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Chapter 4

Preferential Segregation in Maize

The outstanding example of the utilization of heterosis in plant improvement is that of hybrid corn. Extensive studies on maize genetics have clearly demonstrated that chromosome and gene segregation are in accordance with Mendel's laws of segregation and recombination. It would appear, therefore, that any unusual mechanism operating in maize to produce deviations from normal Mendelian behavior should be worthy of our consideration, even though the principles involved have no bearing on the nature or manifestation of heterosis. The purpose of this section is to present data on preferential segregation in maize and to offer a tentative interpretation of this phenomenon.

Two kinds of chromosome 10, the shortest member of the haploid set of ten, are found in populations of maize. The common or normal type gives typical Mendelian ratios when the two homologues are heterozygous for mutant loci. The second kind of chromosome 10, which has been found in a number of races from Latin America and the southwestern United States, also gives normal Mendelian ratios for chromosome 10 loci in plants homozygous for this chromosome. This second or abnormal kind of chromosome 10 differs from the normal chromosome 10 by a large, chiefly heterochromatic segment of chromatin attached to the end of the long arm and also in the chromomeric structure of the distal one-sixth of the long arm (see Fig. 4.1 and Fig. 1 of Plate I). As is illustrated in Figure 4.1 the chromomeres in this region are larger and more deeply staining than are the correspondingly situated chromomeres of the normal homologue.

Although normal Mendelian ratios are obtained for segregating loci in chromosome 10 in both kinds of homozygotes, we were able to show in an earlier paper (Rhoades, 1941) that preferential segregation occurs at mega-

FIG. 4.1—Camera lucida sketch at pachynema of bivalent consisting of one normal and one abnormal chromosome 10. Note the dissimilarity in chromomere pattern in the distal one-sixth of the long arm. The identical chromomere pattern found in the remainder of the chromosomes is not figured here.

FIG. 4.2—Anaphase I of cell illustrated in Figure 4 of Plate I. Some of the disjoining dyads are normal appearing while others have active neo-centric regions.

FIG. 4.3—Metaphase I with eleven dyads. Five of the dyads have precocious neo-centromeres at sub-terminal portions of their long arms.

FIG. 4.4—Anaphase II of cell illustrated in Figure 7 of Plate II. In some of the inverted V-shaped monads the true centric regions are attracted toward the opposite pole.

sporogenesis in plants heterozygous for a normal and an abnormal type of chromosome 10. Approximately 70 per cent of the functioning megaspores possessed the abnormal 10 instead of the usual 50 per cent. The excess of female gametes with the abnormal 10 was not due to lethal factors or to megaspore competition. The disjunction of the two dyads comprising the heteromorphic bivalent at anaphase I, and of the two monads of each dyad at anaphase II, was such that an abnormal 10 chromosome tended to pass with a high frequency to the basal spore of the linear set of four.

The factor or factors responsible for this preferential segregation reside in the chromatin segments which differentiate the two kinds of chromosome 10. Whether the distal one-sixth of the long arm or the large heterochromatic piece of extra chromatin carries the causative genes for preferential segregation has not yet been determined—since these two regions of the abnormal chromosome 10 have never been separated by crossing over. The locus of the gene *R* is in the long arm of chromosome 10. There is approximately 1 per cent recombination between *R* and the end of the long arm in plants heterozygous for the two kinds of chromosome 10; but every crossover distal to *R* occurred to the left of the dissimilar chromomeres in the distal one-sixth of the long arm. Apparently little or no crossing over takes place here, although pairing at pachytene is intimate.

Strictly terminal chiasmata in the long arm have not been observed at diakinesis in heterozygous plants. The close linkage of the *R* locus with the extra segment of abnormal 10 is due to a suppression of crossing over in the end regions of the long arm. E. G. Anderson (unpublished) has studied a reciprocal translocation involving normal 10 with the break distal to *R*, and found 5 per cent recombination between *R* and the translocation point. There is an undetermined amount of crossing over between the translocation point and the end of the chromosome. It should be possible to locate the region or regions in abnormal 10 responsible for preferential segregation by obtaining successively larger terminal deficiencies, but this has not been attempted.

The dissimilarity in chromomere pattern in the distal portion of the long arms of the abnormal and normal chromosomes 10, together with the lack of crossing over in this region, suggest the possibility that the gene content may not be identical in the two kinds of chromosome 10. Inasmuch as plants homozygous for the abnormal chromosome 10 are not noticeably different in growth habit and general appearance from sibs carrying only the normal 10, it would appear that some kind of structural modification was responsible for the suppression of crossing over. To assume that this distal region consists of non-homologous loci in the two types of chromosome would mean that plants with two abnormal 10 chromosomes would be homozygous deficient for certain loci found in the comparable region of normal 10. This appears unlikely.

That a structural difference, aside from the extra chromatin of abnormal 10, exists between the two kinds of chromosome 10 also is indicated by the pairing relationships in plants trisomic for chromosome 10. In plants with two normal and one abnormal chromosome 10, trivalent associations were observed in 251 (60.2 per cent) among a total of 417 microsporocytes. When a chain of 3 was found at diakinesis, the abnormal 10 occupied a terminal position in 90 per cent of the cells. It was united with a normal chromosome 10 by a chiasma in the short arm. A univalent chromosome 10 was found at diakinesis in 39.8 per cent of the pollen mother cells.

