for such an interchange should reflect the actual alternate and adjacent-1 segregation frequencies in the ♂, but adjacent-2 segregation in the ♂ could not be measured. If the three types in the ♂ were in the ratio of 2:1:1, there would be an apparent excess of alternate over non-alternate segregations. The data obtained by Brown (1940) on egg hatch in reciprocal crosses with normals indicates the segregation may be different in the ♂ for some translocations. An example is T3-4 A27 in Table 23. It is probable that T3-4 and T2-4 translocations regularly form chains. Adjacent-2 segregations are rare in 3-4 A27 which has short interstitial segments as well as in 3-4 A2 which has a long interstitial segment (Table 23). This is expected if homologous centromeres disjoin.

The actual frequencies of the types of segregation are somewhat in doubt because of the lack of knowledge about the behavior in the ♂. In the experiments of Dobzhansky (1933), Pipkin (1940), and Brown (1940) they have calculated the frequencies by considering the egg hatch data, and the phenotype frequencies from the heterozygous translocation x heterozygous translocation matings (Table 23). There appears to be an excess of alternate segregations over the total of the other types in the ring-formers with short interstitial segments, about 60:40 or 1.5:1. In the chain formers with short interstitial segments there is probably a much greater excess of alternate segregation, 3.5:1 or higher. It is of interest to note that the first translocations from x-ray treatment in Drosophila were described by Painter and Muller (1929) as having predominantly alternate segregation, a behavior comparable to that in Oenothera.

In mice, survival of Df-Dp gametes from interchange heterozygotes was found by Snell (1946). Matings between mice heterozygous for the same interchange produced offspring from both types of non-disjunctional segregation, i.e. adjacent-1 and adjacent-2, in tests of one interchange.

Interchange heterozygotes with low sterility

A number of plant species have been reported in which a ⊙ 4 may show low spore abortion, e.g. Datura stramonium (Blakeslee 1928), Triticum monococcum (Thompson and Hutcheson 1942, Yamashita 1947), Hordeum vulgare (Smith 1941, Burnham et al. 1953), Lycopersicon esculentum (Barton 1954) and Oenothera (Cleland 1929 and others).

Table 24. Summary of pollen abortion observed for interchanges with different configurations in Datura stramonium. (Bergner et al., 1933, based on Table 2, p. 112-113, Proc. Nat. Acad. Sci. 19).

Configurations at metaphase I	± 50%	± 25%	± 15%	Fertile (Normal)	Total
⊙ 4	0	9	2	17	28
∞ 4 (with a c.o.)	5	1		0	6
⊙ 4 or zigzag	1	0		1	2
Tr 4*	6	2		2	10
⊙ 4 or Tr 4	1				1
⊙ 6	0	2	1	0	3
Tr 6	1				1
"II"	1				1
TOTALS	15	14	3	20	52

* Described as being simple translocations (Tr) following radiation treatment, expected to form chains. Chains with a crossover were referred to by Blakeslee as "necktie" configurations.

In Datura stramonium (Jimson weed) only part of the interchange heterozygotes were described as having partial sterility. Some of the configurations at meiosis were an open or a zigzag ⊙ 4, others were a figure-of-eight with a crossover in an interstitial segment. A summary of the configurations and pollen sterilities of 52 translocation heterozygotes based on the observations reported by Bergner et al. (1933) is given in Table 24. They state this may not be a random sample, since identification in the later work was based on sterility.

As shown in Table 24 in the first two rows of data, most of those that regularly had a figure-of-eight configuration (probably a crossover in an interstitial segment) were 50% sterile, while those with a ring of four without such crossovers were normal or had low sterility. They stated, "'necktie' configurations are usually associated with 50 percent abortion--circles and kite-like configurations may or may not be associated with pollen abortion--configurations of four or more chromosomes usually show the chromosomes arranged on the spindle in zigzag fashion". Datura appears to be a species with predominantly alternate segregation in the rings-of-four. As shown earlier, crossing over in interstitial segments will produce spore abortion even if segregation is all alternate, a maximum of 50% if every meiocyte has one or more such crossovers. Hence in Datura, the interchanges with normal fertility should have short interstitial segments (few cytological crossovers).

