The presence of the bridge at this division indicates that when the broken chromatids resulting from a bridge in meiosis become double, the sister strands are fused at the broken end. She suggests the broken end may not reproduce itself normally, remaining single at that point or that the broken ends fuse. Thus the broken chromatid becomes a dicentric chromosome as shown in Figure 7, page 27. Whether the chromatids are already double at anaphase I or not until after anaphase II, this split is in preparation for Division I of the microspore. Hence this dicentric shows up as a bridge at that division. Of the total spores, 17.4% had the bridge. This behavior of the bridge after breakage has been termed a "bridge-breakage-fusion-bridge cycle". In maize it continues through the gametophyte and endosperm divisions but not in the cells of the embryo or developing plant of most stocks of corn. There the broken end is said to "heal" and the fragment is transmitted as a fragment. McClintock states that in certain genotypes, the bridge-break-fusion cycle continues in those tissues.

In the dividing spores, only part of the fragments were in the cytoplasm as shown in Table 7. Observations indicated that the fragment is not attached to a normal chromatid at anaphase II as it was at times in anaphase I. Its inclusion in the telophase II nucleus may depend on its being near enough so that the matrix materials swell and become confluent thus holding the chromosome and the fragment together in the telophase nucleus. If too far away it forms a micronucleus. This may occur at any division. Barber (1941) reported that when a wall is not formed between nuclei in a binucleate pollen grain, a deficient nucleus can continue to develop, and at the same rate as a balanced nucleus.

Here in the maize inversion heterozygote, the inclusion of the acentric fragment as a micronucleus in a deficient spore should permit that spore to develop normally. The degree of pollen abortion predicted from the observed frequency of bridges should be higher than the observed pollen abortion. From the data in Table 6A, the predicted pollen abortion is 25.2%. The observed value, 28.2, was in excess rather than less. The inclusion of the fragment in a deficiency-carrying tube cell should enable certain ones to produce a normal pollen tube. McClintock states that "A broken chromosome-4, which is deficient for a relatively long region, has been transmitted through the pollen." Such fragments would not be expected to persist through very many cell divisions.

Crossing over and sterility in paracentric inversions in *Drosophila*

As noted earlier, single crossovers and 3-strand and 4-strand doubles within this type of inversion give rise to dicentric and acentric chromatids, each of which has a deficiency and a duplication.

Genetic crossing over

The effect of heterozygous paracentric inversions on recombination will be illustrated by backcross data from two experiments. The first one involves an inversion in chromosome 3 and five marker genes, three within the inversion (Sturtevant 1926). The data are in Table 8.

Table 8. Offspring from the cross: $\frac{\downarrow \quad \text{In} \quad \downarrow}{\text{st sr} + e^s \text{ ro ca} +}$ x st sr e^s ro ca, where the

1 2 3 4 5 6

arrows indicate the inversion points and 1, 2 - - - - 6 are the regions of possible crossing over. (Sturtevant, 1926, from Table 1, p. 698, Biol. Zentralbl. 46).

region of c.o.	genotypes	In	N chromosome	
0	wild type	2214		
0	st sr e^s ro ca		2058	
1	sr e^s ro ca		219	
1	st	238		
2	st sr	4		
2	e^s ro ca		3	
4, 6	st sr e^s		1	
3, 5	e^s ro	1		total 4738

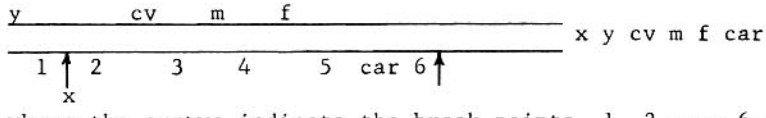
As shown in Table 8, the only recovered crossovers within the inversion were one st sr e^s in a normal chromosome and one e^s ro in an inverted chromosome. These could be explained on the basis of double crossing over occurring in meiocytes in which homologous regions were paired. Sturtevant pointed out that single crossing over would result in "duplication + deficient" chromatids. The standard map values and those from the data in Table 8 are as follows:

	st	sr	e^s	ro	ca
standard map:	18.0	8.7	20.4	9.6	
in inversion heterozygotes	9.6	~~1.7~~	0.02	0.02	

Note the reduced recombination in the st sr region adjacent to the inversion, as well as the very low values within the inversion.

The data for an inversion involving a longer map distance are shown in Table 9.

Table 9. Backcross results from tests of flies heterozygous for an X-ray induced inversion in X of the following constitution:



where the arrows indicate the break points, 1, 2 ---- 6 indicate the regions of possible crossing over and x is a new rough eye character associated with the inversion (Grüneberg 1935, Table 1, p. 164, Jour. Genetics 31).

c.o. events	obs. numbers	c.o. events	obs. numbers
0	819-1060	3-5	23-28
1	3-2	3-6	24-22
2-3	4-4	4-5	3-4
2-4	18-13	4-6	12-11
2-5	5-2	5-6	2-1
2-6	4-2		
3-4	44-64		

total = 2174

Standard map: y 13.7 cv 22.4 m 20.6 f 5.8 car

het. inversion: 0.23 In 2.4 9.8 7.8 3.1 3.6 In

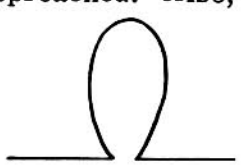
Here also, only double crossovers within the inversion were recovered, but their frequency was much higher than in the previous example.

