THE EFFECT OF ABNORMAL CHROMOSOME 10 ON PREFERENTIAL SEGREGATION AND CROSSING OVER IN MAIZE

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AMONG the more interesting phenomena encountered in the genetic analysis of organisms with an orthodox or classical type of meiosis are those exceptional cases where a modification of the processes controlling segregation leads to a nonrandom distribution of the four chromatids of a bivalent to the functional gametes. In such organisms the formation of equal numbers of gametes with the *A* and *a* alleles by *A a* heterozygotes almost invariably occurs. Sometimes the two classes of gametes are equally represented in the gametic population but are not found in equal numbers in the progeny because of gametic selection, differential viability, etc. These complications confuse but do not contradict the 1:1 segregation of alternative alleles which underlies Mendelian inheritance. Some organisms, particularly the Hemiptera, have acquired in their evolutionary development unusual and even bizarre meiotic modifications which clearly deviate from the normal pattern. We are not concerned here with these successful meiotic modifications although it is possible that the information gleaned from the analysis of exceptional cases in organisms with orthodox meiosis may reveal how the aberrant systems arose.

SANDLER and NOVITSKI (1957) proposed the term meiotic drive for those situations where unequal production of the two classes of gametes occurs in organisms generally having a normal pattern of meiosis. The cytogenetic events at meiosis in maize are of the classical type, but an exception to the rule of a 1:1 recovery of segregating alleles in the female gametes of heterozygous plants was found some years ago (RHOADES 1942, 1952). Subsequent to these early studies on preferential segregation, we have conducted a series of investigations which constitute the basis of this report. Preferential segregation in maize is a good example of meiotic drive.

Evidence will be presented demonstrating (1) that preferential segregation for heterologous chromosomes occurs only when abnormal chromosome 10 (*K10*) is homozygous or heterozygous, (2) that all tested chromosomes of the complement will undergo preferential segregation if the two homologues of the pair differ by the presence and absence of heterochromatic knobs, (3) that crossing over between the centromere and knob to produce heteromorphic dyads is an essential prerequisite to preferential segregation, (4) that the percentage of recovery in the basal megaspore for any allele on the knobbed chromosome can be accurately predicted from the frequency of tetrad ranks, (5) that the degree of

preferential segregation from heteromorphic dyads coming from single and double exchanges is the same, (6) that the *K10* chromosome increases recombination values and brings about more intimate synapsis, (7) that preferential segregation takes place in dyads with a deficient chromatid derived from bridge breakage if the intact chromatid is knobbed and that segregation is random when the normal chromatid is knobless, and finally (8) that the neocentromeres produced by the *K10* chromosome afford a plausible mechanism for preferential segregation.

Preferential Segregation

Preferential segregation was first found in plants heterozygous for the abnormal chromosome 10 (*K10/k10*) where more than 70% of the functional megaspores received the *K10* chromosome (RHOADES 1942). LONGLEY (1945) reported that preferential segregation occurred for both chromosomes 6 and 9 in *K10/k10* plants if the two homologues were unlike in knob constitution. For a more detailed study of the effect of the *K10* chromosome on preferential segregation of other chromosomes, chromosome 3 was chosen because of the information available on the location of certain loci in the long arm. In the following description, cytological and genetical markers in the long arm of chromosome 3 will be cited as being positioned according to a scale where the arm is considered to be of unit length with the centromere represented as the zero point.

Some strains of maize possess a large heterochromatic knob on chromosome 3 at a position 0.6 of the length of the arm from the centromere. Any chromosome 3 having this knob is designated as *K3*, those lacking it as *k3*. A further advantage of this chromosome is that considerable information is available on the physical location of the three loci employed. The glossy-6 (gl_6) locus lies in a proximal position in the long arm somewhere between .1 and .25. The amount of crossing over between gl_6 and the centromere is low, being only 2 to 3%. The liguleless-2 (lg_2) locus lies proximal to the knob but is closely linked to it. Lg_2 serves as an efficient marker in following the knob in segregation studies. The aleurone color factor A_1 is situated in the distal end of the long arm. It lies between positions .75 and .95 and is almost certainly closer to .95 than to .75 judging from recombination values in our unpublished inversion studies.

In *k3 k3* plants 23 to 25% recombination occurs between *Gl* and *Lg* and 36% between *Lg* and *A*. As will be shown, the crossover values in these regions are modified by the presence or absence of the knob lying between *Lg* and *A*. Some undetected double exchanges undoubtedly occur in regions this long but the number of these is not believed to affect the arguments presented. Another reason for selecting these marker genes is that they can all be scored either on the basis of aleurone color, as is the case for *A*, or as seedlings for *Gl* and *Lg*. Since the amount of preferential segregation for a locus is determined from the ratio of its two alternative alleles, it is imperative that traits be used which can be classified either on the kernel or as seedlings grown in the favorable environment of a greenhouse bench. Otherwise the estimate may be disturbed by selective elimination of plants with deleterious genotypes. For example, it is known that field-

grown progenies suffer in particular the partial loss of plants homozygous for the *gl* allele.

Nine combinations are possible with two kinds of chromosome 10 (*K10* and *k10*) and two types of chromosome 3 (*K3* and *k3*). Segregation data from female testcrosses for eight of these have been obtained (items 1 to 12, Table 1). These data clearly demonstrate that preferential segregation for the chromosome-3 alleles occurs only in those combinations having heteromorphic chromosomes 3 (*K3 k3*). A second requirement is the presence of the *K10* chromosome which may be homozygous or heterozygous. In *K10 K10 K3 k3* plants the two chromosomes 10 are randomly segregated but the *K3* chromosome is preferentially recovered; in *K10 k10 K3 k3* plants both chromosomes undergo preferential segregation.

Table 1 contains no data from male testcrosses but extensive tests show that random segregation occurs for chromosome 3 loci in *K10 k10 K3 k3* plants since each of the four chromatids of a tetrad is included in the quartet of microspores. Also listed in Table 1 are data from combinations with structurally modified chromosomes 3 and 9.

If preferential segregation depends upon the occurrence of dyads with one knobbed and one knobless chromatid, it is possible to predict the kinds of exchanges which will lead to preferential segregation at anaphase II. The types of exchanges giving rise to preferential segregation or to random segregation for the three loci are indicated below. Preferential segregation is indicated by the + sign and random segregation by the = sign. The *Gl-Lg* segment is region 1 and region 2 is delimited by *Lg* and *A*. The dominant alleles are in the knobbed chromosome. The linkage phase, however, is immaterial and preferential segregation occurs for recessive alleles when they are on the knobbed homologue.

Type of Exchange	*Gl*	*Lg*	*A*
None	=	=	=
Single in (1)	=	+	+
Single in (2)	=	=	=
2-strand double	=	+	=
3-strand double	=	+	=
4-strand double	=	+	=

As the tabulation above indicates, exchanges in (1), which result in heteromorphic dyads, lead to preferential segregation for the *Lg* and *A* alleles. The dyads from no-exchange tetrads and those from single exchanges in (2) are homomorphic and segregation is random for all loci. The double exchanges form heteromorphic dyads but segregation is preferential only for the *Lg* locus.

Little or no preferential segregation is found for the *Gl* allele present on the knobbed chromosome. This is consistent with the low amount of recombination known to occur between *Gl* and the centromere because only those exchanges in the centromere(C)-*Gl* region will yield heteromorphic dyads with the *Gl* allele

TABLE 1

Testcross data from ears with different combinations of K10 *and* k10 *with normal and structurally modified chromosomes 3 and 9. The bracketed combinations are sibs*

TESTCROSSES INVOLVING STRUCTURALLY NORMAL CHROMOSOMES 3

	Gl Lg A N3 / gl lg a N3 ♀	Gametic classes (0) Gl Lg A	(0) gl lg a	(1) Gl lg a	(1) gl Lg A	(2) Gl Lg a	(2) gl lg A	(1-2) Gl lg A	(1-2) gl Lg a	Σ	Recovery % Gl	% Lg	% A	%Recombination Gl-Lg	Lg-A	Σ
1.	K10 K10 K3 K3	255	260	177	194	181	166	108	108	1449	49.8	50.9	49.9	40.5 ±1.3	38.9 ±1.3	79.4
2.	K10 k10 K3 K3	490	533	379	417	338	321	192	192	2862	48.9	50.2	49.6	41.2 ±0.9	36.4 ±0.9	77.6
3.	K10 K10 K3 k3	326	126	98	277	135	68	36	112	1178	50.5	72.2**	60.0**	44.4 ±1.4	29.8 ±1.3	74.2
4.	K10 k10 K3 k3	777	359	272	661	343	197	108	250	2967	50.0	67.5**	57.9**	43.5 ±0.9	30.2 ±0.8	73.7
5.	†K10 k10 K3 k3	2529	871	663	2060	859	474	309	662	8427	51.7**	72.5**	63.6**	43.9 ±0.5	27.3 ±0.5	71.2
6.	†K10 k10 k3 k3	730	656	425	465	471	493	210	178	3628	50.6	50.8	52.3**	35.2 ±0.8	37.3 ±0.8	72.5
7.	†k10 k10 K3 k3	954	870	514	525	274	326	121	143	3727	50.0	50.9	51.7*	35.0 ±0.8	23.2 ±0.7	58.2
8.	†k10 k10 k3 k3	286	259	122	118	174	159	67	38	1223	53.1	50.4	51.5	28.2 ±1.3	35.8 ±1.4	64.0
9.	k10 k10 K3 K3	167	155	74	64	118	99	31	31	735	53.1	51.0	48.6	26.7 ±1.6	38.0 ±1.8	64.7
10.	k10 k10 k3 k3	274	263	97	89	164	200	40	38	1165	49.4	48.5	51.8	22.7 ±1.2	37.9 ±1.4	60.6
11.	K10 k10 K3 k3	496	239	167	430	223	130	60	155	1900	49.8	68.6**	58.2**	42.9 ±1.1	29.9 ±1.0	72.8
12.	k10 k10 K3 k3	266	262	138	142	72	87	36	44	1047	49.0	50.0	50.7	34.4 ±1.5	22.8 ±1.3	57.2