Oenothera appears to be similar in behavior but the interchanges that have survived in nature have genetically short interstitial segments, and hence little or no sterility from crossovers in those segments. By irradiation of races of Oenothera with 7 chromosome pairs, interchange heterozygotes have been produced which do have long interstitial segments and show partial sterility (Catcheside 1954 and Marquardt 1948).

In Triticum monococcum and T. aegilopoides, interchange heterozygotes show low percentages of pollen abortion, 9% as an average (range of 7 to 17%) for 7 lines with a ⊙ 4 reported by Yamashita (1951); 10 to 15% based on my observations on a group in monococcum furnished by Luther Smith.

In barley, Hordeum vulgare, Smith (1941) reported 66.5% seed set as an average for plants with a ring of four; and that 67.2% of the associations of four at metaphase I of meiosis were alternate. Spore quartet analysis to determine the frequencies of the various segregations is not possible, since two pairs of chromosomes possess a nucleolar organizer. Non-disjunction of one still leaves the other to organize the nucleolus. Spore abortion is variable, but for a group of 27 interchanges in the Mars variety, the average pollen abortion was 29% (Burnham et al. 1954). The characteristic value for each line has not been determined, but the low value probably indicates a considerable excess of alternate over adjacent segregation.

In Collinsia heterophylla, interchanges induced by ionizing radiations displayed a directed orientation of the interchange complex at metaphase I (Garber and Dhillon 1962); whereas those induced by colchicine did not (Soriano 1957).

Effect of interchanges on crossing over

Maize

In the T2-6a heterozygote mentioned earlier as showing wide variation in the position of the cross (page 71), crossing over in the Y-P1 region in the long arm of 6 was only 3.3% whereas in normal stocks it is about 28. For T5-9a (5L.7-9S.3) heterozygotes, and for normal stocks the recombination values between several markers are shown in Table 25.

Table 25. Recombination percentages obtained in stocks heterozygous and homozygous for T5-9a as compared with standard values (Burnham 1934a and unpublished).

Chromosome 9	Standard	Heterozygous T5-9a as ♂	Homozygous T5-9a
yg_2 - sh	23	11	-
sh - wx	20	5	18.6
wx - v_1	12	11	independent
Chromosome 5			
bm - pr	27	32	-
pr - wx	independent	28	23.8

The positions of the genes and the break points in the two chromosomes are shown in the diagrams that follow:

There was considerable reduction in crossing over in the interchange heterozygote, greatest in the sh-wx region, less in the yg_2-sh region (Table 25). There was no reduction in any of the other regions. At pachytene the "cross" was at various positions in the short arm of chromosome 9, never in the long arm (only part of Chromosome 9 is shown). The configuration became T shaped when the "cross" was located farther out on the short arm of 9, and in two instances two "pairs" were seen. The range of the variation is indicated by the horizontal arrows in the above diagrams. The reduction in crossing over in 9 was confined to the short arm, and corresponds to the region in which variable pairing was observed.

Non-homologous pairing appears to explain the reduction. Had it been a result of failure to recover crossovers in the interstitial segments, bm-pr should have shown the reduction and yg-sh-wx should not. Crossing over in the bm-pr region seems to have been increased. Similar comparisons are needed in interchange heterozygotes showing relatively little variation in the position of the "cross", e.g. in T8-9a (Burnham 1934a). In the interchange homozygotes, recombination in the sh-wx region, here at the distal end of the long arm of 5, showed practically no change.

Interchanges involving breaks near the centromere in chromosomes 4 and 6 have shown very little reduction in crossing over near the breaks (Anderson, Kramer and Longley 1955). This region is one in which crossing over normally is low, and hence may show variable pairing and no detectable effect on recombination.