If pairing, as reflected by chiasmata formation, were random among the three chromosomes, the ratio of normal:abnormal chromosomes 10 in the univalent class should be 2:1. Actually the unpaired chromosome was a normal 10 in 28 cells among a total of 166, while in the remaining 138 cells the univalent was an abnormal 10. In individuals again trisomic for chromosome 10, but possessing one normal and two abnormal chromosomes, the percentage of trivalent associations at diakinesis was 57.9 in a total of 513 cells. In the chains of 3, the two abnormal homologues were adjacent members, joined by a chiasma between their long arms, in 70 per cent of the cases. An unpaired chromosome 10 was found in 42.1 per cent of the microsporocytes.

If pairing were random, two times as many abnormal 10's as normal 10's should be found as univalents; but in a total of 216 cells an abnormal 10 was the univalent in 69, while a normal chromosome 10 was the univalent in 147. Chiasma formation among the three chromosomes 10 of trisomic plants clearly is not at random. There is a marked preference for exchanges in the long arm between the two structurally identical homologues. If synapsis usually begins at the ends and progresses proximally, the non-random associations found in trisomic plants become understandable. Normal recombination values for the *li*-g_1 and g_1-*R* regions which lie proximal to *R* (see Table 4.1 for g_1-*R* data) indicate that any suppression of crossing over is confined to the region beyond the *R* locus in disomic plants heterozygous for the two kinds of chromosome 10. It is no doubt significant that differences in chromomeric structure are not found in regions proximal to the *R* locus.

Inasmuch as the *R* locus is closely linked with the extra chromatin of abnormal 10, the ratio of *R*:*r* gametes from heterozygous plants gives a good approximation of the frequency with which the abnormal chromosome passes to the basal megaspore. The genetic length of the long arm of chromosome 10 is such that at least one chiasma is found in the arm. If one chiasma invariably occurs in the long arm of heteromorphic bivalents, each of the two disjoining dyads of anaphase I will possess one normal chromatid and one abnormal chromatid. Preferential segregation would be restricted to the second meiotic division, and occur only if the orientation of the dyad on the spindle of metaphase II were such that the abnormal chromatid passed to

the lower pole of the spindle. Normal segregation would occur in those megasporocytes which had homomorphic dyads.

If the terminal segment of abnormal 10 determines preferential segregation, it follows that loci near the end of the long arm will be preferentially segregated more frequently than loci further removed from the end of the chromosome. From the data in Tables 4.1 and 4.2 it is evident that the distortion from a 1:1 ratio is greater for the *R* locus than for the more proximally situated g_1 locus. The *li* locus which is proximal to g_1 was less affected than g_1.

Longley (1945) reported non-random segregation at megasporogenesis for chromosome pairs other than chromosome 10 when one of the two homologues had a prominent knob and the other was knobless. Segregation was random for these heteromorphic bivalents in plants homozygous for the normal chromosome 10, and non-random if abnormal 10 was heterozygous. He studied preferential segregation of chromosomes 9 and 6. The data for chromosome 9 are the most instructive. Some strains of maize have a chromosome 9 with a knob at the end of the short arm, others have a knobless chromosome 9. The *C*, *Sh*, and *Wx* loci lie in the short arm of this chromosome, with *Wx* nearer to the centromere. *C* and *Sh* are in the distal one-third of the short arm. Approximately 44 per cent recombination occurs between *Wx* and the terminal knob—they approach independence—while *C* and *Sh* are 23 and 26 recombination units distant from the knob.

When plants of knob-*C*/knobless-*c* constitution, which were also heterozygous for abnormal 10, were pollinated by recessive *c*, 64 per cent of the functioning megaspores possessed the *C* allele. The *Sh* locus, close to *C*, showed a similar degree of preferential segregation in comparable tests, but the *Wx* locus was little affected. Such a progressive decrease in effect is expected if the terminal knob on the short arm is instrumental in producing preferential segregation. The part played by the knob of chromosome 9 was wholly unexpected. Obviously this heterochromatic structure can no longer be considered as genetically inert. The data on various loci in chromosomes 9 and 10 prove that the degree of preferential segregation of a locus is a function of its linkage with heterochromatic regions which, in some way, are concerned with non-random segregation.

The data presented above show that alternative alleles are not present in equal numbers among the female gametes when abnormal 10 is heterozygous. We have here an exception to Mendel's first law. Are deviations from Mendel's second law, the independent assortment of factor pairs on non-homologous chromosomes, also occurring? This question is answered by Longley's data where the *C* and *R* loci are both segregating preferentially. In separate experiments he found the *C* locus was included in 64 per cent and the *R* locus in 69 per cent of the functioning megaspores. Assuming that these percentages hold in plants where both are simultaneously segregating, the observed

