short interstitial segments, adjacent 2 segregation ranged from 14 to 36%, the average for the entire group being 25%. Where counts could be made, pollen abortion was about 50% in this group, this representing the total of the two types of adjacents. For eight translocations in which either or both interstitial segments were probably long enough for a high frequency of crossing-over (breaks at 0.6 or greater in the longer chromosomes and 0.7 or greater in the shorter ones), the average was 5.4% of adjacent 2 segregation. One, T6-8a with 17.4%, appears to be an exception. However, another T6-8, (D1), with breaks at about the same points had only 1.1% of adjacent 2. Some unknown factor seems to have been operating in T6-8a. With this one exception, the relationship appears to be that when the interstitial segments are short, both types of adjacents occur with relatively high frequencies, not always equal but their total is roughly 50%. When one or both interstitial segments are long, adjacent 2 segregation is low in frequency.

Chromosome segregation was studied also in plants heterozygous for translocations having one break in the satellite of chromosome 6 and which, therefore, always form a chain of four chromosomes. Two were studied which have short interstitial segments, T1-6b and T5-6b, and one, T3-6b, with one long interstitial segment. In the first two there was less than 1% of adjacent 2 segregation, and in the latter none. This result, therefore, differs from that for the ring-forming translocations since in them, those with short interstitial segments had a relatively high frequency of adjacent 2 segregation. In T5-6c/+ which has one long interstitial segment, 30.6% of chains were observed at diakinesis, and no measurable adjacent 2 segregation. The evidence from these translocation hetero-

zygotes indicates that adjacent 2 segregation from chains is infrequent whether the amount of crossing-over in the interstitial segment is low or high. Adjacent 2 segregation, therefore, occurs rarely except in rings in which no crossing-over has occurred in the interstitial segments.

Discussion and Summary.—In maize translocation heterozygotes which form rings and have short interstitial segments (low crossover frequency), alternate segregation occurs in about 50% of the meiocytes. In them, both types of adjacent segregations occur, not always with equal frequency but the total is roughly 50%. In those with interstitial segments long enough to permit a high crossover frequency there is little or no adjacent 2 segregation. The segregation in rings differs greatly from that in chains. In chains, there is little or no adjacent 2 segregation whether the interstitial segment is long or short (in terms of crossing-over).

There is, therefore, no evidence of any directed segregation in the maize translocations studied, even with greatly different break positions. This, together with Catcheside's observations[3] that segregation is directed even in unequal translocations produced by x-raying an *Oenothera* race with seven pairs suggest that directed segregation may be genetically controlled. Even in a species homozygous for such a genotype, translocations with long interstitial segments should have considerable sterility as a result of crossing-over in those segments, reaching 50% as a possible maximum. According to the literature, the translocations that have survived in *Oenothera* are those with short interstitial segments. These, therefore, would have low sterility from that source. A search for genes in maize affecting segregation is being made by crossing stocks of widely different origin with translocations having short interstitial segments.

Acknowledgment.—I wish to express my gratitude to Dr. Barbara McClintock who originally suggested the problem and furnished the stocks of T5-6c, T6-10b, In5a, and the stock combining T5-6c with In5a. Throughout the course of the experiments, assistance has been furnished from research funds of the University of Minnesota graduate school, for which I am deeply grateful. A sabbatical leave, together with a Gosney Research Fellowship at the California Institute of Technology (1947–1948), made its completion possible. I am indebted to Dr. E. G. Anderson for his generous coöperation. I wish to acknowledge the assistance of Gertrud Joachim, C. H. Li and H. R. Highkin in gathering the pollen sterility and spore quartet data.

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† Gosney Fellow, California Institute of Technology.

‡ Permanent address.

NOTE: Lamm, *Hereditas*, **34**, 280–288 (1948) (Dr. H. H. Kramer called my attention to this paper as mine was being completed), and Kramer and Hanson (*Genetics*, in press) have pointed out that chromatids resulting from single crossovers in the interstitial

segment are recovered only when adjacent 1 segregation occurs, and are lost following alternate segregation. This may be seen by referring to column 2 of table 1 in which the crossover chromatids are marked with an asterisk. The results of multiple crossovers are not indicated. In species with more alternate than adjacent 1 segregation, genetic crossing-over in this region will be reduced. My conclusions in this paper are based on frequencies of cytological crossing-over, and consequently are not altered by these relations.

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EARLY TERTIARY ECOTONES IN WESTERN NORTH AMERICA

BY RALPH W. CHANEY

DEPARTMENT OF PALEONTOLOGY, UNIVERSITY OF CALIFORNIA, AND CARNEGIE INSTITUTION OF WASHINGTON

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Within recent years we have come to realize that the concept of cosmopolitan land floras during past ages is not substantiated by the fossil record of the Tertiary period. Similar floras may be recognized over a wide range in latitude, but differences in their age are always apparent. We now interpret their shifting positions during later geologic time as resulting from migrations largely induced by changes in climate. Varying distance from the equator seems always to have involved sufficiently great differences in temperature to leave its impress on land vegetation. During periods of submergence, when there was wide circulation of winds and ocean currents, more uniform climates appear to have characterized broad areas of the earth; but even at such times the fossil record shows marked differences in forest composition from south to north.

Major units of living vegetation, although largely confined to latitudinal zones, show at their borders a tendency to merge into one another. We may draw forest boundaries on a map with a firm hand, but in nature such lines are commonly blurred. A similar overlapping of floras may be observed in the records of past vegetation. Transitional occurrences of this sort, past and present, are known as ecotones; since they are largely a function of temperature they extend along the parallels, bending southward along mountain ranges in the northern hemisphere, and north on windward shores. Ecotones provide a critical basis for determining the