Barley

As noted earlier, when alternate segregation occurs following crossing over in an interstitial segment, the crossover chromatids pass to the aborted spores. Hence the excess of alternate segregation which characterizes the interchange heterozygotes in barley should result in reduced genetic recombination in regions which include an interstitial segment. The same should be true of interchanges in Datura, Lycopersicon, and Oenothera. For ten interchanges involving chromosome 6 in barley, the gene for orange lemma (o) showed one to two percent of recombination, regardless of the position of the break in 6; another showed 8% (Ramage, unpublished). It is probable that o is at or near the centromere. The amount by which recombination was reduced is not known, but it was probably high in T1-6a, at least, where the break in 6 was in the satellite. Similar

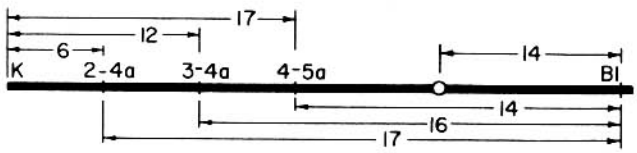
information places a Xantha near the centromere in 5, (Tuleen unpublished).

As suggested by Lamm (1949) and Hanson (1952) the pattern of the reduction in interchange heterozygotes should furnish a clue as to the position of the centromere. Data on linkage between three markers in linkage group 4 and three interchanges involving this chromosome were reported by Hanson (1952). To simplify the picture only the data for hooded (K) vs. normal (k) awns and blue (B1) vs. normal aleurone are presented in Table 26.

Table 26. Recombination values for chromosome 4 in three interchange heterozygotes in barley (Hanson, 1952, from Table 5, p. 96, Genetics 37). T = interchange.

Stock	K-T	T-B1	K-B1 Total	Reduction for K-B1
T2-4a	6	17	23	17
T3-4a	12	16	28	12
T4-5a	17	14	31	9
Normal	-	-	40	-

As shown in Table 26, the greatest reduction in recombination over the entire distance from K to B1 was 17, with T2-4a. As the interchange position moved farther from K and closer to B1, there was less reduction. This indicates that the region in which crossing over was reduced was becoming progressively shorter. This should be the interstitial segment. The results appear to fit best the following positions of the genes, interchanges and centromere:



The recombination values between the three interchanges and the centromere might then be of the order of 3, 2 and 0, respectively, after subtracting 14, the recombination distance from the centromere to B1.

The extent of the reduction imposes an upper limit on the sterility to be expected, as pointed out by Kramer and Swamley (1961). They have presented formulas for calculating the degree of spore abortion, and the recombination values with different assumed frequencies of alternate segregations. These frequencies may be different for crossover and non-crossover meiocytes. Also variable pairing near the break points may account for part of the reduced recombination without any corresponding increase in sterility (Burnham et al. in M.S.). Current experiments to compare recombination values in homozygous and heterozygous interchange lines in relation to the degree of sterility may measure the relative importance of the different possible causes of reduced recombination.

Drosophila

Variability in pairing has been observed in the salivary gland chromosomes of Drosophila inversion heterozygotes (Hoover, 1937, 1938); and it probably occurs also at meiosis. The evidence that fertility in interchange heterozygotes in Drosophila may be of the order of 60 to 70% suggests that alternate segregation is higher than 50%, although 50% may be a spurious result (see page 82). The reduction in crossing over from this source would be in the interstitial segments, whereas that from variable pairing would not be confined to those segments.

The effects of heterozygous translocations on crossing over in Drosophila are summarized in Table 27, based on information included in the descriptions of mutants by

Table 27. Summary of effects of heterozygous translocations on crossing over in Drosophila (from descriptions in Bridges and Brehme 1944).

