THE RELATIONSHIP OF CROSSOVER FREQUENCY TO SYNAPTIC EXTENT AT PACHYTENE IN MAIZE¹

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THE early meiotic prophase and preceding interphase of eukaryotic organisms are stages of the most intense interest to geneticists but also, unfortunately, among the most unyielding to the techniques of chromosome cytologists. Conflicting reports in the literature on the orientation and structure of the chromosome material emphasize the present difficulties of direct observation with either light or electron microscope. While we know on a gross level that chromosomes generally must pair homologously and that replication and crossing over must occur within these stages, it is not known whether chromosomes tend to (or must) complete observable synapsis before crossing over occurs or whether it can immediately follow the homologous meeting of short segments, whether crossing over accompanies or follows replication, or whether both are true, but occur by differing mechanisms.

This study is based on quantitative observations of chromosomes at the earliest meiotic stage in maize during which chromosomes can be accurately traced (pachytene). It is designed to permit inferences on chromosome behavior prior to this stage.

MATERIALS AND METHODS

Over a number of years the cytogenetics of maize backcross progenies of maize-Tripsacum hybrids has been studied. Particular attention has been paid to a derivative stock containing a pair of maize-Tripsacum interchange chromosomes in addition to a normal chromosome 2 (parent constitution of Figure 1). The exchange point was estimated cytologically to have been located at about 25 percent of the total length of chromosome 2 from the end of the short arm (MAGUIRE 1960a) and genetically at about 54 crossover units from the same end of chromosome 2 (MAGUIRE 1964). The interchange chromosomes in this case have exchanged parts partially homologous in that the knob-bearing Tripsacum segment carries fully dominant alleles for three genetic markers found in the corresponding region of the short arm of chromosome 2 of maize, white sheath (*ws*), liguleless (*lg*) and glossy seedling (*gl*) (MAGUIRE 1962a); normal appearing synapsis is usually found at pachytene in disomic plants carrying a 2^T chromosome and a normal chromosome 2, and plants homozygous 2^T2^T are viable although somewhat reduced in vigor (MAGUIRE 1961a). The exchanged segments are considered to correspond in homology only partially, however, since two maize segments for this region pair preferentially in trivalent configuration with almost total exclusion of the Tripsacum segment in those plants where the choice is available (MAGUIRE 1957). Analysis of chromosome distribution patterns at pachytene in plants carrying two 2^T chromosomes and a T² chromosome (where the T² chromosome appears as a univalent) left doubt of the existence of a significant affinity between the corresponding Tripsacum and maize

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segments (MAGUIRE 1961b). Crossing over or secondary exchange between the corresponding maize and *Tripsacum* segments occurs only rarely (MAGUIRE 1960b), but derivatives of such exchange have been found and crossed to give 21-chromosome plants with an array of constitutions differing in duplication or triplication of corresponding chromosome regions, extent and position of segments of the *Tripsacum* chromosome in maize chromosome 2, and extent of segments of maize chromosome 2 in the T^2 chromosome. Chromosome pairing relationships at pachytene and metaphase I were studied and compared in these constitutions. A maize inbred noted for excellence of pachytene configurations (L289) formed the major genetic background of the stocks used (WELLWOOD and RANDOLPH 1957).

Microsporocyte samples were fixed in alcohol-acetic 3:1 mixture and stored under refrigeration until studied in acetocarmine smear preparations. For frequency estimations, slides were systematically scanned under low power, where those cells whose chromosomes appeared sufficiently well spread were selected for study under oil immersion. Only very occasional metaphase cells were rejected as unanalyzable either under low power or oil immersion, but many pachytene cells were clearly unsuited for further study when viewed under low power magnification (because of poor spreading of chromosomes). It is thought probable that no important bias in estimation of trivalent frequencies was introduced by the rejection of these cells although the possibility of error introduced by this technique should be kept in mind. At best in pachytene preparations it was necessary to reject as unanalyzable 10 percent of those cells which were studied under oil immersion. The possible effect of such rejections will be discussed later. Since classification of nonrejected cells was clear and easily accomplished, errors here are considered unlikely. All observations were made by the author. All available plants of each of the new constitutions were studied. Variability within and between plants of like constitutions will be discussed later. Measurements of lengths of pachytene chromosome regions were made from camera lucida drawings traced under uniform conditions. Relative extents of synopsis at pachytene in trivalent configurations where no homologous segments were triplicated were taken as the best indication for extent of homology. Since it is recognized that these measurements are subject to errors introduced by the smear technique and that there may be some haphazard pairing failure of homologous regions as well as some nonhomologous or homeologous pairing, estimations of the cytological location of the exchanges are based on averages of a number of measurements and, where feasible, on such pairing behavior in more than one type of constitution.

It should be pointed out that trivalent formation in this material depends directly on the pairing of the T^2 chromosome (or its derivatives), since any combination of normal chromosome 2 and $2T$ chromosomes (or derivatives) virtually always pair with each other and form one or more chiasmata in the long arm and proximal short-arm regions.