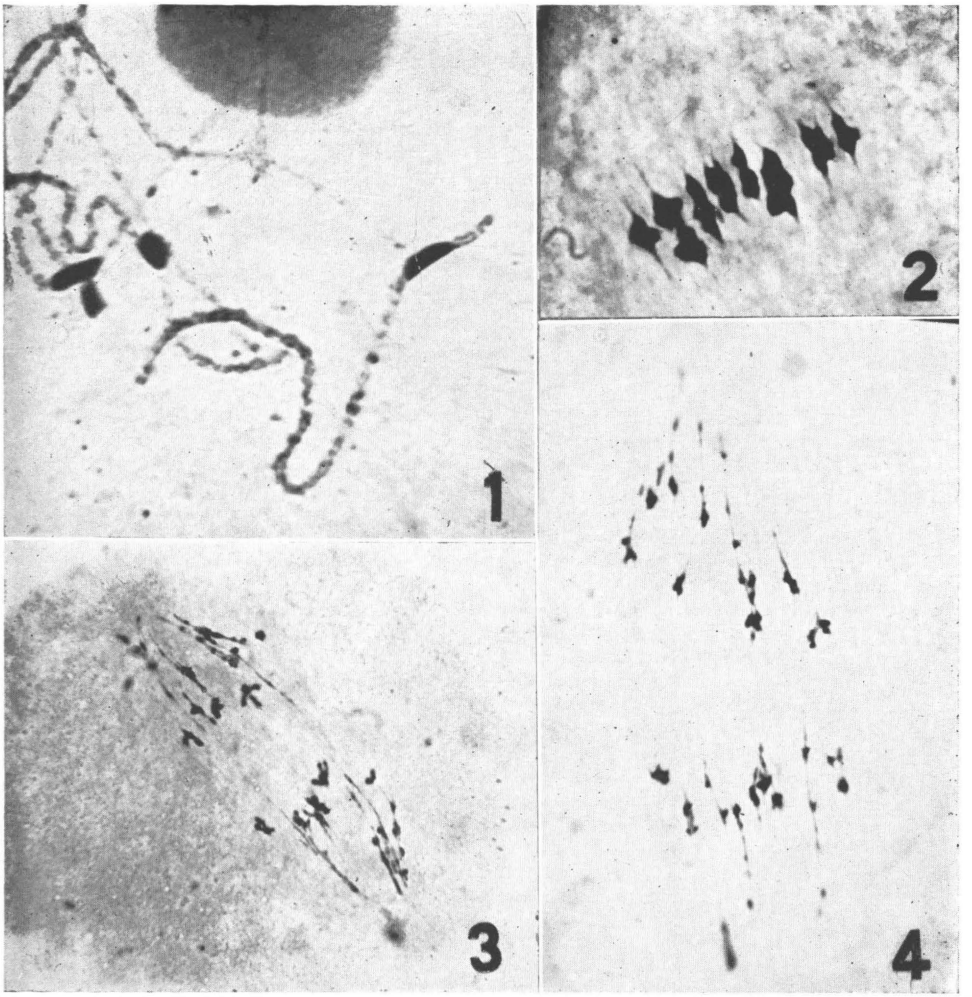

PLATE I: Fig. 1—Pachytene showing homozygous abnormal 10. Carmine smear. The proximal portion of the extra chromatin is euchromatic as is a smaller distal piece. A large and conspicuous knob lies between the two euchromatic portions. Fig. 2—Metaphase I in microsporocyte homozygous for abnormal 10. Carmine smear. The ten bivalents each have their true centric regions co-oriented on the spindle. The onset of neo-centric activity is manifest in the second, sixth, and seventh bivalents from the right. The third and fourth bivalents from the right are somewhat superimposed. Figs. 3 and 4—Anaphase I in microsporocyte homozygous for abnormal 10. Carmine smear. Some of the dyads are undergoing a normal anaphase separation while in others the neo-centric regions are pulling the ends poleward. Note that the normal appearing dyads are slower in their poleward migration. Fig. 4.2 is a drawing of Fig. 4 above.

PLATE II: Figs. 1 and 2—Metaphase II in plant homozygous for abnormal 10. Carmine smear. Precocious poleward movement of neo-centric regions is clearly evident. One dyad has a single neo-centric region (Fig. 4.5, dyad No. 8) while the left-most dyad has a neo-centric region in both long arms (Fig. 4.5, dyad No. 7). This cell was figured in Rhoades and Vilkomerson 1942. Figs. 3 and 4—Anaphase II in plant homozygous for abnormal 10. Carmine smear. Note that the rod-shaped monads with precocious neo-centromeres are the first to reach the poles. Fig. 5—Metaphase II in plant homozygous for abnormal 10. Carmine smear. The only chromosome of the haploid complement which can be recognized at metaphase II is chromosome 6 which has a satellite at the end of the short arm. In this cell the chromosome 6 dyad is the second from the left. That the terminal chromosome of the satellite is actually a small knob is indicated by the formation of neo-centric regions at the end of the short arm. Fig. 6—Early anaphase II in plant heterozygous for abnormal 10. Carmine smear. That the poleward movement of neo-centric regions is less rapid in heterozygous than in homozygous abnormal 10 plants is indicated here by the relatively slight attenuation of the rod-shaped monads. Fig.—7 Late anaphase II in plant homozygous for abnormal 10. Carmine smear. The previously greatly stretched rod monads with precocious neo-centromeres have contracted. Note the inverted V-shaped chromatids. This is the same cell shown in Figure 4.4. Fig. 8—Side view of metaphase I in a normal plant showing the fibrillar nature of the chromosomal fibers. Fixed in Benda, stained with haemotoxylin. Paraffine section. The only chromosomal fibers present are those formed by the true centromeres. Ordinarily chromosomal fibers are not evident in carmine smears since they are destroyed by acetic-alcohol fixation and it is necessary to use special techniques to demonstrate them. Similar fibrillar chromosomal fibers are found at neo-centric regions when proper fixation and staining methods are employed. Fig. 9 (top)—Polar view of metaphase I in normal plant. Fixed in Benda, stained with haemotoxylin. Paraffine section. Note the arrangement of the ten bivalents on the equatorial plate. This microsporocyte was cut slightly above the metaphase plate. The next section, which includes the remaining portion of this cell, is a cross section through the ten sets of chromosomal fibers.