TESTCROSSES INVOLVING REARRANGED CHROMOSOMES 3 WITH K10 AND k10

Gl k Lg a IN3b / gl K lg A IN3b ♀	(0) Gl Lg a	(0) gl lg A	(1) Gl lg A	(1) gl Lg a	(2) Gl Lg A	(2) gl lg a	(1-2) Gl lg a	(1-2) gl Lg A	Σ	Recovery % gl	% lg	% A	% Recombination Gl-Lg	Lg-A	Σ
13. k10 k10 K3 k3	291	275	107	90	120	129	40	41	1093	48.9	50.4	49.7	25.4 ±1.3	30.2 ±1.4	55.6
14. K10 k10 K3 k3	205	412	268	140	105	256	134	81	1601	55.5**	66.8**	54.1**	38.9 ±1.2	36.0 ±1.2	74.9
Gl lg K a N3 / gl k Lg A IN3b ♀	**Gl lg a**	**gl Lg A**	**Gl Lg A**	**gl lg a**	**Gl lg A**	**gl Lg a**	**Gl Lg a**	**gl lg A**	**Σ**	**% Gl**	**% lg**	**% a**			**Σ**
15. k10 k10 K3 k3	493	463	3	1	46	45	7	3	1061	51.7	51.2	51.4	1.3	9.5	10.8
16. K10 k10 K3 k3	1005	985	10	21	120	95	44	63	2343	50.3	51.6	49.7	5.4	13.3	18.7
Gl a k lg IN3a / gl Lg K A N3 ♀									**Σ**	**% gl**	**% Lg**	**% A**	**Gl-Lg**	**Lg-A**	**Σ**
17. k10 k10 K3 k3	1035	1020	138	140	2	10	5	0	2350	49.8	49.9	49.4	12.0	0.7	12.7
18. K10 k10 K3 k3	1086	1562	582	307	26	28	63	36	3690	52.4**	60.6**	59.8**	26.8	4.1	30.9
gl A k Lg IN3a / Gl a k lg IN3a ♀	**(0) gl A Lg**	**(0) Gl a lg**	**(1) gl a lg**	**(1) Gl A Lg**	**(2) gl A lg**	**(2) Gl a Lg**	**(1-2) gl a Lg**	**(1-2) Gl A lg**	**Σ**	**% Gl**	**% Lg**	**% A**			
19. k10 k10 k3 k3	275	254	71	64	174	158	27	40	1063	48.5	49.3	52.0	19.0	37.5	56.5
Gl a K Lg IN3a / Gl a k lg IN3a ♀	**Lg**	**lg**	**Σ**	**% Lg**											
20. K10 k10 K3 k3	1075	659	1734	61.9**											
21. k10 k10 K3 k3	674	647	1321	51.0											

TABLE 1—Continued

	Gametic Classes									Recovery			% Recombination		
Gl Lg K A N3 / a k lg gl IN3c ♀	Gl Lg A	gl lg a	Gl lg a	gl Lg A	Gl Lg a	gl lg A	Gl lg a	gl Lg a	Σ	% Gl	% Lg	% A	Gl-Lg	Lg-A	Σ
22. K10 k10 K3 k3	1426	1281	108	93	110	63	342	473	3896	51.0	54.0**	49.4	26.1	25.4	51.5
a k Lg Gl IN3c / A k lg gl IN3c ♀	(0) a Lg Gl	(0) A lg gl	(1) a lg gl	(1) A Lg Gl	(2) a Lg gl	(2) A lg Gl	(1-2) a lg Gl	(1-2) A Lg gl	Σ	% Gl	% Lg	% A	Gl-Lg	Lg-A	Σ
23. k10 k10 k3 k3	962	1005	619	633	323	330	104	124	4100	49.5	49.8	51.2	23.9 ±0.7	36.1 ±0.7	60.0

TESTCROSSES INVOLVING MODIFIED CHROMOSOMES 9 WITH K10 and k10

k wd Wx N9 / K^m Wd wx (R) 9 ♀	(0) wd Wx	(0) Wd wx	(1) wd wx	(1) Wd Wx	Σ	Recovery % Wd	Recovery % wx	% Recomb. Wd-Wx
24. [K10 k10 $K9^m$ k9]	694	703	2	39	1438	51.6	49.0	2.9
25. [k10 k10 $K9^m$ k9]	506	528	2	13	1049	52.5	50.5	1.4
K^S Wd Wx N9 / k wd wx N9 ♀	(0) Wd Wx	(0) wd wx	(1) Wd wx	(1) wd wx	Σ	% Wd	% Wx	Wd-Wx
26. K10 k10 $K9^S$ k9	3676	2546	2198	1504	9924	59.2**	52.2**	37.3 ±0.2

K^m Yg N9 / K^S yg Tp9 ♀	Yg	yg	Σ	% Yg
27. K10 k10 $K9^m$ $K9^S$	1634	1353	2987	54.7**
K^m Yg N9 / K^S yg N9 ♀	Yg	yg		
28. K10 k10 $K9^m$ $K9^S$	1331	709	2040	65.2**
K^S yg N9 / k wd N9 ♀	yg	wd		% yg
29. K10 k10 $K9^S$ k9	1221	802	2023	60.4**
30. k10 k10 $K9^S$ k9	499	494	993	50.2

K^S Wd N9 / k wd N9 ♀	Wd	wd	Σ	% Wd
31. [K10 K10 $K9^S$ k9]	1040	655	1695	61.4**
32. K10 k10 $K9^S$ k9	2001	1366	3367	59.4**
33. k10 k10 $K9^S$ k9	659	664	1323	49.8
34. k10 k10 $K9^S$ k9	1629	1618	3247	50.1

* Significant deviation from 50%. ** Highly significant deviation from 50%.
† Segregating for the *et* allele 12 map units to the right of *A*. Homozygous *et* kernels are often so scarred and poorly developed that they have a lower germination. This accounts for the somewhat higher percentage of *A* kernels in those combinations where random segregation is expected.

in the knobbed chromatids. Preferential segregation is and should be highest for the *Lg* allele and that for *A* should be lower because the double exchanges preferentially segregate *Lg* but not the *A* allele. The data show that the percentage of recovery for the *Lg* allele, close to the knob, is 71.1%, a value greater than that for the *A* locus (61.1%) which in turn exceeds that for the *Gl* allele (51.1%).

The experimental design can be fairly criticized for the excessive length of the *Gl-Lg* and *Lg-A* regions, but the marker genes used were deliberately chosen because of accuracy of classification of the alternative alleles and because of equal viability for all genotypes under greenhouse environmental conditions. Nevertheless the effect of undetected crossovers must be considered before the varying degrees of preferential segregation can be accepted on the basis of the kinds of exchanges given above. Given below are the consequences of undetected single and double exchanges. The number of triple exchanges should be extremely low

and they are ignored. Preferential segregation is indicated by the + sign and random segregation by the = sign.

Type of Exchange	*Gl*	*Lg*	*A*
Single between (C)-*Gl*	+	+	+
Single between *Lg-K*	=	=	+
Double in (C)-*Gl* and *Gl-Lg*	=	+ (3-strand) = (2 and 4-strand)	+ (3-strand) = (2 and 4-strand)
Double in (C)-*Gl* and *Lg-A*	+	+	=
Double in *Gl-Lg*	=	+ (3-strand) = (2 and 4-strand)	+ (3-strand) = (2 and 4-strand)
Double in *Lg-A*	=	=	=

It is evident from the above tabulation that undetected double and single crossovers could affect the recovery of the three dominant alleles. For example, singles in the (C)-*Gl* region and doubles with one in (C)-*Gl* and one in *Lg-A* result in preferential segregation for the *Gl* locus, which was not the case with any of the detectable crossovers. The consequences of undetected exchanges on recovery of the *Lg* allele will depend on the relative frequencies of the different crossovers. The percentage of *Lg* will be reduced by singles in *Lg-K*, by doubles in *Lg-A*, and to a lesser extent by doubles in *Gl-Lg* and doubles in (C)-*Gl* + *Gl-Lg*. The percentage of *Lg* will be raised by singles in (C)-*Gl* and doubles in (C)-*Gl* + *Lg-A*. The recovery of the *A* allele will be increased by singles in (C)-*Gl* and singles in *Lg-K*, but decreased by doubles in (C)-*Gl* + *Lg-A* and doubles in *Lg-A*. The other exchanges probably will not greatly affect the frequency of *A*.

Thus, it becomes important to obtain some estimate of the frequencies of these different kinds of crossovers. The amount of crossing over between *Gl* and the centromere is approximately 2 to 3% (RHOADES and DEMPSEY, unpublished) and single and double exchanges involving this region should have little effect on the percentage of recovery of the *Gl* allele. The segregation data for the *Gl* locus are consistent with the low level of exchanges proximal to the *Gl* gene. *Lg* and *K* are also closely linked (about 1% recombination, DEMPSEY unpublished) and exchanges in this segment should be rare. No experimental data are available on the frequency of double exchanges in the *Gl-Lg* region, but they should not exceed 4%. Doubles in the *Lg-A* segment also should occur with a frequency of 4% or less. It may be concluded that the effect of undetected exchanges on the preferential segregation of alleles borne on the knobbed chromosome will be negligible.

Contrary to LONGLEY's (1945) claim of preferential segregation of the knobbed homologues in *k10 k10* plants heterozygous for knobbed chromosomes are the extensive data in Table 1 where segregation is random for heteromorphic chromosomes 3 in *k10 k10* plants.

The Dependence of Preferential Segregation Upon Crossing Over

According to the hypothesis that preferential segregation occurs only when

heteromorphic dyads are produced, and it is the knobbed chromatid which segregates preferentially at anaphase II, those loci closer to the knob would undergo a higher degree of preferential segregation than would more distant loci. There is abundant evidence in Table 1 that this is so.

Reduction in crossing over by Transposition 9–3: One test of the hypothesis that preferential segregation is dependent upon crossing over is the determination of the ratios of genes in the short arm of chromosome 9 from plants in which the amount of recombination is greatly reduced compared to that in sibs. Such a test was made using a chromosome 9 with a short interstitial segment from the long arm of chromosome 3 transposed into its short arm between the *Sh* and *Wx* loci (*Tp9*). The presence of the transposed piece reduces the amount of crossing over in the short arm of chromosome 9 to less than one-third the normal amount (RHOADES 1958 and unpublished). Sib plants of three classes, all heterozygous for *K10*, were obtained. One class was of the constitution Transposition 9/Normal 9(*Tp9/N9*). The chromosome 9 with the *Tp* had a small terminal knob (K^s) on 9S and carried the yellow green-2 (yg_2) allele, while the N9 had a much larger knob (K^m) and the *Yg* allele (item 27, Table 1). The second class of plants was of N9/N9 constitution. One chromosome 9 had the prominent knob and the *Yg* allele, the other possessing the small knob and the *yg* allele (item 28, Table 1). The third class had two N9's, one with the small knob and the *yg* allele, and the other with the *wd* mutation (a small terminal deficiency that is allelic to yg_2) which is wholly devoid of a knob (item 29, Table 1). All three classes were heterozygous for *K10* and had heteromorphic chromosomes 9. Preferential segregation for chromosome 9 is known to occur in *K10 k10* plants when the two chromosomes differ in knob size (KIKUDOME 1959). Studies of preferential segregation were made in the *K10* plants. The first two classes were pollinated by *yg* plants and the ratio of *Yg*:*yg* plants obtained. The third class was pollinated by *wd* pollen and the *yg*:*wd* ratio determined. In backcrosses of K^m *Yg* N/K^s *yg Tp9* plants, in which crossing over is greatly reduced in 9S, the *Yg* seedlings constituted 54.7% of the offspring. Plants of K^m *Yg* N9/K^s *yg* N9 constitution with normal crossing over in 9S gave 65.2% of *Yg* plants. Individuals of K^s *yg* N9/*k wd* N9 genotype, again with free crossing over, produced 60.4% *yg* seedlings. Control data from closely related plants of K^s *yg* N9/*k wd* N9 constitution and homozygous for *k10* gave 50.2% *yg* plants (item 30, Table 1).