RESULTS

Secondary exchanges between maize and Tripsacum segments: So far, one product of each of three secondary exchanges has been recovered for further crosses. For convenience these are referred to as I, II and III in the description which follows. The probable origin of each of the three exchanges is diagrammed in Figure 1.

Exchange I was detected cytologically in a 21-chromosome plant in which the terminal knob from *Tripsacum* was located on the T^2 chromosome. The reciprocal recombinant was not found. No genetical tests of the position of the exchange are yet available. On the basis of measurements in pachytene trivalent configurations of plants carrying this T^2 chromosome and two normal chromosomes 2 it is estimated that this secondary exchange occurred between the T^2 chromosome and normal chromosome 2 at a point approximately 11 percent of the total cytological length of chromosome 2 from the end of its short arm. Note that the

FIGURE 1.—Diagrammatic representation of the probable course of three secondary exchanges (involving maize and *Tripsacum* chromosomes) and the products recovered. Maize chromosome 2 is represented by a solid line, the *Tripsacum* chromosome by horizontal and vertical dashed lines. The different symbols for different parts of the *Tripsacum* chromosome serve only to distinguish the parts separated by the original interchange. Open circles indicate the approximate locations of centromeres.

FIGURE 2.—Diagrammatic representation of the chromosomal constitutions of the various types so far derived by combining the available primary and secondary maize-Tripsacum interchange chromosomes and normal chromosome 2. The various chromosomal regions are represented in the same way as in Figure 1. Roman numerals designate recovered derivatives of exchanges I, II and III of Figure 1.

shorter knob-bearing *Tripsacum* segment of an exchange-I type chromosome can be shifted to a standard chromosome 2 by normal crossing over between homologous maize segments.

Exchange II was detected genetically among the test-cross progeny of a disomic plant which was heterozygous for the knob-bearing *Tripsacum* segment substitution, with dominant *Ws Lg Gl* in the *Tripsacum* segment and recessive *ws lg gl* in maize chromosome 2. The secondary exchange chromosome recovered carried the terminal knob and dominant *Ws* and *Lg* alleles from *Tripsacum* and recessive *gl* from maize chromosome 2. An exchange was therefore thought to have been located between *lg* (at 11) and *gl* (at 30) on the genetic map of chromosome 2. When this secondary exchange chromosome was combined with the original maize-*Tripsacum* interchange chromosomes (constitution illustrated in Figure 2e), and measurements of pachytene trivalent configurations were made, it was estimated that this secondary exchange occurred at a point about 12 percent of the total length of chromosome 2 from the end of its short arm. Thus exchanges I and II appear to have occurred at very nearly the same point (or conceivably at precisely the same point). Note that the short knob bearing *Tripsacum* segment in the exchange-II type chromosome can also be shifted to the T² chromosome by normal crossing over. Such a crossover product has been found and studied in the constitutions illustrated in Figure 2g and h. Plants of constitutions 2h and 2i differed in that the smallest chromosome of Figure 2h carried a type-II exchange while that of Figure 2i carried a type-I exchange, but their pairing relationships were found to be remarkably similar (as described later). This is considered further evidence of the similarity of the location of exchanges I and II.

Exchange III was detected genetically among the progeny of a plant of the parent constitution illustrated in Figure 1-III. Phenotypically the plant in which exchange III was found was *ws lg Gl* and contained 21 chromosomes of the usual relative lengths but lacked the terminal knob of the *Tripsacum* segment. Since the *Tripsacum* segment normally carries *Ws Lg Gl* alleles and was the only source of these dominants in this line, *Ws* and *Lg* had apparently been lost by exchange. However, the pairing relationships (to be described later) of the derived chromosome are most easily explained if it is supposed that the secondary exchange which produced it involved nonhomologous regions. It has been noted in the past (MAGUIRE 1960a) that in 21-chromosome plants carrying the original interchange chromosomes (Figure 2a) the two segments derived from *Tripsacum* may remain univalent at pachytene or may pair nonhomologously. Any single exchange between these two segments which involved the *Tripsacum* segment of the long arm of T² would yield a dicentric 2^T chromatid lacking a substantial region of the short arm of chromosome 2 and an acentric T² chromatid. The acentric would be lost at anaphase and the dicentric would be expected to form a bridge which on breaking, might yield new types of T² chromosome. These would probably be viable in some gametes because the missing chromosome-2 material would be present in another chromosome. Although no such altered T² chromosomes have been recovered, bridges and fragments are occasionally found at anaphase in sporocytes of some 21-chromosome plants carrying the original maize-*Tripsacum* interchange chromosomes and a normal maize chromosome 2. Non-

homologous exchanges, however, between the knob bearing *Tripsacum* segment and the short arm of the T^2 chromosome would be expected to yield new mono-centric T^2 and Q^T chromosomes. The viability of the new 2^T chromosome, in the absence of additional chromosome 2 material for the terminal region, would depend upon the extent and importance of the missing segment. The postulated derivation of the type-III exchange is diagrammed in Figure 1-III.