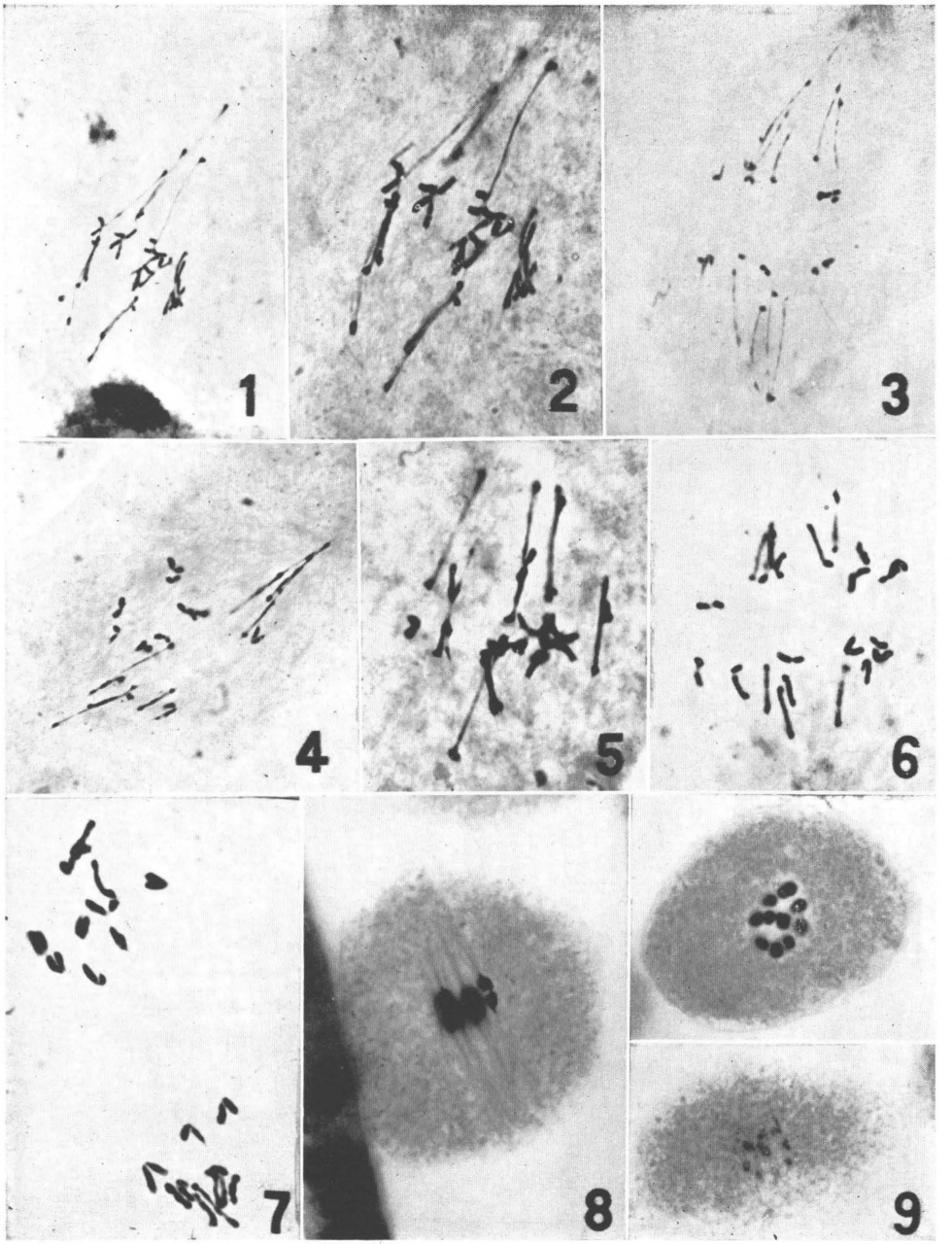
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TABLE 4.1

LINKAGE DATA FROM THE CROSS OF *G r* ABNORMAL/*g R* NORMAL × *g r* ♂♂

Linkage Phase	Constitution of Chromosomes					Ratio of *R*:*r* on Ear
Repulsion	(o) *G* *r*	(o) *g* *R*	(x) *G* *R*	(x) *g* *r*	Total	
	243	138	29	49	459	186*R*:326*r*
	102	86	9	13	210	136*R*:319*r*
	150	114	18	20	302	145*R*:288*r*
	396	50	7	59	512	169*R*:588*r*
	154	81	11	29	275	120*R*:277*r*
	169	90	21	30	310	127*R*:223*r*
	215	61	24	77	377	102*R*:338*r*
	231	79	35	81	426	133*R*:358*r*
	1660	699	154	358	2871	1118*R*:2717*r*

% *R* in total = 29.7 % *g* in total = 36.8 29.2% *R*
% *R* in non-crossover classes = 29.6
% *R* in crossover classes = 30.1
G − *R* recombination = 17.8%

TABLE 4.2

LINKAGE DATA FROM THE CROSS OF *G r* NORMAL/*g R* ABNORMAL × *g r*

Linkage Phase	Constitution of Chromosomes					Ratio of *R*:*r* on Ear
Repulsion	(o) *G* *r*	(o) *g* *R*	(x) *G* *R*	(x) *g* *r*	Total	
	12	87	13	1	113	182*R*: 42*r*
	38	96	29	6	169	188*R*: 59*r*
	35	86	33	7	161	230*R*: 74*r*
	39	107	21	9	176	241*R*: 77*r*
	124	376	96	23	619	841*R*:252*r*

% *r* seeds in total = 23.8
% *r* seeds in non-crossover classes = 24.8
% *r* seeds in crossover classes = 19.3
G − *R* recombination = 19.2%

frequencies of F_2 phenotypes can be compared with those calculated on the assumption of independent assortment. The two values agreed very closely, indicating little or no deviation from the law of independent assortment. His data, from plants where loci in chromosomes 9 and 6 are both segregating preferentially, likewise permit such a conclusion to be drawn.