The data in Table 1 support the hypothesis that formation of heteromorphic dyads via crossing over is an essential antecedent to preferential sergegation. Preferential segregation is reduced when crossing over is decreased.

Reduction in crossing over by the (R)9 chromosome: Confirmatory evidence was obtained using a rearranged chromosome 9, which almost completely suppresses crossing over in the short arm. Plants heterozygous for the (R)9 chromosome and for the *wd* and *wx* markers were testcrossed as the female parent. Sib plants with and without *K10* were available. As shown in Table 1 (item 25), there was an extremely low amount of crossing over between *wd-wx* in the homozygous *k10* plants, and the contrasting alleles for the two segregating loci were each recovered in 50% of the progeny. Although plants heterozygous for

a knobbed 9 and a knobless 9 (*wd*) undergo preferential segregation if *K10* is present, no marked deviation from a 1:1 ratio for *Wd*:*wd* would be expected in *K10* plants containing the (R)9 chromosome if crossing over was a requisite for preferential segregation. On the other hand, the usual percentage of preferential segregation in *K9 k9* heterozygotes should occur if preferential segregation of *K9* chromatids to the basal megaspore is unrelated to recombination and is due to some intrinsic property of the knobs. The data obtained from both *K10 k10* and *k10 k10* backcrossed individuals (items 24 and 25) show a very close fit to a 1:1 ratio for both the *wd* and *wx* loci, while in the plants with normal chromosomes 9 and *K10 k10* preferential segregation for the *Wd* allele occurred (item 26, Table 1).

Reduction in crossing over by inversion heterozygotes: Substantiating data were also obtained from studies of plants heterozygous for three paracentric inversions involving the long arm of chromosome 3. The breakpoints of *In 3a* are 3L .4 and .95, the breakpoints of *In 3b* are 3L .25 and .75, while *In 3c* involves nearly the whole long arm with the proximal break near the centromere and the distal one near the tip. The knob at .6 is included in the inverted segment of all three inversions. Gl_6 is in the proximal uninverted segment of *In 3a* while Lg_2 and A_1 are included in this inversion. *Lg* is in the inverted region of *In 3b*, *Gl* is in the proximal segment, and *A* is in the distal noninverted segment. All three loci are in the inverted portion of *In 3c* with the proximal breakpoint between the centromere and *Gl* and the distal breakpoint beyond the *A* locus.

The testcrosses of *K10 k10* plants singly heterozygous for the three inversions, for the large knob in 3L, and for the *Gl Lg A* loci gave the following percentages of recovery in the megaspores of the alleles carried by the knobbed chromosome 3 (items 16, 18, 22, Table 1).

	% *Gl*	% *Lg*	% *A*
In 3a/N	52.4	60.6	59.8
In 3b/N	50.3	51.6	49.7
In 3c/N	51.0	54.0	49.4

The varying percentages of recovered alleles in the three inversions are intelligible on the assumption that heteromorphic dyads are required for preferential segregation. Only the salient points will be briefly indicated. Except for the *In 3a* data, a full account will be postponed until a subsequent paper when plants homozygous for the three inversions will be compared with the heterozygotes. With *In 3a*, crossing over is frequent in the proximal segment between *Gl* and the proximal breakpoint. This leads to preferential segregation for alleles distal to the crossover. *Lg* and *A* are recovered with nearly the same frequency since they are separated only by rare double crossovers in the inversion loop. In *In 3b* heterozygotes, heteromorphic dyads are produced following single exchanges in the proximal region and by certain of the double exchanges. The genetic data show that these exchanges are infrequent although when they do occur the reciprocal classes are not equal. *In 3c* is a long inversion and double exchanges within the loop are frequent. When one exchange occurs to the right and one to

the left of *Lg*, heteromorphic dyads result and preferential segregation for *Lg* is found. Since one of the two exchanges usually separates *Lg* and *A*, the *A* locus shows random segregation (49.4%) while the *Lg* allele was found in 54.0% of the ovules.

Conclusion: All of these experiments have in common a reduction in crossing over ranging from almost complete suppression, as in the (R)9 case, to variable but reduced amounts. The degree of preferential segregation found in the different combinations is wholly consistent with the hypothesis that the formation of heteromorphic dyads by crossing over between the centromere and knob is an essential prerequisite for preferential segregation.

Preferential Segregation from Different Exchanges

The question next considered is whether or not the degree of preferential segregation to the basal megaspore is identical from heteromorphic dyads produced in tetrads with a single exchange between knob and centromere and in those arising from double exchanges with one proximal and one distal to the knob. The answer was obtained from three testcrosses listed in Table 1 (items 3, 4, and 11) involving plants of *K10 K10* or *K10 k10 Gl Lg K A/gl lg k a* constitution. The *K10* population (item 5) with a total of 8427 individuals was not deemed appropriate for analysis because of the semilethal effect of the segregating *et* allele which is 12 crossover units distal to *A*. The population with 2967 plants (item 4 of Table 1) is analysed below. A similar analysis was made for the other two populations.

The genetic data were translated into tetrad frequencies from the combined totals of the noncrossover classes, the singles in (1), etc., on the assumption of no chromatid interference. The only exchanges considered are singles in (1) and (2) and doubles in (1-2). This transformation gave 0.6% no-exchange tetrads, 39.0% with a single exchange in (1) (*Gl-Lg*), 12.4% with a single exchange in (2) (*Lg-A*), and 48.0% with exchanges in (1) and (2). The contributions to the complementary crossover and noncrossover chromatid classes expected from these tetrads can be calculated if the degree of preferential segregation can be determined for any complementary pair of crossover classes and the amount found is assumed to hold for all heteromorphic dyads irrespective of their origin. If the fit between the calculated chromatid frequencies and those observed is close, it may be argued that the same amount of preferential segregation occurs in heteromorphic dyads derived from either single or double exchanges.

The 2- and 3-strand double exchanges are the source of double-crossover chromatids. All dyads from these exchanges are heteromorphic for the knob lying distal but closely linked to the *Lg* allele. The *Gl lg A* doubles are knobless. Three hundred and fifty-eight doubles were found, of which 250 (70%) were *gl Lg a*. Complementary single crossovers in (1), in (2), and both types of noncrossover chromatids are produced by 3-strand doubles. In calculating the expected proportions of the genotypes from double exchanges it is assumed that the knobbed and knobless complementary strands for each class are recovered in the same 70:30 ratio as were the double-crossover chromatids. Four-strand double ex-

changes give rise to heteromorphic dyads composed of singles in (1) and (2). Tetrads with single exchanges in (1) also form heteromorphic dyads and again the assumption of 70% preferential segregation is made in allocating the products to the crossover and noncrossover classes. No-exchange tetrads and those with single exchanges in (2) produce homomorphic dyads and segregation should be random.

Table 2 gives the calculated and observed values for the three populations analyzed. The agreement between the estimated frequencies of the various chromatid classes based on the above postulations and those observed is extremely good. The assumptions made appear to be justified and the conclusion is drawn that heteromorphic dyads derived from single exchanges undergo the same degree of preferential segregation as do those from double exchanges.

Since the degree of preferential segregation is the same for single- and double-exchange tetrads, it follows that the ratio of singles in (1) should give estimates of preferential segregation which are similar to those obtained from only double crossovers. There are several ways of arriving at this estimate from singles. The different ways of determining preferential segregation give essentially the same value. This is true for the population analyzed above and also for the other two populations entered in Table 2. A value of 71.4% is obtained for the data of item 4, Table 1, by using the observed genetic ratio of single crossovers in region 1. The number of knobbed and knobless double crossovers should equal the number of singles coming from double exchanges. When the contribution of the doubles is subtracted from the appropriate single-crossover classes in order to ascertain

TABLE 2

Comparison of observed percentages of chromatid classes with those calculated from tetrads on the assumptions that the degree of preferential segregation is the same in all heteromorphic dyads and that segregation is random in homomorphic dyads

	(0) *Gl* *Lg* *A*	(0) *gl* *lg* *a*	(1) *Gl* *lg* *a*	(1) *gl* *Lg* *A*	(2) *Gl* *Lg* *a*	(2) *gl* *lg* *A*	(1-2) *Gl* *lg* *A*	(1-2) *gl* *Lg* *a*
K10 k10 K3 k3								
Item 4, Table 1 $\Sigma = 2967$								
Observed percentage	26.2	12.1	9.2	22.3	11.6	6.6	3.6	8.4
*Calc. 70% pref. seg.	25.5	12.9	9.4	22.1	11.5	6.7	3.6	8.4
†Calc. 71.3% pref. seg.	25.9	12.4	9.0	22.5	11.7	6.5	3.4	8.6
Item 11, Table 1 $\Sigma = 1900$								
Observed percentage	26.1	12.6	8.8	22.6	11.7	6.8	3.2	8.1
*Calc. 72.1% pref. seg.	26.3	12.4	8.8	22.6	11.8	6.8	3.2	8.1
†Calc. 71.3% pref. seg.	26.3	12.4	8.8	22.6	11.8	6.8	3.2	8.1
K10 K10 K3 k3								
Item 3, Table 1 $\Sigma = 1178$								
Observed percentage	27.7	10.7	8.3	23.5	11.4	5.8	3.1	9.5
*Calc. 75.7% pref. seg.	27.4	11.0	7.7	24.1	11.8	5.4	3.1	9.5
†Calc. 72.7% pref. seg.	26.3	12.1	8.8	23.0	11.3	5.9	3.6	9.0

* Preferential segregation based on ratio of double crossovers.
† Preferential segregation based on ratio of adjusted singles in region 1.

the number of crossovers coming only from single exchanges in region 1, the ratio of adjusted singles gives 71.3% preferential segregation. Finally, estimates of single crossovers in (1) can be had from the calculated tetrad frequencies. This method gives 70.8%.

It is of interest that preferential segregation appears to be a little higher in *K10 K10* plants than in *K10 k10*, but the one *K10 K10* population is the smallest of the three populations analyzed and sampling errors may be responsible for the difference. However, preferential segregation in *K10 K10 K9 k9* (61.4% for *Wd*) plants is also slightly higher than that in *K10 k10 K9 k9* sibs (59.4%) (items 31, 32, Table 1). The degree of preferential segregation in each of the *K10 K10* populations is not significantly higher than that in their respective *K10 k10* sibs, but it becomes so if the two populations are considered together. It may well be that homozygous *K10* compounds have a higher level of preferential segregation but the data in hand do not afford a firm conclusion because the difference was only significant at the .05 level.

Single exchanges in region 2 produce homomorphic dyads. Random segregation should occur producing equal frequencies of the two complementary crossover classes. The sums of the observed crossovers in region 2 for the three populations are 701 *Gl Lg a* and 395 *gl lg A*. The deviation from 1:1 is due to the contribution of single crossovers in (2) from 3- and 4-strand doubles which lead to preferential segregation. If there is no chromatid interference, the number of knobbed chromatids coming from double exchanges which are single crossovers in region 2 should equal the number of knobbed double-crossover chromatids while the knobless crossovers in (2) and knobless double-crossover chromatids should occur with the same frequency. If the 517 *gl Lg a* and 204 *Gl lg A* double crossovers are subtracted from the appropriate knobbed and knobless classes of singles in (2), the remainders, 184 and 191, represent the crossovers in (2) coming from single-exchange tetrads. Equal numbers are expected and realized.