Only a small number of measurements of synaptic extent in pachytene trivalent configurations involving the exchange-III chromosome are available. An estimate based on these measurements would place the exchange point at approximately 15 percent of the total length of chromosome 2 from the end of the short arm, but it is emphasized that this is a comparatively unreliable estimate.

Pairing behavior in derived and original constitutions: By intercrossing plants carrying the various chromosomal arrangements the constitutions illustrated in Figure 2 have been constructed. Each of these types has been studied intensively

TABLE 1

*Data on pairing relationships of plants of the various constitutions
(correspondingly designated in Figure 2)*

Consti- tution	Plant No.	Metaphase trivalent frequency				Pachytene trivalent frequency					Pachytene pairing		
		No. of cells	%	Average (%)	SD	No. of cells	%	Percent unclas- sified	Aver- age (%)	SD	No. of cells	% chr. 2 and/ or 2^T paired	Origin of pairing (% from end)
a*	.		..	90	5.6	59/60	98	56	..	..	115	20	..
b*	.		..	70	9.1	133/205	65	64	..	..	54	15	..
c	1	91/100	91	..	..	68/70	97	31	..	..	..	..	..
	2	153/167	92	..	..	212/213	100	36	..	..	..	..	..
	3	101/104	97	..	..	101/102	99	22	..	..	..	..	..
	.		..	93	3.2		..	..	99	1.0	24	20	..
d	1	6/101	6	..	..	0/14	0	33	..	..	..	..	..
	2	15/105	14	..	..		..	..	..	..	..	..	..
	3	16/100	16	..	..		..	..	..	..	..	..	..
	4	20/100	20	..	..		..	..	..	..	..	..	..
	5	20/100	20	..	..		..	..	..	..	..	..	..
	6	57/367	16	..	..	104/883	12	16	..	..	10	15	11
	7	26/163	16	..	..	17/61	28	33	..	..	..	..	..
	8	10/105	10	..	..	42/183	23	49	..	..	..	..	..
	9	20/161	12	..	..	59/214	27	26	..	..	..	..	..
	10	52/314	17	..	..	11/76	14	53	..	..	..	..	..
.	.		..	15	4.4		..	..	17	10.8	..	..	..
e	1	96/221	43	..	..	114/245	47	12	..	..	14	12	13
	2	202/360	56	..	..	77/126	61	29	..	..	9	10	11
	3	49/100	49	..	..		..	..	..	..	..	..	..
	4	90/206	44	..	..	63/153	41	34	..	..	..	..	..
	5	50/131	39	..	..		..	..	..	..	..	..	..
	6	64/126	51	..	..		..	..	..	..	..	..	..
	7	46/90	51	..	..		..	..	..	..	..	..	..
.	.		..	48	5.8		..	..	50	10.1	..	..	..
f	1	61/368	17	..	..	54/307	18	20	..	..	5	11	16

TABLE 1--Continued

Consti- tution	Plant No.	Metaphase trivalent frequency				Pachytene trivalent frequency				Pachytene pairing			
		No. of cells	%	Average (%)	sd	No. of cells	%	Percent unclas- sified	Aver- age (%)	sd	No. of cells	% chr. 2 and/ or 2 ⁺ paired with T ²	Origin of pairing (% from end)
g	1	67/100	67	..	..	34/57	60	68	..	..	3	23	..
h	1	71/100	71	..	..	7/11	64	54	..	..	9	16	..
i	1	60/100	60	..	..	39/59	66	52	..	..	5	20	..
	2	154/206	75	..	..	..	..	..	..	..	..	..	..
	3	79/97	81	..	..	..	..	..	..	..	..	..	..
j	..	..	..	72	11.3	..	..	..	..	..	..	..	..
	1	99/100	99	..	..	..	..	..	..	..	..	..	..
	2	97/100	97	..	..	..	..	..	..	..	..	..	..
	3	97/100	97	..	..	..	..	..	..	..	14	23	..
	4	95/100	95	..	..	..	..	..	..	..	..	..	..
	5	95/100	95	..	..	18/18	100	64	..	..	..	..	..
	6	88/100	88	..	..	..	..	..	..	..	..	..	..
	7	89/100	89	..	..	..	..	..	..	..	..	..	..
	8	97/100	97	..	..	..	..	..	..	..	..	..	..
	9	94/100	94	..	..	..	..	..	..	..	..	..	..
k	10	96/100	96	..	..	..	..	..	..	..	..	..	..
	..	..	..	95	3.6	..	..	..	..	..	..	..	..
	1	136/190	72	..	..	27/37	73	52	..	..	3	15	..
	2	71/100	71	..	..	..	..	..	..	..	..	..	..
	3	66/100	66	..	..	..	..	..	..	..	..	..	..
	4	64/100	64	..	..	12/18	67	70	..	..	1	23	..
	5	62/100	62	..	..	22/38	58	55	..	..	..	..	..
	6	61/100	61	..	..	14/29	48	66	..	..	..	..	..
	7	54/100	54	..	..	..	..	..	..	..	2	15	..
	8	54/100	54	..	..	..	..	..	..	..	..	..	..
	9	52/100	52	..	..	15/34	44	64	..	..	3	11	..
	10	52/100	52	..	..	..	..	..	..	..	..	..	..
	11	49/100	49	..	..	..	..	..	..	..	..	..	..
12	36/100	36	..	..	15/47	32	56	..	..	..	..	..	
..	..	..	58	10.2	..	..	..	54	15	..	..	..	