In my 1942 paper on preferential segregation the statement was made that the chromosomes in plants with the abnormal chromosome 10 formed extra chromosomal (half spindle) fibers at regions other than the true centromere region. Rhoades and Vilkomerson (1942) found these supernumerary chromosomal fibers were produced only in plants homozygous or heterozygous for the abnormal 10, and that sister plants homozygous for the normal 10 had chromosomal fibers originating solely from the localized centric region in an orthodox manner (see Fig. 8 of Plate II). Although the abnormal chromosome 10 was clearly responsible for the formation of these neo-centric regions, they were not restricted to this chromosome since many of the nonhomologous chromosomes had supernumerary chromosomal fibers. The abnormal chromosome 10 is thus responsible for the formation of neo-centric regions, as well as for preferential segregation. Since 1942, a considerable body of data has been obtained bearing on the behavior of abnormal 10. Some of the more pertinent observations have suggested a cytological mechanism for the phenomenon of preferential segregation.

The unorthodox formation of supernumerary chromosomal fibers from neocentric regions is limited to the two meiotic divisions. (For a description of normal meiosis in maize see Rhoades, 1950.) The first meiotic division is in no way exceptional until metaphase I is reached. Normal appearing bivalents are co-oriented on the spindle figure in a regular manner with the half spindle fibers, arising from the true centric regions, extending poleward. Normally these fibers effect the anaphase movement of the disjoining dyads with the localized centromere region leading the journey to the spindle pole. However, in plants with the abnormal 10, chromosomal fibers arise from distal regions of the chromosome while the bivalents are still co-oriented on the spindle at metaphase I. The neo-centric regions are drawn poleward more rapidly than the true centric regions. Consequently the distal ends, instead of being directed toward the spindle plate during anaphase I, lead the way to the pole.

The appearance of many disjoining dyads at anaphase I suggests that their poleward migration is due largely, even exclusively, to the fibers originating from the neo-centric regions. The primary centric region appears to play no active role even though it possessed chromosomal fibers at metaphase I when the tetrad (bivalent) was co-oriented. At mid-anaphase there is no indication of the presence of these fibers in many of the dyads with the precocious neo-centric regions.

Figure 4.5 and Figures 3 and 4 of Plate I illustrate some of the observed

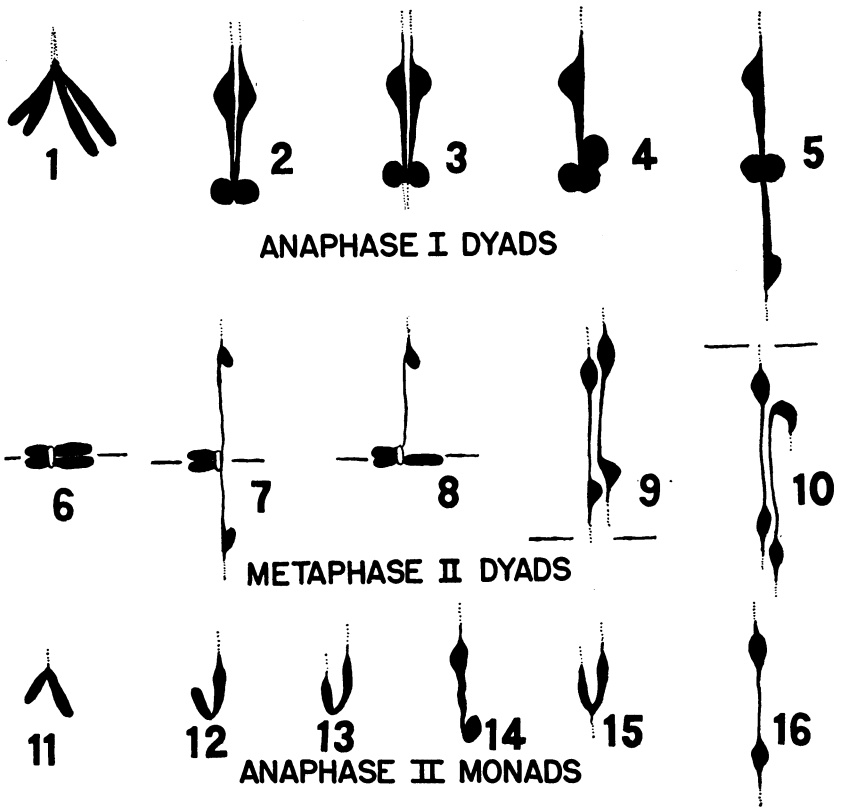

FIG. 4.5—All figures are from carmine smears of homozygous abnormal 10 plants. Figures 1–5 represent various configurations found at anaphase I. Figure 1 is a normal dyad with chromosomal fibers formed only at the true centric region. In Figure 2, two arms have formed neo-centric regions. The true centric regions appear to be inactive. Figure 3 shows a dyad with two neo-centric regions and an active true centric region whose chromosomal fibers are directed away from the nearest pole. Figure 4 is a dyad with a single neo-centric region. In Figure 5 the two neo-centric regions are directed to opposite poles. Figures 6–7 illustrate various metaphase II dyads. The location of the equatorial plate is represented by horizontal lines. Figure 6 is essentially normal with no formation of neo-centromeres. Figure 7 is a dyad with two neo-centric regions directed toward opposite poles. There is a single neo-centric region in Figure 8. Figure 9 is a dyad which is displaced from the equatorial plate. The true centric region has divided to form two independent monads. Each monad has formed two neo-centric regions which are oriented toward opposite poles. In Figure 10 one of the monads has its two neo-centromeres directed to opposite poles. Figures 11–16 are illustrations of monads found at anaphase II. Figure 11 is a normally disjoining monad. In Figure 12 a single neo-centromere is evident. Figure 13 shows two neo-centric regions. Figure 14 has a single neo-centromere which was active at metaphase II. In Figure 15, chromosomal fibers have arisen from two neo-centric regions and also from the true centric region. The true centric region and the neo-centromeres are acting in opposite directions. Figure 16 shows a monad with two neo-centric regions which are directed toward opposite poles. This type of monad is derived from those shown in Figure 9.