The percentage of preferential segregation from heteromorphic dyads always exceeds that of the recovery of the *Lg* allele (or the knob) in the testcross progeny because tetrads with single exchanges in region 2 and with no exchanges result in equal numbers of the *Lg* and *lg* alleles in the basal megaspore. However, the percentage of preferential segregation from heteromorphic dyads never reaches 100%. Even when heteromorphic dyads are produced, preferential segregation does not occur invariably.

Chromatid interference will affect the calculations of tetrad ranks from the genetic data and their subsequent transformation to chromatid classes. Not a great deal is known about chromatid interference in maize, but the half-tetrad analysis of diploid eggs produced by plants homozygous for the elongate gene (*el*) gave a 2:1 ratio of the combined 2- and 4-strand doubles to one class of 3-strand doubles involving the long arm of chromosome 3. The other 3-strand class was not detectable (RHOADES and DEMPSEY 1966). No chromatid interference is also indicated in the studies of *In 3a* by RHOADES and DEMPSEY (1953). Scant though the data be, the assumption of no chromatid interference is probably justified and no serious error is introduced into the calculations. Indeed, all

of the data manipulation gave results which were consistent on the assumption of no chromatid interference.

It can be concluded from the above analysis that heteromorphic dyads derived from single exchanges in (1) and from doubles in (1) and (2) have the same degree of preferential segregation. However, the available data for chromosome 3 shed no light on the possibility that the position of the exchange may influence the segregation pattern. The critical exchange producing preferential segregation is in region 1. Over 40% recombination occurs in this region in *K10* plants, and it is not possible to define the position of exchanges within such a long interval. The magnitude of preferential segregation represents an average value derived from all exchanges and gives no indication of any possible effect of exchange position within this segment. However, tentative conclusions can be reached by analyzing the extensive testcross data on four linked genes in the short arm of chromosome 9 (given in Table 2, KIKUDOME 1959) if the ratio of adjusted complementary single crossover chromatids or the ratio of the observed singles is used to determine the amount of preferential segregation following exchanges in each region. The validity of this manipulation has been established above. The estimate from singles is preferable to that from doubles because of the low number of double-crossover chromatids.

The results of this analysis, shown in Table 3, reveal a progressive decrease in the degree of preferential segregation. Single exchanges in region 3, which is nearest the centromere, gave 73.4% preferential segregation, those in region 2 and those in region 1, 67.5 and 66.1% respectively. The degree of preferential segregation determined from the ratio of observed singles in each region gave the same seriation. The significance of this observation is not understood, but a differential accumulation of centromere substance may be involved. If this relationship between exchange position and centromere can be shown to hold for

TABLE 3

Data from Table 2 of Kikudome (1959) on preferential segregation of chromosome 9

K10 k10 K^m Yg C Sh Wx / K^s yg c sh wx × yg c sh wx

	(0) *Yg C Sh Wx*	(0) *yg c sh wx*	(1) *Yg c sh wx*	(1) *yg C Sh Wx*	(2) *Yg C sh wx*	(2) *yg c Sh Wx*	(3) *Yg C Sh wx*	(3) *yg c sh Wx*	(1-2) *Yg c Sh Wx*	(1-2) *yg C sh wx*	(1-3) *Yg c sh Wx*	(1-3) *yg C Sh wx*	(2-3) *Yg C sh Wx*	(2-3) *yg c Sh wx*
No.	7854	3290	1509	797	494	248	2258	849	23	19	70	51	25	14
Percent	44.9	18.8	8.6	4.6	2.8	1.4	12.9	4.8	0.1	0.1	0.4	0.3	0.1	0.1

Adjusted singles in region 1: $1509 - (23 + 70) = 1416$ $\frac{1416}{2143} = 66.1\%$ pref. seg. in (1)

Adjusted singles in region 2: $797 - (19 + 51) = 727$, $494 - (23 + 25) = 446$ $\frac{446}{661} = 67.5\%$ pref. seg. in (2)

Adjusted singles in region 3: $248 - (19 + 14) = 215$, $2258 - (70 + 25) = 2163$, $849 - (51 + 14) = 784$ $\frac{2163}{2947} = 73.4\%$ pref. seg. in (3)

other chromosomes, it will play an important role in reaching an understanding of the interaction between knobs and centromeres.

Preferential segregation occurs only when heteromorphic dyads are formed, but its degree may vary following exchanges in a specific chromosome segment when differences in knob size exist. KIKUDOME (1959) found that the recovery of the *Wd* allele varied from 68.8% in *K10 k10* $K^L9/k9$ plants to 59.1% in *K10 k10* $K^s9/k9$ plants (K^L9 is a large terminal knob and K^s9 is a small knob) even though the same amount of recombination took place between the *Wd* and *Wx* loci and equal numbers of heteromorphic dyads were formed. The degree of preferential segregation is influenced by the difference in knob size exhibited by the chromatids of the dyad; the greater the difference, the greater is the degree of preferential segregation.

The Effect of Abnormal Chromosome 10 on Crossing Over

That abnormal chromosome 10 (*K10*) increases crossing over was first reported in 1957 by RHOADES and DEMPSEY. This conclusion has been confirmed by subsequent investigations in this laboratory as well as by KIKUDOME (1959). The data given in Table 1 were subjected to an analysis for the effect of *K10* on recombination in different kinds of chromosome 3 bivalents. Data from plants with the same genotype, insofar as their constitution for chromosomes 10 and 3 is concerned, were combined. Also grouped together were plants with *K10 K10* and *K10 k10* because they had the same behaviour in testcrosses. Plants with two knobbed chromosomes 3 (*K3 K3*) and those with two knobless 3's (*k3 k3*) are designated in Table 4 as homomorphic and those with one knobbed and one knobless chromosomes 3 (*K3 k3*) are labelled heteromorphic. The analysis of the different combinations of the *K10 k10*, *K3* and *k3* chromosomes includes a comparison of the calculated tetrad ranks, the observed chromatid ranks, and the amount of recombination found for the two marked regions, *Gl-Lg* and *Lg-A*, in the long arm of chromosome 3.

The comparison of calculated tetrad ranks in plants with homomorphic chromosomes 3 (*K3 K3* or *k3 k3*) discloses that the plants with *K10* have more tetrads with double exchanges and slightly more with single exchanges in region 1 but fewer tetrads with single exchanges in (2) and with no exchanges than do *k10 K3 K3* and *k10 k3 k3* plants. *K10 K3 K3* individuals differ from *K10 k3 k3* in having fewer single exchanges in (2) and more double-exchange tetrads. The same amount of recombination (37%) in the *Lg-A* region for the two combinations is obtained because the smaller frequency of single exchanges in (2) found in *K3 K3* plants is almost exactly compensated for by an increase in number of doubles. It is noteworthy that the frequency of recombination between *Lg* and *A* is nearly identical in all of the four classes with homomorphic chromosomes 3 (Table 4). There occurs, however, in these combinations a highly significant increase in recombination for the proximal *Gl-Lg* region in *K10 K3 K3* and *K10 k3 k3* compared to the value found in *k10 K3 K3* and *k10 k3 k3* as is shown in the following tabulation.

TABLE 4

Calculated frequencies of tetrad ranks and observed percentages of chromatid ranks from the combined totals of progenies of similar genotypes. Data from Table 1

	Calculated tetrad rank frequencies				Observed strand rank frequencies				Observed recombination percentages				Segregation percentages		
	(0)	(1)	(2)	(1-2)	(0)	(1)	(2)	(1-2)	*Gl-Lg*	*Lg-A*	Σ	*Coin.*	*%Gl*	*%Lg*	*%A*
Homomorphic chromosomes 3 *(K3 K3* or *k3 k3)*															
K10 K3 K3 Σ = 4311	—0.8	26.4	18.8	55.6	35.7	27.1	23.3	13.9	41.0	37.2	78.2	0.92	49.2	50.5	49.7
K10 k3 k3 Σ = 3628	—1.2	27.6	31.8	42.8	38.2	24.5	26.6	10.7	35.2	37.3	72.5	0.82	50.6	50.8	52.3*
k10 k3 k3 Σ = 2388	6.0	20.2	43.0	30.8	45.3	17.8	29.2	7.7	25.5	36.8	62.3	0.83	51.3	49.5	51.6
k10 K3 K3 Σ = 735	4.5	19.6	42.2	33.6	43.8	18.2	29.5	8.4	26.6	37.9	64.5	0.84	53.1	51.0	48.6
Heteromorphic chromosomes 3 *(K3 k3)*															
K10 K3 k3 Σ = 14,472	2.4	40.6	10.2	46.8	39.5	32.0	16.8	11.7	43.7	28.5	72.2	0.94	51.1	71.1	61.1
k10 K3 k3 Σ = 4774	13.0	40.8	17.4	28.8	49.3	27.6	15.9	7.2	34.8	23.1	57.9	0.90	49.7	50.7	51.5
													%gl	*%lg*	*%A*
K10 In 3b K3 / *In 3b k3* Σ = 1601	3.8	24.2	18.4	53.6	38.5	25.5	22.6	13.4	38.9	36.0	74.9	0.96	55.5	66.8	54.1
k10 In 3b K3 / *In 3b k3* Σ = 1093	18.4	21.2	30.8	29.6	51.8	18.0	22.8	7.4	25.4	30.2	55.6	0.96	48.9	50.4	49.7

* Based on single population of 3628 in which *et* was segregating.

			Percentage increase in recombination for the *Gl-Lg* region
K10 k3 k3	vs.	*k10 k3 k3*	38
K10 K3 K3	vs.	*k10 k3 k3*	61
K10 K3 K3	vs.	*k10 K3 K3*	54
K10 k3 k3	vs.	*k10 K3 K3*	32

Plants heterozygous for the knob on the long arm of chromosome 3 give a somewhat different pattern. As in the homomorphic classes there were more tetrads with double exchanges, fewer single-exchange tetrads in (2) and fewer with no exchanges in *K10* than in *k10* plants. They were unlike in that the homomorphic tetrads had more single exchanges in region 1 in *K10* than in *k10* individuals while in heteromorphic *K3 k3* plants equal frequencies of single exchanges in (1) occur in *K10* and *k10*.

The observed amount of recombination for region 2 in *k10 k3 k3* plants is considerably higher (an increase of 59%) than that in *k10 K3 k3*. The lower amount in *K3 k3* heterozygotes is due to the heterozygous knob located within region 2 which is known to produce asynapsis of the adjacent homologous segments. The decrease in region 2 is, however, accompanied by a compensatory increase (36%) in the *Gl-Lg* region. Comparison of recombination values for the *Gl-Lg* and *Lg-A* regions from *K10 K3 k3 and K10 k3 k3* combinations likewise reveals a compensatory increase in the *Gl-Lg* region in *K3 k3* plants when the value for the *Lg-A* interval is lowered because of knob heterozygosity. The reduction in the *Lg-A* interval in *K10 K3 k3* individuals is less than that in *k10 K3 k3* and it appears that the *K10* chromosome is able to partially overcome the decrease due to knob heterozygosity. It has been our experience that a decreased amount of recombination in one segment of a chromosome is commonly accompanied by an increase in adjacent regions of the arm. It is not known whether or not this compensatory increase extends to regions on the other side of the centromere.