* Reported in MAGUIRE 1960a. Pachytene values for constitutions a and b were from single plants; metaphase values were the average of 60 plants for constitution a, and of 17 plants for constitution b.

as to pachytene and metaphase trivalent frequencies, and position and extent of synapsis in pachytene trivalent configurations. The data from these studies are summarized in Table 1; the constitutions are those correspondingly designated in Figure 2.

In all cases where measurements from as many as seven plants of a given type were available, the distributions of metaphase and pachytene trivalent frequencies were tested for deviation from normality. In each case these distributions were found not to deviate from the normal at the 10 percent level. Standard deviations were estimated where as many as three plants were available. These standard deviations were commonly larger for pachytene than metaphase measurements. In the case of constitution 2k involving exchange type III, the range

of variation among plants in both pachytene and metaphase trivalent frequencies was exceptionally large suggesting the possibility of undetected differences among plants in constitution, perhaps due to further exchanges in their parent. This progeny was also unusual in having a very high transmission frequency of the T^2 chromosome (16/19) through the female parent and is the subject of further study in this respect.

The values of metaphase and pachytene trivalent frequencies given in Table 1 represent in many cases pooled data from several anthers of a plant. From those cases where more than one anther of a plant were studied, trivalent frequencies found at pachytene and metaphase are listed in Tables 2 and 3. Again frequency variations are often greater at pachytene than at metaphase. It can be seen that in some cases plants with apparent low variability at metaphase show high variability at pachytene. Thus, unless there is some leveling off mechanism inherent in the process of chiasma formation, the best explanation for the greater pachytene variability would seem to be greater sampling error there due to the com-

TABLE 2

*Pachytene trivalent frequencies of different anthers within plants of several of the constitutions**

Constitution	Plant No.	Anther No.	Pachytene trivalent frequency		Constitution	Plant No.	Anther No.	Pachytene trivalent frequency			
			Number of cells	Percent				Number of cells	Percent		
c	2	1	58/58	100	e	1	5	13/25	52		
		2	48/48	100			6	14/32	44		
		3	35/35	100			7	24/44	55		
		4	42/43	98			e	2	1	14/20	70
		5	29/29	100					2	19/29	66
	3	1	75/76	99	3	14/23	61				
2		26/26	100	4	24/41	59					
d	8	1	14/51	27	5	6/13	46				
		2	4/15	27	4	1	1/12	8			
		3	5/36	14		2	9/27	33			
		4	14/45	31		3	3/12	25			
		5	2/16	13		4	9/13	69			
		6	3/20	13	5	14/32	44				
	9	1	4/13	31	6	14/30	47				
		2	4/13	31	7	10/18	56				
		3	5/12	42	f	1	1	13/39	33		
		4	6/19	32			2	11/38	29		
		5	7/14	50			3	3/12	25		
		6	12/38	32			4	1/14	7		
		7	2/15	13			5	2/22	9		
		8	8/31	26			6	3/23	13		
9	5/29	17	7	2/35	6						
10	3/17	18	8	5/76	7						
e	1	1	14/38	37	9	14/48	29				
		2	5/28	18	i	1	1	17/28	61		
		3	12/28	43			2	16/22	73		
		4	32/50	64							

* Data are listed for only those anthers with more than ten analyzable cells.

TABLE 3

Metaphase trivalent frequencies of different anthers within plants of several of the constitutions

Constitution	Plant No.	Anther No.	Metaphase trivalent frequency		Constitution	Plant No.	Anther No.	Metaphase trivalent frequency	
			Number of cells	Percent				Number of cells	Percent
c	2	1	29/32	91		6	1	26/47	55
		2	124/135	92			2	38/79	48
e	1	1	41/100	41	f	1	1	20/133	15
		2	55/121	45			2	10/56	18
	2	1	148/260	57			3	31/179	17
		2	54/100	54	g	1	1	38/60	63
	4	1	46/106	43			2	29/40	73
		2	44/100	44	i	2	1	78/106	74
	5	1	9/20	45			2	76/100	76
		2	41/111	37					

paratively small size of pachytene samples and the rejection of a much larger proportion of cells as unanalyzable. A test for normal distribution of pachytene trivalent frequencies among anthers of a plant (e1) from which seven were studied (each with 25 or more cells classified) showed no significant deviation from the normal at the 10 percent level. Such a distribution provides some justification for the supposition that rejection of unanalyzable cells introduces no important sampling bias. It should be continually borne in mind, however, that the smaller pachytene samples may give comparatively unreliable estimates of trivalent frequency.