anaphase I configurations. Chromosomal fibers may arise from one or both of the long arms of each dyad at late metaphase or early anaphase I. Although it was not always possible to differentiate between long and short arms, the neo-centric regions in general appear to be confined to the long arm. When both long arms of the two chromatids of a dyad possessed a neo-centric region, the chromosomal fibers arising from these centric regions were usually directed toward the same pole. Occasionally they were oriented to opposite poles thus causing a great attenuation. In such cases, however, those chromosomal fibers nearest to one pole were powerful enough to overcome the oppositely directed force of the second neo-centromere. Despite the great complexity of configurations at anaphase I resulting from interacting and conflicting half-spindle fibers arising from both the true and neo-centric regions, the end of anaphase I usually finds ten dyads at each pole. Sometimes, however, greatly stretched chromosomes undergo breakage. This breakage doubtless accounts for the higher pollen abortion (about 10 per cent) found in homozygous abnormal 10 plants as contrasted to the lower (0–5 per cent) pollen abortion of normal sibs.

Even though one or two arms of some dyads are markedly stretched at anaphase I, the ensuing telophase is normal. All four arms of each dyad contract to form a spherical mass of chromatin which is loosely enveloped by the lightly-staining matrical substance. The chromonemata uncoil during interphase and early prophase II finds each daughter cell with ten, long X-shaped dyads of typical appearance. The two chromatids comprising each dyad are conjoined by the undivided primary centric region. There is no indication of neo-centric regions, although some of the long arms possessed chromosomal fibers at the preceding anaphase.

The onset of metaphase II sometimes occurs before the dyads have undergone their usual contraction. Occasionally chromosomal fibers arising from neo-centric regions in the long arms are found at late prophase II. These precociously acting fibers produce an extension of the long arms before any spindle is visible. This observation is of singular importance. Some authorities believe that the centromere region is attracted (whatever this term may signify) to the spindle pole. Here we have a movement produced by the chromosomal fibers of neo-centric regions in the absence of an organized spindle. The way in which these neo-centric fibers act can only be conjectured, but no interaction between centric regions and spindle pole is essential. It is, indeed, probable that the only role of a bipolar spindle is to provide a structural frame which channels the chromosomes to the spindle poles. Clark's (1940) studies on divergent spindles are pertinent in this respect.

The objection may be raised that the chromosomal fibers of neo-centric regions are not comparable to those arising from the true centric region. I do not believe this is a valid criticism. Not only are both kinds of fibers concerned with chromosome movement, but, as will be shown in a later section,

the fiber-producing activity of the neo-centric regions is a product of the true centric region.

The appearance of neo-centric fibers in prophase II is not the rule. Usually the dyads come to lie with the true centric region on the spindle plate at metaphase II before any pronounced activity of neo-centric regions is apparent. Before the primary centric region divides, thus permitting a normal anaphase, chromosomal fibers again arise near the distal ends of the long arms of some dyads. These newly formed fibers move the long arms poleward while the dyad is still held on the metaphase plate by the undivided true centric region. This poleward movement is so rapid that the ends of the chromosomes may reach the spindle poles before the true anaphase occurs. Eventually the true centric region becomes functionally split, and the two monads fall apart and pass poleward. It is evident from Figures 4.4 and 4.5 and Figure 7 of Plate II that the configurations of the disjoining monads (chromatids) at anaphase II are greatly different from normal.

Neo-centric activity, as shown by formation of additional chromosomal fibers, occurs in plants both homozygous and heterozygous for the abnormal 10, but it is much more striking in homozygous plants. Plants trisomic for abnormal 10 were not greatly different from homozygous disomic sibs.

Precocious chromosomal fiber formation by neo-centromeres at metaphase II appears in general to be confined to the long arms of the dyads, although it is often difficult to differentiate between two unequal arms when one is stretched poleward. Some chromosomes have arm ratios so extreme that the distinction between long and short arms is clear, and in these chromosomes the precocious fibers at metaphase II arise from the long arms. It is perhaps significant that, with the exception of the terminal knob on the short arm of chromosome 9, all remaining knobs in our material were situated in the long arms. (Chromosome 6 had two small knobs in its long arm but a maximum of one knob was present in the other chromosomes.) Correspondingly, only one of the two arms of any chromatid had neo-centric activity at metaphase II.[1] The number of dyads with precocious spindle fibers, as judged by the number of arms pulled poleward at metaphase II, varied 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 metaphase II. Those with four knobs had four such dyads. That is, a strong correlation exists between knob number and the number of dyads with neo-centric activity at metaphase II.

A further observation of some interest was that in plants homozygous for all knobs both homologous arms of a dyad usually were pulled poleward at metaphase II; while in plants heterozygous for some knobs many of the dyads had only one arm with neo-centric activity (see Figure 4.5 and Figures

1. With the possible exception of chromosome 6. See Figure 5 of Plate II.

1 and 2 of Plate II). It is not unreasonable to assume that dyads with both homologous arms exhibiting neo-centromeres at metaphase II carried a knob in each chromatid, while dyads with one neo-centromere consisted of one knobbed and one knobless chromatid. Such heteromorphic dyads would arise from heteromorphic bivalents by a crossover between the true centromere and the knob. We believe that only knobbed chromatids have active neo-centromeres at metaphase II, and that knobless ones are normal at this stage. Unfortunately, knobs cannot be recognized at metaphase II, and the validity of the above assumptions rests upon indirect but convincing evidence.