The same relative proportions of tetrad and chromatid ranks are found in *K10* and *k10* plants homozygous for *In 3b* as in plants with uninverted chromosomes. The differences in recombination for the two marked regions reflect the changed lengths of these segments. There was a 53% increase in *K10* plants for the *Gl-Lg* region and a 19% increase in crossing over for the *Lg-A* region. The data for homozygous *In 3b* agree in general with those from normal chromosomes 3 which are heteromorphic for the knob and need not be discussed further.

It may be concluded with some certitude that the *K10* chromosome not only induces a significant increase in crossing over in the proximal *Gl-Lg* segment of chromosome 3 but also tends to reverse the decrease in the *Lg-A* region produced by the heterozygous knob. The enhancement in crossing over by *K10* in chromosomes 3 which are devoid of structural rearrangements is highly significant but not spectacular. However, the data in Table 1 from plants heterozygous for three different paracentric inversions in the long arm of chromosome 3, which are described in an earlier section, show a much more dramatic ability of *K10* to increase recombination values in structural heterozygotes where crossing over is greatly reduced. The amount of detected crossing over between *Gl* and *Lg* in

In 3b heterozygotes carrying *K10* is four times that in *k10* sibs (items 15 and 16, Table 1). The only crossover strands ordinarily recovered from exchanges within the inverted segments are those from double exchanges since the crossover chromatids involved in single exchanges go to form the dicentric bridge and acentric fragment. A minimal estimate of recombination within the loop can be obtained from the percentage of pollen or ovule abortion. *K10* plants heterozygous for *In 3b* had approximately 70% more aborted pollen than did *k10* sibs.

The increase in recombination in *In 3a* heterozygotes induced by the *K10* chromosome is particularly instructive because the genetic data for the *Gl-Lg* region are paralleled by cytological observations. Between two and three times as much recombination between *Gl* and *Lg* is observed in *In 3a* heterozygotes with *K10* as in those homozygous for the *k10* chromosome (items 17 and 18, Table 1). Certain double exchanges with one crossover in the proximal uninverted segment, in which occur nearly all of the exchanges producing crossovers between *Gl* and *Lg*, result in anaphase I microsporocytes with an acentric fragment and no dicentric bridge. The frequency of such configurations in *K10* plants was three times that found in *k10* individuals. The increase found in the genetic data is in agreement with that observed cytologically. Further evidence of enhanced recombination in *K10* plants comes from the sixfold increase for the *Lg-A* region in *K10* compared to the value in *k10* plants.

The number of double-crossover chromatids recovered from *K10 In 3c* heterozygotes is five times that in *k10* individuals. Comparable data on crossover enhancement have been obtained from translocation studies. There exist data from diverse experiments that are consistent in demonstrating the ability of the *K10* chromosome to greatly enhance crossover values in structural heterozygotes. In the case of structural homozygotes for chromosome 3 the enhanced crossover values were in proximal segments, but this localization does not appear to hold for structural heterozygotes. No explanation is offered concerning the localization of increased crossover values by *K10* to the proximal *Gl-Lg* region of structural homozygotes, but in the case of *In 3b* heterozygotes the solution is believed to lie in the more intimate pairing of the inverted and normal chromosomes that we have observed in sporocytes with the *K10* chromosome (Figure 1). This correlation of the intimacy of pachytene pairing with recombination frequency will be

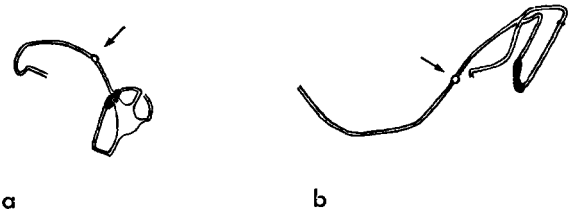

FIGURE 1.—Pachytene pairing of normal chromosome 3 and an *In 3b* chromosome. A large knob is present in both chromosomes. The centromere is indicated by an arrow. (a) From a plant carrying normal chromosomes 10. (b) From a plant heterozygous for abnormal chromosome 10. These camera lucida drawings illustrate the more intimate pairing produced by *K10* chromosome in inversion heterozygotes.

considered in another paper in which our present observations and those from other studies will be presented in relation to their bearing on the time and mechanism of crossing over.

It was concluded that the enhancement of crossing over by *K10* was restricted to the proximal *Gl-Lg* region in plants with homomorphic chromosomes 3. The *Gl-Lg* region consists in large part of heterochromatic, deeply staining chromomeres. Crossing over in *k10* plants within segments adjacent to the centromere is much lower per unit of pachytene length than is that occurring in euchromatic regions. If the increase in recombination induced by *K10* in the proximal regions of chromosome 3 holds for similar regions of other chromosomes of the complement, then the *K10* chromosome can be utilized to release cryptic genetic variability by promoting genetic exchange in regions which normally tend to be inherited en bloc. Little is known about the genetic function of heterochromatin in maize but it has been suggested that loci situated therein are of importance in the inheritance of quantitative traits. If true, greater genetic variation should be a consequence of more frequent recombination. The *K10* chromosome may prove to be of some value by breaking tight linkages in heterochromatic regions.

Preferential Segregation from Deficient Dyads

In *K10* plants with two structurally normal chromosomes 3 heterozygous for the knob in the long arm, the knobbed chromatid was preferentially recovered from dyads which were heteromorphic for the knob in approximately 70% of the basal megaspores. In dealing with the *In 3a* data it is possible to consider the segregation pattern in dyads having one intact chromatid and a deficient one arising from breakage of the AI dicentric bridge produced by crossing over in the inversion loop.

The high amount of ovule abortion in *K10 k10 In 3a*/N3 plants is much greater than that which can be ascribed to 4-strand doubles in the loop. Double bridges are found in only 2% of the microsporocytes and should be of equal frequency in megasporocytes since the amount of crossing over is the same in reciprocal crosses. The data demand that the deficient chromatids arising from breakage of single bridges are often included in the basal megaspores which subsequently abort. This is in contradistinction to the behavior of dicentric bridges from inversion crossing over in Sciara where the two chromatids comprising the bridge are excluded from the egg nucleus (CARSON 1946). If the normal chromatids of a deficient dyad, irrespective of whether it is knobbed or not, invariably moved to the basal pole, there would be little ovule abortion. The question remains of the segregation ratio in dyads composed of a deficient chromatid and a normal knobbed one as well as in those having a deficient chromatid and a normal knobless one.

The following conclusions may be tentatively adduced from an analysis of the *K10 k10 In 3a*/N3 data of Table 1 (item 18). (1) In a dyad with a deficient chromatid and a knobbed intact chromatid, the knobbed strand moves to a terminal pole 70% of the time. This value of 70% recovery from deficient dyads

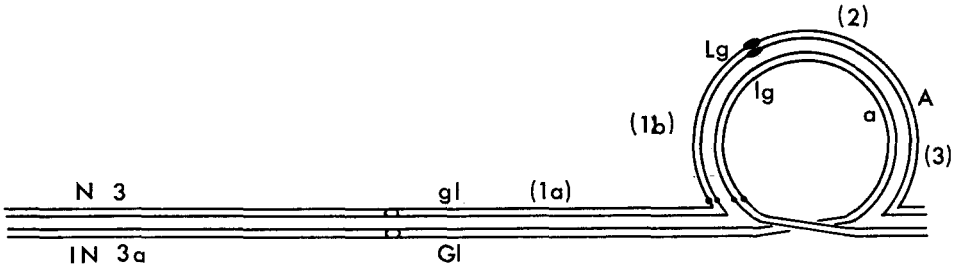

FIGURE 2.—Diagram of pairing in a N3 *gl Lg K A / In 3a Gl a k lg* heterozygote. The crossover regions are designated (1a) from *gl* to the proximal breakpoint of the inversion, (1b) from the proximal breakpoint to *Lg*, (2) from *Lg* to *A*, and (3) from *A* to the distal breakpoint.

appears to hold irrespective or whether or not the deficient chromatid is knobbed or knobless. The same value is found with nondeficient heteromorphic dyads. (2) Random segregation occurs in deficient dyads having a knobless intact chromatid; this is apparently true regardless of the presence or absence of a knob in the deficient chromatid.

The argument behind the above conclusions is as follows. The *Gl-Lg* crossovers listed in item 18 of Table 1 (*Gl Lg A* and *gl lg a*) arise from single exchanges in (1a), from 3- and 4-strand doubles in (1a-1b), (1a-2), and (1a-3), and from 2- and 3-strand doubles in (1b-3). The crossover regions mentioned here are identified in Figure 2 in which a heterozygous *In 3a* bivalent is diagrammed. Dicentric bridges are not produced by single exchanges in (1a) but, since heteromorphic dyads result, 70% of the basal megaspores receive a knobbed chromatid of *Gl Lg A* constitution. All of the 3-strand doubles in (1a-1b), (1a-2), and (1a-3) yield one dyad with a dicentric loop, which forms a bridge at AII, and one with two normal chromatids of which one is knobbed and the other knobless. This latter dyad undergoes 70% preferential segregation of the knobbed chromatid. However, both dyads resulting from 4-strand doubles in these regions have one normal and one deficient chromatid. Half of the deficient dyads will contain a normal chromatid with a knob and in these cases 70% preferential segregation of the knobbed over the deficient chromatid is assumed. When the intact chromatid is knobless, segregation should be random. In a deficient dyad with an intact knobless chromatid and a knobbed deficient one, the knob derived from the bridge would not have undergone a poleward orientation at AI and it would have no effect in controlling AII segregation, which would be random for the two chromatids. From 4-strand doubles giving such dyads, a ratio of 70 *Gl Lg A*: 50 *gl lg a* chromatids should be obtained or 58.3% recovery of the knobbed crossover strand, while the average recovery from combined 3- and 4-strand doubles is 63.6% (70 *Gl Lg A*: 40 *gl lg a*).

The 2-strand doubles in (1b-3) form no dicentric bridges and 70% preferential segregation should occur while the 3-strand doubles give a 70:50 ratio of *Gl Lg A* to *gl lg a* chromatids. The frequency of *Gl Lg A* from all (1b-3) doubles should be 63.6% (i.e., 70:40).

All double exchanges which yield crossovers between *gl* and *lg* only should, on

the assumptions made, produce a ratio of 70 single crossovers with a knob to 40 which are knobless. Doubles in regions (1b-2) and (2-3) do not produce *Gl Lg A* or *gl lg a* chromatids. The single exchanges in region 1a give 70% preferential segregation. It follows that the observed percentage of *Gl-Lg* crossovers with knobbed chromatids should lie between 70% and 63.6%. The 65.5% found is in good accord with the value expected on the basis of the postulated behavior of deficient dyads.