Since plants of all chromosomal constitutions had similar genetic backgrounds and all were grown under similar conditions, for purposes of broad comparisons the averages are taken as approximations of values of pachytene and metaphase trivalent frequencies to be expected for each of the constitutions in their current background (Table 4).

TABLE 4

Summary of pachytene and metaphase trivalent frequencies for all constitutions

Constitution	Average pachytene trivalent frequency (percent)	Average metaphase trivalent frequency (percent)
a	98	90
b	65	70
c	99	93
d	17	15
e	50	48
f	18	17
g	60	67
h	64	71
i	66	72
j	100	95
k	54	58

A striking feature throughout the trivalent frequency data is the similarity of metaphase and pachytene estimates. When these are placed on ordinate and abscissa scales respectively, and points are plotted for those individual plants where estimates (based on at least 25 cells) of both pachytene and metaphase trivalent frequencies are available, it can be seen that their distribution is approximately linear with a Y intercept near the origin and a slope of about 1 (Figure 3). A linear regression of metaphase trivalent frequencies on pachytene trivalent frequencies gives a Y intercept of -0.72 percent and a slope of $+0.99$ (product-moment correlation coefficient $r = +0.97$). By the traditional view, in those constitutions where fewer than 50 map units in the T^2 chromosome are available for homologous pairing, metaphase trivalent frequency would be expected to lag below pachytene trivalent frequency even if synaptic extent were always maximal. Actually, even in those cases where about 50 map units of homology may be available, synaptic extents are often substantially less than this (MAGUIRE 1960a, 1964). Further, unless the processes of synapsis and chiasma formation in this arm of the trivalent configuration are strongly causally related (or both are strongly linked to some other factor) other variables might be expected to influence them differently.

The most meaningful individual comparison of pachytene pairing to metaphase chiasma frequency in this study can be made in the case of data from several anthers with unusually clear pachytene cells from a plant of constitution 2e. Here it was possible to classify 90 percent of 116 cells at pachytene for

FIGURE 3.—Metaphase I trivalent frequency as a function of pachytene trivalent frequency. Points are plotted for each plant where both measurements of frequency are based on at least 25 cells. A line of slope 1, Y intercept 0, is drawn for comparison to the linear regression line.

presence of a trivalent or univalent; 52/104 (or 50 percent) contained a trivalent. In the unlikely event that all 12 of the unclassified cells actually contained a trivalent, the pachytene trivalent frequency was 55 percent. Forty-three percent of 221 metaphase cells from this plant contained a trivalent, thus the minimum frequency of cells with at least one chiasma per pachytene trivalent (in the appropriate arm) is inferred to have been about 43/55 or 78 percent. If, as seems more likely, the trivalent frequency in the 12 unclassified cells did not differ greatly from the rest of the sample, then the minimum frequency of cells with at least one chiasma per pachytene trivalent (in the appropriate arm) was about 43/50 or 86 percent. The extent of homologous pairing available for crossing over in the pertinent region of these trivalent configurations is estimated to have included a maximum of 29 genetic map units; this estimate is based on synaptic extents in constitutions 2d, 2e and 2f and an estimate of 54 genetic units within the maize segment originally transferred to the T^2 chromosome (MAGUIRE 1964). The estimate of 29 map units is probably an overestimation since it assumes a uniform cytological distribution of the genetic map although it is known to be somewhat concentrated distally. But if as much as 29 genetic map units are available on the average in homologous pairing in trivalent configurations, a maximum expectation of chiasma frequency in them would be 58 percent where an actual *minimum* found was estimated at 78 percent.

It seems unlikely that pachytene trivalent frequencies were generally underestimated as a result of sampling error for the following reason. In plants of those chromosomal constitutions where high metaphase trivalent frequencies were found, correspondingly high frequencies were found at pachytene. If unclassified cells tend to contain a disproportionately high frequency of trivalents, lower frequencies might be expected here, since the short chromosome is comparable cytologically, and in some cases identical to that of constitutions with lower metaphase trivalent frequencies. The same sort of classification difficulties in pachytene cells might be expected throughout.