Two types of disjoining monads are found at anaphase II, those which are rod-shaped and those which are V-shaped. Monads which had one arm extending poleward at metaphase II are rod-shaped. They are the first to reach the pole. Indeed distal portions of such chromatids already had arrived there during metaphase II owing to the early action of their neo-centromeres. The V-shaped monads of anaphase II are derived from those chromatids devoid of neo-centromeres at metaphase II. The poleward migration of some monads is first begun by the chromosomal fibers emanating from the true centric region, but shortly after anaphase is initiated chromosomal fibers may arise from the ends of both arms. These terminally placed fibers, which are directed to the same pole, propel their ends poleward with such rapidity that the ends first overtake and then pass the centric region in the course of anaphase migration. Consequently these monads reach the poles as inverted V-shaped chromosomes (see Fig. 4.4). The spindle fibers from the true centric region now are directed toward the spindle plate rather than to the pole—they have reversed their orientation. This would be impossible if chromosomal fibers were of a thread-like structure. It is more likely that these fibers represent nothing more than lines of force emanating from the centromere. Inverted V-shaped chromatids are not invariably found at anaphase II.

Some monads have chromosomal fibers only at the true centric region and move poleward in a normal fashion. Either neo-centric regions are not present, or else arise too late to be effective. It should be emphasized that a fundamental distinction exists between the rod and inverted V chromatids found at anaphase II. The rod-shaped monads come from dyads with neo-centric activity at metaphase II. Their supernumerary chromosomal fibers arise from one arm. Their sub-terminal location suggests they may arise adjacent to the knob, but this is merely a conjecture. The later-formed extra chromosomal fibers of the inverted V chromatids, which are knobless, are terminal and arise from both arms.

If a dyad is oriented on the spindle plate at metaphase II before the onset of precocious neo-centromere activity, the supernumerary chromosomal fibers arising from the knobbed arm of the chromatid situated slightly above the spindle plate are directed toward the upper (nearest) pole, and those from the bottom chromatid go to the lower pole—they are co-oriented (see

Fig. 4.3). No such regularity is found in those infrequently occurring dyads which are longitudinally displaced from the spindle plate at metaphase II. Their true centric regions divide prematurely. Consequently, the two chromatids of these displaced dyads no longer remain conjoined, but fall apart to become independent monads which lie side-by-side, parallel with the longitudinal axis of the spindle.

The neo-centric activity which these monads now manifest is similar to that found at anaphase II for those monads derived from normally oriented dyads lacking precocious neo-centromeres at metaphase II, in that neo-centromeres may arise from the ends of both arms. When this occurs, the orientation of the two neo-centromeres of each monad is usually to opposite poles, but sometimes both ends of a monad are directed toward the same pole. Although the monads from displaced dyads have neo-centromeres at the end of each arm, one end being attracted to the nearest pole and the other to the more distant pole, normal disjunction usually occurs. This requires one monad to move away from the nearest pole toward which one of its ends is attracted, and to pass to the more distant pole, while the other monad goes to the nearest pole. It is difficult to interpret this phenomenon in terms of strength of attraction as a function of distance from centromere to pole.

The formation of neo-centric regions requires the presence of the abnormal chromosome 10. In its absence, no such regions are found. It appears highly probable that heterochromatic knobs located on other chromosomes also are concerned in the formation of precocious centric regions at both meiotic metaphases, since the cytological observations show a correlation between number of knobs and number of precocious centric regions. Knobless arms later form neo-centric regions, but not until anaphase movement has already been initiated by the true centric region.

It is possible that maize chromosomes possess latent centric regions which are activated by the abnormal 10. It has been demonstrated, however, that the true centric region is involved in the formation of neo-centromeres. Plants homozygous for abnormal 10 and heterozygous for the long paracentric inversion in chromosome 4, studied by McClintock (1938) and Morgan (1950), were obtained. Both the normal and inverted chromosome 4 carried a large knob in the long arm which is included in the inverted segment. Single crossovers within the inversion give rise to two non-crossover monocentric chromatids, one dicentric chromatid which forms a bridge at anaphase I, and an acentric fragment. The knobbed acentric fragment lies passively on the spindle with no indication of spindle fiber activity. Neo-centromeres arise from the same chromatin segments comprising the acentric fragment when they constitute a portion of a whole chromosome 4. It follows that the true or primary centromere plays an essential role in the production of neo-centromeres.

The localized centromeres of maize chromosomes are concerned with the

elaboration of fiber-producing material. Normally this unique substance is confined to the true centric region, hence chromosomal fibers arise solely from this part of the chromosome.

It is our belief: (1) that these centric regions produce an over-abundance of fiber-forming material if abnormal 10 is present in the nucleus; (2) that a portion of this substance escapes from the confines of the centric regions and moves distally along the chromosome to produce supernumerary chromosomal fibers; and (3) that the knobs either stimulate centric activity or else cause the excess fiber-forming substance to move preferentially along knob-bearing arms so that neo-centric activity is first manifested by these arms.