If it be assumed that intact knobbed chromatids invariably were recovered from deficient dyads and that random segregation occurred in deficient dyads with a knobless normal chromatid, an average percentage of recovery of knobbed *Gl-Lg* crossovers would be 68% from double-exchange tetrads. In the single-exchange tetrads, a knob recovery of 70% occurs. The observed percentage of recovery of the knobbed single-crossover chromatids should therefore lie between 68 and 70, depending upon the relative frequencies of single exchanges in (1a) and of the appropriate doubles. The 65.5% observed is in agreement with the first hypothesis but the demonstration can hardly be said to be rigorous.

A similar analysis of the (1b-2) and (2-3) double-crossover chromatids likewise reveals that a 70:50 segregation ratio for deficient dyads gives a better fit to the observed frequencies than does a 100:50 ratio. As in the case of the single crossovers in (1a), these data are best accounted for by the poleward orientation at AI of the knobbed normal chromatids and the persistence of this orientation at MII, possibly as a consequence of neocentric activity.

The segregation pattern at AII does not appear to be affected by the knobbed or knobless constitution of the deficient chromatid derived by breakage of the dicentric bridge. The latter statement holds for deficient dyads with a normal knobless chromatid which give random segregation at AII. Whether or not the deficient chromatid is knobbed or knobless appears to be immaterial.

A small fraction of the duplication-deficiency (Dp Df) chromatids, those coming from bridge breakage close to one of the two centromeres, hence not greatly deficient, are able to form viable embryo sacs. The argument for the segregation patterns in deficient dyads was based on the observed frequencies of the complementary crossovers between *Gl* and *Lg*. It is essential to determine if an unequal contribution to these classes could come through Dp Df chromosomes which appear to be crossovers in (1). Since single exchanges within the inverted segment are much more numerous than are doubles, only the contribution of single exchanges to the formation of Dp Df strands will be considered here. The contribution of the comparatively infrequent double exchanges is negligible.

Dp Df chromosomes of *gl lg a* and *Gl Lg A* constitution arise from single exchanges in regions 1b and 3. Single exchanges in (1b), between the proximal breakpoint of the inversion and the *Lg* locus, lead to the formation of a dicentric bridge carrying the *gl lg a Gl* alleles reading from one centromere to the other. The two intact chromatids are noncrossovers but one is knobbed. Rupture of the bridge between the *a* allele and *Gl* yields a Dp Df *a lg gl* strand which in some instances might form viable megaspores. Operating against its recovery in a basal megaspore is the fact that the Dp Df *a lg gl* deficient strand has a knob-bearing

intact chromatid as its partner and the knobbed strand will be preferentially recovered. Consequently, few if any viable deficient strands from bridge breakage with the *gl lg a* alleles are anticipated.

Single exchanges in region 3, the segment from the *A* locus to the distal breakpoint of the inversion, form dicentric bridges carrying the *gl Lg A Gl* alleles in the order listed. Breaks at either end of the bridge between the *gl* allele and one centric region and between the *Gl* allele and the other centric region produce Dp Df strands of *gl Lg A Gl* constitution. Bridge breakage between *gl* and *Lg* will also give strands with the three dominant alleles. Not being grossly deficient, a portion of these could be recovered and contribute to apparent *Gl Lg A* single crossovers. When breakage occurs between *Lg* and *gl* or between *gl* and the centromere, the Dp Df strand will be associated with a knobless normal strand and a few *Gl Lg A* Dp Df chromatids may be recovered. The frequency of single exchanges in (3) cannot be estimated from the present data but it is known to constitute a relatively small portion of the inverted segment (RHOADES and DEMPSEY 1953). Any contribution of simulated crossovers to region 1 classes from Dp Df chromosomes should have no marked effect on the ratio of the complementary *Gl Lg A* and *gl lg a* classes.

One might predict that the knobbed intact chromatid of a deficient dyad should show a higher degree of preferential segregation than the knobbed chromatid of a heteromorphic dyad consisting of two normal chromatids, but according to the analysis presented above, no difference exists. The explanation may lie in the behavior of the deficient chromatid following rupture of the bridge. Each portion of the broken bridge may literally snap back towards its centric region in much the same way as does an elastic fiber under tension which is suddenly severed. Support for this concept comes from BAJER's (1963) observations of bridge breakage in living cells. After release from the bridge, the dyad would undergo the same degree of preferential orientation as a heteromorphic dyad with two normal chromatids.

The conclusion that in *K10 k10 In 3a k3*/N3 *K3* plants preferential recovery of knobbed normal chromatids occurs in deficient dyads and that segregation is random when the intact chromatid is knobless is amenable to testing. There should be a lower percentage of ovule abortion in *K10 k10 In 3a k3*/N3 *K3* plants than in *K10 k10 In 3a k3*/N3 *k3* individuals where there is no preferential segregation of chromosome 3. The amount of crossing over in the two combinations should be essentially equal judging from the data on structurally normal chromosomes 3. Single exchanges in (1b), (2), and (3) in *K3 k3* plants should lead to 40% ovule abortion while the same exchanges in *k3 k3* megasporocytes produce 50% ovule abortion. From all double exchanges in *K3 k3* plants, 45% of the basal megaspores receive a deficient chromatid while in *k3 k3* plants the fraction would be 50%. If the degree of preferential segregation from deficient dyads with a knobbed intact chromatid exceeds 70%, there will be a correspondingly greater difference in ovule abortion. On the other hand, if these dyads underwent random segregation there would be no difference in ovule abortion between *K3 k3* and *k3 k3* plants.

Neocentromeres and Preferential Segregation

In this section earlier cytological observations on neocentromere formation are reviewed and further cytological studies are presented. The concordance between the preferential segregation of knobbed chromatids and their neocentric activity is discussed. The hypothesis is advanced which seeks to account for preferential segregation on the basis of polarized orientation due to neocentromeres. Finally, certain discordant observations are considered.

In addition to its ability to induce preferential segregation and to increase recombination, the *K10* chromosome causes the formation of neocentromeres during the two meiotic divisions (RHOADES and VILKOMERSON 1942; RHOADES 1952). Cytological observations on neocentromeres in *K10 K10* plants are given in the 1952 paper. A brief summary is as follows.

The first meiotic division is in no way exceptional until metaphase I. Bivalents are co-oriented on the spindle in the usual manner with half-spindle fibers, originating from the true centric regions, facing poleward. Normally these fibers effect anaphase movement of the disjoining dyads with the localized centromere region proceeding first to the pole. However, in plants with the *K10* chromosome, chromosomal fibers arise from distal portions of certain chromosomes at the time the bivalents are still co-oriented on the spindle at MI. The neocentric regions move poleward more rapidly than the true centric regions. The distal ends of the chromatids, instead of being directed toward the spindle plate during AI, lead the way in the poleward migration. Chromosomal fibers may arise from one or both of the long arms of each dyad at late metaphase or at early anaphase. When both long arms of the two chromatids of a dyad possess a neocentric region, the chromosomal fibers arising from these centric regions are usually directed towards the same pole.

The ensuing telophase is normal. All four arms of each dyad contract to form a spherical mass of chromatin. The chromonemata uncoil during interphase and early PII finds each daughter cell with ten long X-shaped dyads of typical appearance with the two chromatids of each dyad conjoined by the primary centric region. There is no indication of neocentric activity at this time. The onset of MII may sometimes occur before the dyads have undergone their usual contraction. Precocious fibers arise near the ends of certain chromatids and these arms are stretched toward the poles before the true centromere region divides. The number of dyads with precocious spindle fibers, judged by the number of arms pulled poleward at MII, varies in different strains. The maximum number in some plants was seven, in others five, etc. Plants with seven knobbed chromosomes had a maximum of seven dyads with arms stretched poleward at MII. Those with five knobs had five such dyads. A convincing demonstration of the relationship between knobs and neocentromere activity came from the analysis of a plant with homozygous knobs on nine chromosomes (one of these was *K10*) and one pair which was knobless. Pachytene studies disclosed that chromosome 9 was knobless, that chromosomes 2, 4, 5, 6, 7, 8, and 10 were homozygous for a knob in their long arms, and that chromosomes 1 and 3 were homozygous for knobs in both their short and long arms. If neocentromeres are formed only at knobbed regions, one dyad at MII should have no neocentric activity, seven should have neocentric regions only in their long arms with the short arms behaving normally, and two of the dyads should have neocentromeres in both their short and long arms. As Figure 3 illustrates, this is precisely the situation observed. The two dyads with the short and long arms of each chromatid extended poleward are clearly two of the longer chromosomes and presumably are chromosomes 1 and 3, the longest and third longest of the complement. The normal appearing dyad is obviously one of the smaller chromosomes and it may be assumed to be chromosome 9, the second shortest.

Circumstantial though the evidence is, it may be reasonably concluded that precocious neocentric activity at both AI and MII is directly related to the hetero-

FIGURE 3.—Metaphase II in a plant homozygous for abnormal chromosome 10. One dyad (chromosome 9) has no knobs and shows no neocentric activity; seven dyads have knobs in their long arms and these arms are extended poleward; and two dyads (chromosomes 1 and 3) possess knobs in the long and short arms and neocentromeres are present in both arms.

chromatic knobs. In other strains we have observed dyads at AI and MII with only one arm of the four exhibiting neocentric activity. This is understandable if they arose from a bivalent heterozygous for a knob in which an exchange had taken place between the knob and centromere, thus forming heteromorphic dyads consisting of one knobbed and one knobless chromatid. Owing to its neocentric activity, the knob-bearing chromatid comes to lie nearest the pole at the end of anaphase.

The genetical studies prove that segregation is random in *K10* plants when the chromosomes of a pair are both knobbed or knobless, that preferential segregation depends upon an exchange producing heteromorphic dyads, and that it is the knobbed chromatid which is preferentially recovered in the basal megaspore. Because the genetical results and the cytological observations (even though the latter were made in microsporocytes) paralleled one another so strikingly, the following working hypothesis was presented (RHOADES 1952) to account for preferential segregation on the basis of neocentromere formation.

In maize the two spindles of the second meiotic division of the megaspore mother cell are arranged in tandem. The basal megaspore of the linear set of four develops into the female gametophyte, the remaining three aborting. In plants heterozygous for knobbed and knobless homologues, one arm of some of the disjoining dyads at AI possesses precociously acting chromosomal fibers not evident in the homologous arm. It is believed that the knobbed arms form these neocentric regions while knobless arms do not. The rapidity with which neocentric regions pass poleward brings those chromatids possessing them to the pole in advance of the knobless arms. In a dyad consisting of one knobbed and one knobless chromatid, the knobbed chromatid would come to lie closer to the pole while the knobless partner would face the spindle plate. Preferential segregation would

occur if this orientation persists until the second metaphase so that the knobbed chromatids face the two terminal poles and the knobless chromatids are oriented towards the two inner poles. Accordingly preferential segregation would take place only when there has been an exchange between the knob and the primary centric region in a heterozygous bivalent. It follows that the level of preferential segregation would be directly related to the amount of crossing over in the knob-centromere interval. On this hypothesis it is the orientation of the disjoining dyads at AI which is decisive in determining preferential segregation. The presence or absence of neocentric activity at MII is of no consequence since the orientation has been previously established at AI. This view is supported by our studies on deficient dyads where random segregation at MII was indicated for heteromorphic dyads in which the deficient knobbed chromatid came from a dicentric bridge.