When estimated frequency of trivalents at metaphase, which may in turn be taken as a fairly reliable estimate of the pachytene frequency, is plotted against estimated cytological extent of homology in the T^2 chromosome for regions present in either or both of the other chromosomes, points for the various chromosomal constitutions are distributed in an interesting pattern (Figure 4). Points for constitutions 2a and 2c, which are alike in extent of homology in the T^2 chromosome and alike in that no parts are present in triplicate, but differ in the distribution of corresponding parts between the other two chromosomes, have very similar metaphase trivalent frequencies. Points for constitutions 2b, 2g, 2h and 2i which are like the two mentioned above in extent of homology in the T^2 chromosome but unlike them in having some regions present in triplicate (regardless of the extent of the triplicated region) form a closely grouped cluster at lower metaphase trivalent frequencies. Notice that constitutions 2h and 2i are reciprocal to constitution 2g in the sense that sections present in duplicate and triplicate are reversed with respect to terminal and intercalary regions. Constitution 2e with approximately half maximum homologous extent in the T^2 chromosome (but no regions present in triplicate) has a metaphase trivalent frequency less

FIGURE 4.—Average metaphase I trivalent frequency as a function of probable cytological extent (percent chromosome 2) of homology in the T^2 chromosome to the representatives of chromosome 2 present in each complement. The letter at each point designates a constitution of Figure 2.

than constitutions 2a and 2c by about the same fraction as that by which constitution 2d and 2f are less than that of the 2b, 2g, 2h and 2i cluster. This cluster has maximum homologous extent in the T^2 chromosome and regions present in triplicate. Direct comparisons to the others of constitutions 2j and 2k are probably unjustified since their nature, though not thoroughly understood, is thought to differ fundamentally. Homologous pairing of entirely different regions may be involved as well as homeologous pairing. Nevertheless, when these two are compared, the one with the supposed greater extent of common homology shows markedly greater metaphase trivalent frequency.

If these findings are taken at face value, it appears that metaphase trivalent frequency tends to be reduced in those constitutions where regions are present in triplicate. RHOADES (1931, 1936), DOBZHANSKY (1935) and GRELL (1964) have found decreased crossover frequencies in duplicated regions of maize or *Drosophila*. GREEN (1962), however, found crossing over to be increased in the vicinity of small tandem duplications, and there have been numerous reports of increased crossover frequency in polyploids. In this study it seems that the extent of the region present in triplicate can be reduced by approximately half without affecting trivalent frequency, whereas reduction of the extent of the homologous region in the T^2 chromosome by half reduces trivalent frequency by large and similar fractions in those cases where there are homologous regions present in triplicate and where only duplicated regions are present. Beyond these suppositions it appears that the location of the terminal knob is unimportant, that terminal or intercalary position of a triplicated region has no important effect on triva-

lent frequency, and that common homology to the T^2 chromosome may be divided between the 2^T chromosome and a normal chromosome 2 (as in constitution 2c) without reduction in trivalent frequency. In plants of constitution 2c, where homology to the modified T^2 chromosomes was approximately equally divided between the normal chromosome 2 (intercalary region) and a 2^T chromosome (terminal region), pairing was more frequent between the homologous terminal regions, and from here it commonly extended into the intercalary region non-homologously, at the exclusion of pairing-partner change and homologous pairing. This suggests that pairing of the modified T^2 chromosome may be more readily initiated terminally than centrally.

DISCUSSION

Correlation of metaphase and pachytene trivalent frequencies: Alternate interpretations are possible for the close similarity of pachytene and metaphase trivalent frequencies described above. A 1:1 relationship of true chiasma frequency to gross-level crossover frequency is considered highly probable; aside from widespread support for such a view in the literature, a comparison of observed genetic recombination and chiasma frequencies in material of constitution 2a strongly supported an assumption of close correspondence between the two frequencies in this material (MAGUIRE 1964). Presumably either crossing over (and therefore chiasma formation) is established before the generalized synapsis of pachytene occurs (in such a way that both pachytene and metaphase trivalent frequencies are dependent on crossover frequencies), or in the synaptic configurations of the types described above, crossing over generally occurs in the pertinent arm of the trivalent regardless of the extent of genetic map available in the synapsed regions. The former interpretation supports the views of PRITCHARD (1960) who proposed that crossing over generally occurs in short "effectively paired" segments before synapsis is widespread. It is difficult at present to conceive of a mechanism by which crossing over could occur in completed synaptic configurations in such a way that the shape of the configuration would be a more important determiner of crossover frequency within it than the extent of pairing. However, such a system has not been ruled out, and would be required if the discovery of some DNA replication at pachytene is to be interpreted as the result of regular crossing over by some sort of copy or repair mechanism at this stage (WIMBER and PRENSKY 1963). This second alternative could also be more easily reconciled with the hypothesis that inter- and intra-chromosomal crossover effects in heterozygous aberration constitutions, polysomics and polyploids are a result of localized increased supply of a scarce substance required for intimate pairing (see RAMEL 1962). There is unfortunately very little data on actual relative extents and frequencies of pachytene homologous pairing to accompany the genetic evidence of such crossover effects. RHOADES (1936) found approximately the same trivalent frequencies at pachytene and metaphase in maize carrying an extra fragment chromosome composed of the entire short arm of chromosome 5, in spite of the fact that this fragment was frequently synapsed for only a portion of its length at pachytene. EINSET (1943), however, found markedly higher trivalent

frequencies at both pachytene and diplotene than at diakinesis and metaphase in a maize trisome 3 plant. The apparent complete loss (not terminal accumulation) of a number of chiasmata between diplotene and diakinesis and the reported ease of classification of pachytene cells suggest that EINSET's data may have been complicated by the presence of nonhomologous pachytene pairing and the difficulty of classification of diplotene cells.