The failure of the acentric fragment to form chromosomal fibers suggests that the postulated movement of the material from the true centric region occurs after crossing over has taken place. If it happened prior to pachytene, the regions which later constitute the acentric fragments would receive some of this fiber-producing substance which subsequently could form spindle fibers. In support of the above interpretation is the observation that small aggregations of a substance similar in appearance to that located in the true centric region are sometimes found near the distal regions of some chromosomes at metaphase I and metaphase II. This observation is subject to various interpretations. But in conjunction with the behavior of acentric fragments, it strengthens the hypothesis that the production of neo-centromeres is intimately related to the presence or activity of the primary centric region. It is obvious that the presumed movement of the products of the centromere along the arms of the chromosome has a bearing on the kinetic theory of Position Effect.

Evidence has been presented that the abnormal chromosome 10 produces the phenomenon of preferential segregation, and that it also causes the formation of neo-centromeres. Are these two phenomena related—does preferential segregation occur as a consequence of neo-centric activity? While no definite answer can be given at this time a tentative hypothesis has been developed. Sturtevant and Beadle (1936), seeking to account for the absence of egg and larvae mortality following single crossovers in paracentric inversions in Drosophila, postulated that the crossover chromatids were selectively eliminated from the egg nucleus. The two spindles of the second meiotic division in Drosophila eggs are arranged in tandem. Following a crossover within the inverted segment, the tetrad at metaphase I consists of two non-crossover chromatids, a dicentric and an acentric chromatid.

They assumed that the chromatin bridge arising from the dicentric chromatid, when the homologous centromeres pass to opposite poles at anaphase I, ties its two centromeres together. The spatial arrangement thus produced is such that the two monocentric chromatids lie nearer the two poles than does the dicentric chromatid.

The persistence of this relationship into the second division results in a

non-random orientation on the metaphase II spindles. The monocentric, non-crossover chromatids are free to pass to the two terminal poles, while the two centromeres from the dicentric chromatid are directed to the two inner poles. Consequently, at anaphase II the terminal poles each receive a non-crossover chromatid. Since the egg nucleus arises from the innermost terminal pole it would contain a non-crossover chromatid with a full set of genes. The correctness of this ingenious hypothesis was established by Darlington and La Cour (1941) in Lilium and Tulipa and by Carson (1946) in Sciara.

It is possible that a somewhat similar mechanism is operating in Zea to produce preferential segregation. In maize, as in Drosophila, the two spindles of the second meiotic division of megasporogenesis are arranged in a linear order. The basal megaspore of the linear set of four develops into the female gametophyte, the remaining three aborting. We know that in plants heterozygous for knobbed and knobless chromosomes, one arm of some of the disjoining dyads at anaphase I possess precociously-acting chromosomal fibers not present in the homologous arm. There is reason to believe that the knobbed arms form precocious neo-centromeres while knobless arms do not. Owing to the rapidity with which neo-centric regions pass poleward at anaphase I, those chromatids with neo-centromeres reach the pole in advance of knobless arms lacking neo-centromeres. 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 one would face the spindle plate.

In order to account for preferential segregation, it is necessary to assume that this orientation persists until the second metaphase, and that it results in the knobbed chromatids facing the two terminal poles while the two knobless ones would be oriented toward the two inner poles. On such a mechanism, preferential segregation would occur only when a crossover takes place between the knob and the true centromere in a heterozygous bivalent. The extent of preferential segregation would be a direct function of the amount of crossing over in the knob-centromere region.

Such an explanation can only be considered as a working hypothesis. It can be critically tested, however, and such experiments are being conducted by Jean Werner Morgan, who also participated in the studies reported here. They include varying the crossover distance between knob and centromere by translocation and inversion, testing for preferential segregation of heteromorphic chromosomes other than chromosome 10 in plants homozygous for abnormal 10, determining neo-centric activity in chromatids with knobs in both the long and short arm, etc. I prefer not to mention her incomplete findings at this time, since to do so would detract from continued interest in her work.

The phenomenon of preferential segregation is by no means confined to maize. Sturtevant (1936) found a non-random segregation of three chromo-

somes IV in Drosophila. Bridges, in Morgan, Bridges, and Sturtevant (1925), established that the distribution of the chromosomes in triploid Drosophila was not according to chance. Beadle (1935) reported that crossing over in triploid Drosophila near the centromere region between one member of attached -*X*'s and a free *X* chromosome was correlated with autosomal disjunction. Lower crossover values were found in 1*X* 2*A* and $\widehat{XX}$ 1*A* combinations than in 1*X* 1*A* and $\widehat{XX}$ 2*A* gametes. This non-random distribution indicates a correlated orientation of non-homologous chromosomes on the equatorial plate.

In Sciara the paternal set of chromosomes moves away from the pole of the monocentric spindle of the primary spermatocyte. The two sister *X* chromosomes pass to the same pole at the second spermatocyte division (Metz, 1938). Schrader (1931) observed a non-random orientation in Protortonia which led to selective distribution in secondary spermatocytes. Catcheside (1944), in an analysis of Zickler's data on spore arrangement in the Ascomycete *Bombardia lunata*, found that certain genes were preferentially segregated. Not all of the above examples are strictly comparable to the situations found in maize, Sciara, and Bombardia. In the latter cases a specific spindle pole receives a certain chromosome or set of chromosomes, while in the Drosophila cases particular chromosomes pass preferentially together, but presumably at random, to either pole.

The neo-centromeres arising from chromosome ends, reported in rye by Prakken and Muntzing (1942) and Östergren and Prakken (1946), closely resemble those found in maize. In both maize and rye the neo-centric regions are found on arms possessing knobs (heterochromatin), and the poleward movement of neo-centromeres is precocious in both plants. Unfortunately, nothing is known about preferential segregation in rye, but it should occur if our hypothesis is correct.