Earlier observations on the involvement of the primary centric region in neocentric formation (RHOADES 1952) have been extended. *K10 K10* plants were obtained heterozygous for several paracentric inversions. Both the normal and inverted chromosomes were knobbed and in all cases the knob was included within the inversion. Single exchanges in the inverted segment produce a dicentric bridge and an acentric fragment which carries the knob. The two chromatids not involved in the exchange are knobbed and neocentric regions arising in them at the knob regions are evident at AI. The behavior of the knobbed acentric fragment is instructive. Observations were made with *In 7a*, *In 4a*, and *In 3a* heterozygotes. The knobbed acentric fragments pass, often in advance of the rest of the dyads, to one or other of the two poles. The attenuated appearance of their poleward ends suggests that they possess chromosomal fibers. In *K10 K10* and *k10 k10* plants with knobless acentric fragments produced by inversion crossing over, the acentric fragments showed no indication of centric activity at AI. They remained on the spindle plate while the dyads were disjoining. In the second meiotic division the knobbed acentric fragments from all three inversions were never observed to congress onto the MII spindle and were found lying at one of the spindle poles. There was no indication of chromosomal fiber formation by the acentric fragments at the second meiotic division. Inasmuch as the knobbed fragments were included in the nuclei organized at telophase I, it is not likely that their behavior in the second division can be ascribed to the onset of the degeneration typically undergone by lagging acentric fragments. The level of neocentric activity of knobbed fragments, judged by their movement on the AI spindle relative to that of the normally disjoining dyads, was less in *K10 k10* than in *K10 K10* sporocytes. It may be supposed that the region of the true centromere elaborates the substance(s) essential for the formation of chromosomal fibers and in *K10* plants this material moves from the centric region laterally along the arms. Upon reaching the knob, some sort of interaction occurs and chromosomal fibers are produced at or adjacent to the knob. These are the neocentric regions. In the case of knobbed inversion heterozygotes, centric substances are believed to move along the chromatid to the knob before the fragment is formed or divorced from its centric region. The fiber-forming substance accumulating at the knob is

able to produce sufficient neocentric activity at AI so the fragment moves poleward. The passive behavior of the knobbed fragment in the second meiotic division is interpreted to indicate that no more fiber-forming material is available, having been depleted and not replenished because the knob is no longer associated with a centromere. These observations on behavior of knobbed acentric fragments differ from the earlier report on *In 4a* where the AI movement was not seen. In the present, more extensive studies these fragments did migrate to the pole in the first division but manifested no kinetic activity in the second division. In all instances the same conclusion is reached relative to the role of the true centromere in neocentric formation.

The neocentromere hypothesis is attractive and appears to be consistent with the genetic data presented here. There exist, however, certain discordant observations which must be satisfactorily accounted for before full acceptance of the hypothesis is granted. (1) If preferential segregation is caused by the attenuation of knobbed arms toward the spindle poles at AI, the strength of neocentric activity, as expressed by the degree of attenuation, could affect the level of preferential recovery of the knobbed chromatid. Neocentric activity is much more pronounced in *K10 K10* than in *K10 k10* microsporocytes. It might be expected, therefore, that preferential segregation would be higher in *K10 K10* plants. The data showed no marked discrepancy in the amount of preferential segregation between *K10 K10* and *K10 k10* although it is slightly higher in *K10 K10* plants. It is conceivable that a threshold exists in the degree of neocentric activity required for polarized orientation at AI and that *K10 k10* plants induce an amount sufficient to reach this threshold even though observed neocentric activity is often low. Further, our observations and those of others on neocentromeres have been made at microsporogenesis and it may well be that megasporocytes would differ in extent of neocentric activity. Unfortunately, meiosis in the ovules is technically difficult and tedious to study. Nothing at all is known about neocentric activity in female flowers save that it was present in the single AI found.

(2) Another possible criticism of the hypothesis is that the bridge produced in plants heterozygous for paracentric inversions in chromosome 3 fails to impose a persistent orientation and assortment is random from dyads with a deficient chromatid and an intact normal chromatid in these *k10 k10* plants. Why should the orientation established at AI by neocentromeres in the same chromosome be stronger than that due to a bridge which directs the two normal chromatids toward the poles? Following rupture of the bridge, chromosome 3 dyads may experience a free rotation of the chromatids so that preferential orientation is lost, while the effect of neocentromeres, although not operating with 100% efficiency, apparently persists longer. When a bridge is formed in *K10* plants, the orientation due to the *K10* chromosome appears to be imposed on that from inversion crossing over as was mentioned in an earlier section. Although the loss of selective orientation in plants heterozygous for paracentric inversions in chromosome 3 remains to be explained, it does not constitute a serious objection to the neocentromere hypothesis.

(3) There remain, however, two sets of observations more difficult to reconcile. Studies by EMMERLING (1959) on the *K°10* chromosome, a modified *K10* chromosome lacking the heterochromatic knob and distal euchromatic segment of *K10*, gave 52.7% recovery of the *K°10* chromosome from *K°10/k10* heterozygotes. Reduced neocentric activity in *K°10/K°10* plants was seen at MII (no mention is made of its occurrence at AI), but there was little neocentric activity in *K°10/k10* MII cells. The *K°10* chromosome induced preferential segregation of heteromorphic chromosomes 9, but the percentage of recovery of the *K9* homologue (53.2%) was much less than the 68.0% from *K10 k10 K9 k9* plants. The combination *K10 K°10* gave 64.8% recovery of the *K10* chromosome. These data are not inconsistent with a reduced strength of neocentric activity resulting in reduced preferential segregation. However, her investigations on the *K^s10* chromosome, which lacks the distal euchromatic end and approximately half of the heterochromatic knob of *K10*, gave anomalous results. Random recovery of *K^s10* occurred in *K^s10/k10* heterozygotes, indicating the equivalence of the two chromosomes in determining the segregation pattern, but there was only 54.3% recovery of the *K10* chromosome from *K10/K^s10* plants compared with the 70.2% found in *K10/k10* individuals. Additional evidence of the aberrant behavior of the *K^s10* chromosome comes from *K^s10 k10* combinations having heteromorphic chromosomes 9 which gave 53.2% recovery of *K9*. Thus, the *K^s10* chromosome was able to induce a moderate degree of preferential segregation of the knobbed chromosome 9 although itself undergoing random segregation. A low degree of neocentric activity was observed at MII in *K^s10/K^s10* sporocytes. Although pachytene studies revealed no structural derangements in the *K^s10* chromosome, a reduced transmission (35.2%) of the *K^s10* took place through the pollen of *K^s10/k10* plants while a 1:1 male transmission ratio occurred in *K°10/k10*. The *K^s10* chromosome should be subjected to further study using marker genes distributed throughout the long arm in order to determine if there is any reduction in crossing over. Perhaps consideration should be given to the fact that *K^s10* has a knob derived from a fracture of the *K10* knob, and its aberrant behavior may be due in some unknown way to its lacking the distal portion of the *K10* knob. The data on the *K°10* chromosome are not in conflict with the neocentromere hypothesis of preferential segregation, but the *K^s10* observations are difficult to account for on this or any other basis. EMMERLING's data contain too many ambiguities, as she recognizes, to be decisive in arguing for or against the hypothesis.

(4) What might seem a potential invalidation of the neocentromere hypothesis is found in KIKUDOME's (1961) observations on a modified form of chromosome 10 designated *K^t10*. This chromosome was discovered by TING (1958), who believed on the basis of its morphology that it originated from a translocation between a *k10* and a supernumerary B chromosome. The structure of this chromosome is uncertain because of contradictory statements concerning its length, but KIKUDOME found that it did not segregate preferentially when heterozygous with *k10* nor did it induce preferential segregation in heteromorphic chromosome

9 bivalents. However, homozygous $K^{t}10$ plants had a low level of neocentric activity. It may be, as KIKUDOME suggests, that the neocentromeres are not strong enough to cause a preferential orientation.

Implicit in all of these arguments is the assumption that the degree of neocentric activity observed in microsporocytes truly reflects the situation in megasporocytes and in particular that at the first meiotic division because it is then that the orientation occurs which later leads to preferential segregation. Laborious though it will be, a final decision on the essential correctness of the neocentromere hypothesis will come only when more is known about neocentromere formation in megasporocytes.

DISCUSSION

In oogenesis and megasporogenesis where only one of the four nuclei resulting from meiosis gives rise to the egg nucleus and the other three are eliminated, unequal numbers of *A* and *a* eggs would be produced if the *A* chromosome were preferentially segregated to the spindle pole from which the female gamete arises. Such is the situation described in this paper. The regular elimination of three of the four meiotic products in the female meiocytes provides a situation which should favor the occurrence of nonrandom segregation since all that is needed is a mechanism for the preferential orientation of a bivalent on the spindle. Four spermatids or four microspores are produced from each male meiocyte. If all are functional, as is presumably true for the four microspores and until recently believed to be true for the four spermatids of Drosophila, it is more difficult to visualize a meiotic mechanism other than differential chromosomal loss which would give rise to unequal proportions of the gametic classes. Despite the seeming advantage afforded by female meiosis, more cases of meiotic drive have been found in Drosophila males than in females.

The only type of meiotic drive in female Drosophila is the preferential recovery in the egg pronucleus of the physically shorter member of a heteromorphic pair. Occurring in stocks with diverse kinds of structural changes, the preferential segregation of the shorter chromosome depends entirely on dissimilarity in size. SANDLER and NOVITSKI call this behavior chromosomal meiotic drive since no specific genetic factors are involved; it is the relative lengths of the heteromorphic homologues, irrespective of their gene constitution, that is responsible.

On the other hand a number of different systems are known in male Drosophila. These include the sex-ratio condition in *D. pseudoobscura* (GERSHENSON 1928; STURTEVANT and DOBZHANSKY 1936), in *D. athabasca* and *azteca* (STURTEVANT and DOBZHANSKY 1936), and in *D. affinis* (NOVITSKI 1947) where the progenies of sex-ratio males consist almost entirely of daughters. NOVITSKI (1947) found a recessive gene in *D. affinis* which reversed the usual situation, giving an excess of sons (male sex-ratio). STURTEVANT and DOBZHANSKY (1936) reported that the X chromosomes in sex-ratio male *D. pseudoobscura* underwent an extra replication. The four X chromatids were distributed among the four spermatids, hence all sperm was X-bearing and only female offspring were produced. The Y chromosome degenerated and was eliminated. These cytological observations

were consistent with the genetic data and the mechanism underlying sex-ratio appeared to be resolved.