General considerations of synapsis and crossing over: The view is widely held that synapsis begins by chance meeting of moving homologues at a stage when chromosomes are in a greatly extended state (the typical very early meiotic prophase), perhaps aided by a bouquet formation or remnant anaphase orientation. It has often been pointed out, however, that this view is faced with many difficulties. In the first place it can be questioned whether there is ever substantial movement of greatly attenuated chromosomes. It has been observed that in *Neurospora* (where karyogamy usually immediately precedes meiosis) that synapsis is initiated between chromosomes which are extremely condensed (McCLINTOCK 1945; SINGLETON 1953). Other important movements appear to occur during condensed stages, and available evidence indicates that there is as a rule no more than slight stirring of nuclear contents during interphase and very early prophase stages (EVANS and BIGGER 1961; RHOADES 1961). There is some evidence that even during pachytene there may be little, if any, change of positions of chromosomes with respect to each other (MAGUIRE 1961b). The assumption of relic anaphase orientation helps little since available evidence in maize argues against usual initiation of synapsis at the centromere region (McCLINTOCK 1931; RHOADES 1936, 1940; TABATA 1962; MAGUIRE 1962), and it is difficult to see how the specific pairing of certain aberration configurations could be promoted by such orientation (as pointed out by FABERGÉ [1942]). Another difficulty of the traditional model is the rarity of the interlocking synaptic configurations which might be expected if synapsis can be randomly initiated independently at widely separated points along each chromosome (SMITH 1942). Furthermore, examples of zygotene stage cells are difficult to find and interpret. Observations have been limited to fixed and stained material, and in many (or perhaps all) cases where clear partial pairing has been seen, synapsis may actually have reached its maximum extent (DARLINGTON 1935; MAGUIRE 1960b). MOENS (1964) has recently reported evidence that synapsis may be completed in interphase in tomatoes, that the pachytene stage then follows (with bivalents at first clumped, then well spread). Between pachytene and diplotene there is a diffuse stage easily confused with traditional zygotene and leptotene during which homologous arms and then centromeres separate.

It was proposed by SMITH (1942) that synapsis may be initiated as a rule in the preceding anaphase or telophase. BOSS (1954) reported somatic chromosome pairing in anaphases of newts, and *Drosophila* chromosomes are said to reassociate homologously at every mitotic anaphase (SONNENBLICK 1950). No direct observations of the premeiotic mitosis in maize are available owing to the difficulty of studying the material, but there are two lines of evidence that premeiotic homologous pairing may occur: (1) The position of a terminal knob may oc-

casionally differ between homeologues or homologues among the sporocytes of a single plant indicating either that a premeiotic exchange has occurred or that early meiotic pairing, exchange, separation and repairing have all occurred before pachytene (MAGUIRE 1961c). (2) In material containing occasional plasmodial sporocytes, extra nuclei with *incomplete* complements nevertheless generally contain only bivalents, as SNOAD (1954) found in *Helianthemum* (MAGUIRE, unpublished). These extra nuclei are thought to have resulted in each case from the separation of components of a complete nucleus. No stages of such separation have been found among thousands of appropriate meiotic cells examined. If a separation occurred premeiotically, it must be assumed that homologues were paired at this time or were somehow consistently distributed to the same nucleus.

The major findings of the present paper which must be taken into account in the construction of a model for synapsis and crossing over are: (1) Pachytene trivalent frequency is sufficiently similar to metaphase trivalent frequency throughout the array of available chromosomal constitutions to suggest either that existence of homologously paired trivalent configurations at pachytene is dependent upon prior crossing over (as it presumably is at metaphase) or that crossing over almost always follows trivalent formation (in this material), regardless of the extent of genetic map available in homologous pairing. (2) The frequency of trivalent formation is depressed in the presence of homologous regions in triplicate in a way which is relatively insensitive to the length of the triplicated regions. (3) The frequency of trivalent formation nevertheless increases with the extent of homology in the T^2 chromosome to either or both of the other two chromosomes.