Breaks Transloc. between Chrom. 1, 2, and 3	Segments Translocated	Segments Interstitial	Crossing over	No. of transloc.
both near cent.	both long	both very short	nearly normal in both chromosomes	5
both distal	both short	both long	greatly reduced near breaks	3
one near cent., other distal	one long, one short	one short, one long	nearly normal, reduced near breaks	2
Transloc. with 4				
near cent. in other	long*	short*	much reduced	3
" " " "	long	short	nearly normal	3
distal in other	short	long	much reduced	5
distal in other	short	long	nearly normal	2

* refers to the segment of the other chromosome involved in the translocation.

Bridges and Brehme (1944), and on data from Dobzhansky and Sturtevant (1931), Brown (1940) and Pipkin (1940).

In Table 27, row 1 includes the 1-2, 1-3, and 2-3 interchanges in which both breaks were near the centromere; and consequently both interstitial segments were short. In the five of that type, all showed nearly normal crossing over in both chromosomes, in the arm in which the break occurred as well as in the non-translocated arm of the same chromosome. It is known that in Drosophila there are regions adjacent to the centromeres which physically are long, but show little crossing over. Hence, interchanges with a break in this region may show variable pairing and yet show little or no reduction in crossing over. When the breaks were distal, as shown in row 2 of Table 27, recombination was greatly reduced near the breaks. When the break was near the middle of an arm; crossing over was reduced in the entire arm, greatest in the regions nearest the break. When the break was still closer to the end, crossing over was greatly reduced near the break but rose to nearly normal in regions farthest away, even in the same arm. This suggests that variable pairing accounts for most of the reduction. If it were caused solely by a great excess of alternate segregation, reduced recombination would be expected in the entire interstitial segment and only there.

Of the translocations with chromosome 4 in Drosophila, (chain-formers probably) shown in the lower portion of Table 27, certain ones with a short interstitial segment showed reduced, others nearly normal recombination; and the same was true for those with long interstitial segments; with about the same number of interchanges in each. No explanation can be offered by this writer. Further study is needed.

Dobzhansky (1931) proposed "competition in pairing" as the explanation for the observed decrease in crossing over. The initiation of pairing at different distances from the interchange point as mentioned earlier will account for the occurrence of non-homologous association, a form of competition which in turn affects crossing over. The extent of the abnormal association is variable.

Aberrant crossing over

The pairing behavior described above also offers an opportunity for an abnormal expression of the crossover process, crossing over between parts paired non-homologously. Several aberrants among the progeny of plants that were trisomic (2n + 1) for a normal or for an interchanged chromosome were analyzed by McClintock (1933, pages 226-232). Her analysis and the photographs are conclusive evidence of their origin by exchanges in regions with non-homologous association. Stadler (1935) presented evidence that it may occur in Zea, also Green (1959a) in Drosophila. If it occurred in interchange heterozygotes, deficiences and duplications for regions adjacent to the break points should be produced or dicentric and acentric chromatids in certain cases, as e.g. in the non-homologously associated segments in Fig. 20B. Experiments using appropriately placed marker genes should permit the detection of such crossovers which then might be analyzed cytologically. McClintock has pointed out that crossing over in regions with non-homologous pairing may be the source of a number of fragment, ring and other aberrant chromosome types.

Methods of detecting interchanges

As described earlier, in many species of plants individuals heterozygous for an interchange show partial sterility of pollen and ovules. This is recognized usually by reduced seed set and in mature pollen as empty grains.

In plant species in which interchange heterozygotes may not be partially sterile, cytological examination at meiosis is needed, or stocks carrying genetic markers in the different chromosomes might be used as described below for mice. The first interchange in Pisum was detected during the course of linkage studies (Hammarlund 1923, 1928 and Håkansson 1929). Later it was found to have 50% sterility.