A second well studied case of meiotic drive in *melanogaster* is that of Segregation-Distortion (SD), a genetic factor located close to the centromere in the right arm of chromosome 2 (SANDLER, HIRAIZUMI and SANDLER 1959). Males heterozygous for SD give progenies in which nearly all offspring carry the SD chromosome, the normal homologue not being transmitted by the sperm. The unusual breeding results could be accounted for if the SD chromosome caused the loss of its homologue by inducing breaks in it followed by sister chromatid reunion with formation of a dicentric chromatid and an acentric fragment. Dicentric bridges were purportedly observed as expected. The hypothesis appeared to be correct.

A third example of meiotic drive is the production of unequal proportions of complementary gametic classes from males with a deficient *Ins(1)sc^{4}-sc^{8}* X chromosome and a normal Y (nonrandom segregation). In those spermatocytes where the X and Y are paired, normal disjunction is expected to form equal numbers of X and Y sperm. If the distribution of the X and Y to the poles is random following pairing failure, equal numbers of X, Y, XY, and nullo sperm are expected. The XY and nullo classes would be recognizable as coming from nondisjunction. However, the complementary types arising from both normal disjunction and nondisjunction were clearly of unequal frequencies. Twice as many X chromosomes were recovered as were Y's and the frequency of nullo sperm was in marked excess of the XY class. In order to account for the genetic data it was hypothesized that meiotic loss occurred when the X and Y were unpaired and that the unpaired Y was much more often lost than was the unpaired X (SANDLER and BRAVER 1954; ZIMMERING 1963).

The ingenious explanations for sex-ratio, for SD, and for nonrandom segregation are now highly suspect, largely due to the illuminating cytological studies of PEACOCK. In 1957, NOVITSKI and I. SANDLER concluded that not all sperm of Drosophila are regularly functional. This revolutionary suggestion was not enthusiastically received because both aneuploid eggs and sperm from translocation heterozygotes were known to function irrespective of their chromosomal constitution. Nevertheless, it proved to be a fruitful suggestion and provided the keystone in unraveling the causes of male meiotic drive. PEACOCK and ERICKSON (1965) demonstrated that exactly half of the sperm was regularly nonfunctional, and that one of the two telophase I poles would give rise to two functional sperm and the other to two nonfunctional sperm. The correct explanation for Segregation-Distortion lay not in chromosome breakage as postulated, but in the preferential assortment of the SD chromosome to the functional sperm. Cytological studies of the sex-ratio condition in *D. pseudoobscura* and *athabasca* disclosed that, contrary to earlier reports, the X did not divide twice as claimed; instead, the two X chromatids were included in the two functional sperm, the Y chromosome degenerated, and the two nullo sperm were nonfunctional (NOVITSKI, PEACOCK and ENGEL 1965). PEACOCK's (1965) cytological studies on nonrandom segregation in *D. melanogaster* males with the deficient X chromosome *Ins(1)sc^{4}-sc^{8}*

revealed, again contrary to accepted dogma, that chromosome loss did not occur when the X and Y were unpaired, that univalent X and Y chromosomes usually passed to the same pole, and that the unequal recovery of the complementary classes from both disjunctional and nondisjunctional AI could be readily accounted for by the nonrandom segregation of the X and Y chromosomes, whether paired or not, into functional and nonfunctional sperm. Thus, in all three systems of meiotic drive discussed above a new, and in all likelihood correct, explanation has come from careful and thorough cytological studies. The usefulness of good cytological studies in elucidating complex genetic phenomena was never better illustrated.

Meiotic drive presupposes two separable phenomena: (1) a mechanism for the directed orientation of a bivalent on the MI spindle and (2) the loss of a portion of the meiotic products. If all of the meiotic products are functional, the nonrandom orientation at MI would not ordinarily be recognizable unless the two poles of the spindle could be distinguished. This is possible in the ascomycetes where asci giving 4:4 segregations for a spore-color mutant fall into two classes, depending on the location of the four wild-type spores into the proximal or distal end of the ascus. Polarized segregation favoring the class with distally located wild-type spores has been reported (CATCHESIDE 1944; MATHIESON 1956). However, BERG (1966) believes the inequality of complementary ascus types is due to unconscious visual selection against the class with mutant spores at the tip since they resemble immature asci. Thus, while directed segregation may occur in the ascomycetes, it has not yet been unequivocally demonstrated.

One of the interesting attributes of the *K10* chromosome is its ability to increase recombination values in other chromosomes of the complement. In the homomorphic chromosome 3 bivalents *(K3 K3* and *k3 k3)* the increase was confined to the proximal *Gl-Lg* region; there was no enhancement for the more distal *Lg-A* interval. However, when recombination was reduced in the *Lg-A* segment because of knob heterozygosity, there was an increase for this region in *K10 k10* plants compared to *k10 k10*. The most striking effect of *K10* on crossing over occurred in structural heterozygotes where a several-fold increase was found for some regions. The cytological studies of pachytene pairing in inversion heterozygotes disclosed a more intimate pairing of the inverted segments and of both the proximal and distal uninverted regions in *K10* sporocytes. The correlation between close pachytene synapsis and increased crossing over may be interpreted to indicate that the time of crossing over is in meiotic prophase. Although increased crossing over is understandable when a more intimate pairing occurs at pachytene, the enhancement found in the *Gl-Lg* region of homomorphic bivalents is less comprehensible. It may be that the closeness of pairing in *k3 k3* or *K3 K3* sporocytes is not the optimum attainable for maximum recombination and that this is increased in *K10* plants. It is conceivable that the heterochromatic regions are not as tightly coiled in *K10* sporocytes and that this facilitates the intimate pairing essential for the occurrence of exchanges. The possibility that pairing is unaffected but exchange frequency is higher in *K10* plants is not consistent with the tighter pairing in structural heterozygotes.

Reduction in crossing over is easier to achieve than is enhancement. With rare exceptions, if any, all structural heterozygotes in maize have lower crossover values for regions included in or immediately adjacent to the structural change. However, a compensatory increase often takes place in those segments more removed from the aberration. Our data are consistent with the suggestion that there is a finite amount of energy (enzymes concerned with chromosome breakage and reunion?) for recombinatory processes and if not utilized in one portion of the chromosome it will be available to other regions where an increase in crossing over will ensue (SCHULTZ and REDFIELD 1951).

No specific genes capable of producing enhanced crossover values other than those in the differential portion of the *K10* chromosome have been found in maize with the possible exception of the asynaptic gene *(as)* where higher crossover values were found in the functional haploid gametes owing to more frequent double exchanges (DEMPSEY 1959). The validity of this case, however, is clouded because the functional gametes may represent a selected group coming from those sporocytes with a higher than normal number of exchanges. HINTON (1966) finds that the recessive *c3G* allele of *D. melanogaster* produces, when heterozygous, a significant increase in crossing over in both the X and third chromosomes. The higher values, which were not localized, were due to fewer noncrossover strands, no change in singles, and more double- and triple-crossover strands. Flies heterozygous for the multiply inverted third chromosome with the *Ubx* marker gene gave the same pattern of enhanced recombination as did the *c3G* heterozygotes.

JESSOP and CATCHESIDE (1965) reported a recessive gene *(rec)* in Neurospora which increased by tenfold interallelic recombination at the histidine-1 locus, but had no discernible effects on nonallelic recombination between the flanking markers. SMITH (1966) found a second *rec* gene in Neurospora that increased nonallelic recombination, but interallelic recombination was relatively unaffected. These cases of genic control of crossing over offer some promise as experimental material for the eventual solution of pairing and recombination mechanisms at the molecular level.

Although the hypothesis that neocentral activity of the knobbed chromatids is responsible for preferential segregation provides a satisfactory mechanism for the nonrandom recovery of knobbed chromosomes, it is possible that the two phenomena are not causally related. Preferential segregation to specific poles occurs in the well analyzed cases of meiotic drive in Drosophila where there is no cytological mechanism known to bring about nonrandom orientation of the bivalent on the spindle. Since this is so in Drosophila, it is conceivable that some unknown mechanism other than neocentric activity is operating in maize. However, considerable evidence has been developed in support of the neocentromere hypothesis. Neocentric regions arising from knobbed arms appears to be fully capable of producing preferential segregation in heteromorphic dyads if the extent to which the knobbed arms undergo precocious AI movement is sufficient to insure their polarized orientation on the MII spindle. The neocentromere hypothesis is appealing in that it offers a mechanism based on a cytologically observable phenomenon;

in no other case of meiotic drive has a specific mechanism been proposed which has a basis in a cellular component.

The situation in maize is unique in that all of the chromosomes will undergo preferential segregation if they are in heteromorphic pairs and if the *K10* chromosome is present. It is also clear that the genetic factors responsible for all of the *K10* effects reside in that portion of the *K10* chromosome by which it differs from a normal 10.

Finally, no matter what the eventual decision may be regarding the validity of the neocentromere hypothesis, the conclusions reached from our cytogenetic studies on the role of *K10* in preferential segregation, on the necessity for heterozygosity of knobs, on the dependence of preferential segregation on crossing over to produce heteromorphic dyads, and on the increase in recombination values will all remain unaffected by any vicissitudes which befall the neocentromere hypothesis.

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SUMMARY

Preferential segregation for other chromosome pairs depends upon abnormal chromosome 10 *(K10)*. If it is homozygous or heterozygous they will undergo preferential segregation when they differ by the presence and absence of heterochromatic knobs. Random segregation occurs in *K10* plants homozygous for knobbed or knobless chromosomes 3. The knobbed chromatids in *K3 k3* plants are preferentially recovered in the basal megaspores. Preferential segregation occurs only when heteromorphic dyads are produced by crossing over between the knob and centromere. Somewhat more than 70% of the time, the knobbed chromatid from these dyads passes to the basal megaspore. Random segregation takes place in homomorphic dyads. When crossing over was reduced by heterozygous structural changes, a concomitant reduction was found in the degree of preferential segregation. The degree of preferential recovery of the knobbed chromatid is directly related to the frequency of heteromorphic dyads. The degree of preferential segregation for loci positioned along the long arm of chromosome 3 can be accurately predicted if the exchange frequency is known. The liguleless-2 locus situated close to the knob in the long arm had the highest degree of recovery (71%). The nearly random segregation ratio found for the *Gl* and *gl* alleles is expected, since the *gl* locus is known to be closely linked to the centromere. Approximately 61% of the megaspores carried the *A* allele which lies distal to the knob. The same degree of preferential segregation was found in the chromosome 3 data for heteromorphic dyads from single and from double exchanges. However, an analysis of KIKUDOME's data on chromosome 9, where the crossover regions were shorter than those in chromosome 3, revealed that preferential segregation was higher following single exchanges in proximal regions than in

more distal ones. The enhanced recombination values found in *K10* plants are attributed to the more intimate pachytene pairing observed in microsporocytes. This observation supports the hypothesis that recombination occurs during the meiotic prophase. The evidence suggests that preferential segregation takes place in deficient dyads if the intact chromatid is knobbed, and that it is random when the normal strand is knobless, irrespective of the knobbed constitution of the deficient chromatid derived from bridge breakage. The cytological studies on neocentric activity in *K10* plants are reviewed, new observations are reported, and the hypothesis relating the neocentric activity found in *K10* plants to the preferential segregation produced by *K10* is critically considered.

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