These results are also difficult to reconcile with the traditional model which invokes initiation of synapsis by random meeting of homologous points followed by zipping up. In some cases an anticipation of difficulties to be encountered in parts as yet unpaired would seem to be required at the beginning of the process. DOYLE (1963) found evidence for not only a reduction in multivalent formation with the inclusion of inversion heterozygosity in polyploids and polysomics of maize, but also preferential pairing among bivalents of chromosomes sequentially matched, beyond that conceivably explainable as a result of multivalent failure. Although his findings are also difficult to fit to a chance meeting model, they may be at least partially accounted for by the additional assumption of persistence of a general alignment of the premeiotic anaphase by which homologous units within the arm heterozygous for the inversion would be brought closer together when in similar sequence. On the other hand, in the present study relative location of the centromere of the T^2 chromosome (and derivatives) is presumed constant throughout the various constitutions with respect to the positions of the component units of the chromosome.

A model which seems simplest and consistent with all observations is as follows:

General alignment of homologous chromosomes or segments is generally achieved or initiated during the anaphase which precedes meiosis. This possibility

is susceptible to check by direct observation. In some fixed and stained material, e.g. Trillium, a general alignment or loose pairing of homologues can be seen at early meiotic prophase, in the absence of apparent polarization. The general alignment phase may be nonsaturated in that more than two members (when present) may participate at any point (FABERGÉ 1942). This alignment is followed (sometime before pachytene) by intimate two-by-two pairing of short segments (PRITCHARD 1960; HOLLIDAY 1964) which are spaced at a considerable distance in any particular configuration, perhaps owing to coiling or kinking relationships (MAGUIRE 1960c), with chiasma interference the observed consequence. The overall frequency of these effectively paired segments along a chromosome pair is (other things being equal) proportional to the extent of available homology in alignment. (This frequency is, of course, also influenced by a number of factors such as coiling or kinking relationships of the chromosomes involved or their chemical environment, which may in turn vary for any particular region with external conditions, presence or absence of certain genes, overall dosage effects, supply of certain substances, etc.) In heterozygous aberration configurations, alignment is interrupted in immediate regions of partner exchange. There exists what may be formally interpreted as an element of resistance to the formation by a chromosome of effectively paired segments with two other chromosomes (partner change) when three chromosomes share a region of common homology. ("Resistance" does not vary in the present study with extent or position of the triplicated region.) Crossing over occurs only within regions of effective pairing (PRITCHARD 1960; HOLLIDAY 1964). The process may be polarized in time within these regions, in keeping with requirements of recent genetic fine-structure data (see STADLER and TOWE 1963). Present knowledge places no firm restrictions on the stage of its occurrence from interphase to diplotene, except that it must accompany or succeed replication. Its completion could require an extended interval.

The nonhomologous pairing (first noted by McCLINTOCK 1933, and familiar to all maize cytogeneticists who have worked with material in which some chromosome regions exist without matching potential pairing partners) might be expected to follow homologous pairing in time. This frequently observed nonhomologous pairing of maize chromosomes does not ordinarily persist past pachytene, but might nevertheless be related to the post-crossover "distributive pairing" postulated by GRELL (1962) to account for certain cases of nonrandom distribution in *Drosophila*.

In the above speculations the assumption of the nonsaturated general alignment phase was preferred to that of a long-range specific pairing force, paradoxically insensitive to quantity of material in some ways, yet sensitive to it in others. Present findings (unless artifact in nature) render some such consideration necessary. For simplicity, all of the variability in crossover frequency and distribution was assigned to frequency and distribution of effective pairing segments, although variability of occurrence of crossing over within these would be expected to introduce an additional element of complexity.

It is emphasized that pachytene stage chromosomes may not represent an ex-

panse of raw material in which crossovers are formed with a gross frequency generally proportional to synapsed length, as usually supposed, but may already contain crossovers or their potential or established precursors.

SUMMARY

Chromosome pairing relationships at pachytene and metaphase I were studied and compared in an array of 21-chromosome constitutions containing maize-Tripsacum interchange chromosomes. These constitutions differed in duplication or triplication of corresponding chromosome regions, in extent and position of segments of a Tripsacum chromosome in a maize chromosome and in extent of segments of a maize chromosome in the extra Tripsacum chromosome.

Pachytene trivalent frequency was found to be sufficiently similar to metaphase trivalent frequency throughout the array of chromosomal constitutions to suggest either that existence of trivalent configurations at pachytene is dependent upon prior crossing over (as it presumably is at metaphase) or that crossing over almost always follows trivalent formation (in this material) regardless of the extent of genetic map available in homologous pairing.

The frequency of trivalent formation appears to be depressed by the presence of homologous regions in triplicate in a way which is relatively insensitive to the length of these triplicated regions. However, the frequency of trivalent formation nevertheless increases with the extent of homology in the extra chromosome to either or both of the other two chromosomes which it may partially match.

It appears that the location of the terminal knob is unimportant, that terminal or intercalary position of a triplicated region has no important effect on trivalent frequency, and that common homology to the extra chromosome may be divided between the other two semi-matching chromosomes without reduction in trivalent frequency.

Theoretical implications are discussed.

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