In higher animals the lethal effect shows up in the zygotes, since deficient gametes function. Egg hatch in Drosophila (Table 23) and litter size in mice (Snell 1935, Hertwig 1941 and Carter et al. 1955) are reduced. In mice, one procedure was the following: males from a stock with three dominant marker genes (markers in linkage groups 11, 6 and 9) plus sex as a marker in group 20 were X-rayed and mated with females carrying five recessive genes (markers in linkage groups 1, 2, 3, 5 and 8) (Carter et al. 1955). The F_1 males were mated with a number of normal females and counts obtained on the number of lethal embryos. By setting up probabilities for different ratios of lethal and normal embryos, the lines were separated into normals, probable, and possible heterozygotes. For the possible heterozygotes, this test might be repeated before studying linkage. The males that were probably heterozygotes were then backcrossed to the multiple recessive. Counts in the progeny detected linkage between markers in the different groups. Cytological studies were made of those showing linkage (Slizynski 1957).

The first interchanges in Drosophila were identified by genetic methods, by testing males for linkage between genes that marked the different chromosomes (Muller and Altenburg 1930, Dobzhansky 1929). For example, males heterozygous for Bristle (B1) a dominant marker in chromosome 2 and Dichaete (D) a dominant marker in 3, were X-rayed and mated to untreated attached -X ($\widehat{XX}$Y) females homozygous for a recessive marker in 4, eyeless-2 (ey2). Male offspring carrying both dominants were backcrossed with untreated eyeless-2 females and counts were made on the segregations in their progeny. Because of the lack of crossing over in the male, the progeny of any male which carried a translocation involving any two or more of the chromosomes (X, 2, 3 or 4) would show no recombination between those markers. In one experiment, two of the B1 D

males from one experiment produced the following progeny when crossed with wild type:

	Wild type	B1	D	B1D	Total
A	361	0	0	361	722
B	424	0	0	390	814

It is obvious that there was complete linkage between B1 and D, indicative of a translocation between chromosomes 2 and 3. If the original X-rayed males had been mated to normal XX females, and the male offspring selected for testing, interchanges between Y and any of the other three chromosomes could have been detected.

Cytological examination at meiosis was difficult because of the extremely small nuclei. Observations of the somatic chromosomes at metaphase in nerve ganglia (Dobzhansky 1931), and in ovarian tissue (Painter and Muller 1929) were easier because of their greater length. The frequent tendency for parallel orientation of the homologues reflected the new positions of the end segments in the interchange heterozygotes. With the discovery of the usefulness of the salivary gland chromosomes, it was possible to locate the cross-shaped configurations with reference to the banding patterns and thus determine precisely the breakage positions. This is difficult or impossible when the breaks are in heterochromatic regions, since these regions are represented by few or no bands in the banding pattern.

Interchanges may be detected by examining the chromosomes at metaphase in somatic tissue and comparing their morphology with that of normal individuals. It is being used on white blood cells and various other cells in man.

Methods using genetic markers also serve to identify the linkage groups and the chromosomes involved in the interchange.

Methods of identifying the chromosomes involved in interchanges

The chromosomes involved in the interchanges in maize were identified by various means. Some of the methods used or that could be used are listed and described below. Not all can be used for every species; the ones to be used depend on the previous cytogenetic information on the species, the stocks available, and the behavior of interchanges in that species. The possible methods are:

Method 1. Test for linkage between partial sterility and marker genes as described earlier (page 70). In self-pollinating crops such as barley, F_2 and F_3 data are relatively easy to use to determine recombination values. Formulas for the calculation have been derived (Hanson and Kramer 1950).

Method 2. In the interchange heterozygote, test for linkage between markers in different linkage groups, as described in the previous section.

Method 3. Identification of the chromosomes involved in the cross-shaped pachytene configuration in the interchange heterozygote, as described on page 67. This method is usable only if the chromosomes stain and spread well enough to permit recognition of the cross-shaped configuration. Identifications of the chromosomes that have exchanged segments are easier if the centromeres can be identified, if there are knobs or other distinctive markers, and if the relative arm lengths and the positions of these distinctive