In studies of chromosome behavior and interchromosomal effects on crossing over the statement frequently made is that inversions were introduced to "eliminate crossing over" in the chromosomes carrying them. Since single crossovers are not recovered, the observed genetic recombination is not a reliable criterion of the frequency of cytological crossing over. This point must be considered when interpreting the results of those experiments (see also page 48).

The recombination values between the y gene outside the inversion and the genes within the inversion based on the data in Table 9 are as follows:

	No.	%
y - x	5	0.23
y - cv	52	2.39
y - m	249	11.45
y - f	140	6.44
y - car	78	3.59

Note that the highest recombination value is between y and the mid-gene of the inversion, and that the values decrease as each end of the inversion is approached. Also, as Gruneberg states it, "In an inverted section there are -- always two points which give the same recombination value with a given gene beyond that inversion." A linkage map showing these recombination values would take the form of a loop in which the two inversion points are together at the base:



Cytological crossing over and sterility

Cytological observations on pairing indicate considerable asynapsis and non-homologous pairing in regions adjacent to the inversion. In short inversions this may occur more frequently, also failure to pair as an inversion loop configuration may be more frequent. This also should lead to decreased cytological and genetical crossing over. In Drosophila the cross: heterozygous inversion ♀ x normal would be expected to produce lethal zygotes if single crossovers occurred within the inverted segment. The reciprocal cross should not. Contrary to expectation, egg counts from Drosophila females heterozygous for paracentric inversions indicated high hatchability; as reported by Gershenson (1935), Stone and Thomas (1935), Beadle and Sturtevant (1935), Sturtevant and Beadle (1936), and Sidorov et al. (1936). The writers in the first two papers concluded that single crossovers were rare. Beadle and Sturtevant, as an alternative explanation of the high egg fertility, proposed that in a linear quartet of cells resulting from meiosis (the egg and polar bodies from oogenesis in Drosophila are essentially in a linear quartet), the bridge at anaphase I constitutes a chromatid tie which orients the two crossover chromatids to the inner two nuclei, and the two non-crossover ones toward the end nuclei. Since the functional cell is an end one, the single crossovers would not be recovered and egg fertility would be high. Also, four-strand double crossovers would result in double bridges, and if the same orientation occurs, the nuclei at the ends of the quartet should receive no strands representing that chromosome. In paracentric inversions in the X chromosome, these would be no-X eggs and in properly marked parents should result in patroclinous XO males. In support of their chromatid tie explanation, Beadle and Sturtevant (1935) showed that for inversions with a low frequency of recovered doubles patroclinous males were rare and were higher in frequency in those having more recovered doubles. If double exchanges within the inversion are at

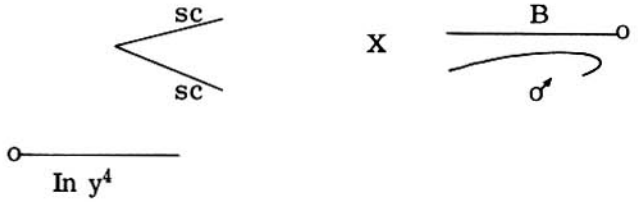
random, a ratio of 3 recovered doubles: 2 patroclinous males is expected.* The observed numbers for five different inversions were 422:275 which is close to the 418:279 expected for the 3:2.

A lack of reduction in ovule fertility might be expected from a similar chromatid tie effect in higher plants heterozygous for paracentric inversions, since megasporogenesis results in a linear quartet of megaspores. Bridges at anaphase II and four-strand doubles should result in deficient megaspores and account for some ovule abortion.

EXPERIMENTAL DETERMINATION OF THE FREQUENCY OF CYTOLOGICAL CROSSING OVER. Special experiments were set up to measure the frequency of cytological crossing over within the inverted regions in inversion heterozygotes in Drosophila, and hence to determine if a special mechanism is needed to explain the high observed egg hatch (Beadle and Sturtevant 1935, Sturtevant and Beadle, 1936). Two methods were used:

1. Attached-X females carrying an inversion in one of the two X-'s. This was used also by Sidorov et al. (1936).
2. Intercrosses between stocks with different inversions.

Method 1. To introduce the inversion into the attached-X chromosome, a triploid female carrying scute in both attached X chromosomes and the inversion in the free X was crossed to Bar (B) males, as follows:



The crossover that introduced the inversion into the attached-X would also introduce the normal allele of scute. Hence, diploid females that were not scute should have had the attached-X carrying the inversion and y^4 (inseparable from In). They were selected and other markers added. In one experiment, cr f v y^2/cr In y^4 attached -X females were crossed to t v f males. The meiotic configuration in these females and the chromosomes from different single crossovers are shown in Fig. 13. The closed -X chromosome from single crossing over is deficient for the segment distal to In and duplicate for the interstitial segment (between In and the centromere). The closed -X plus the male X chromosome produced females that could be recognized (+y $+^{cr}$). Single exchange was calculated to be 90.8%, i.e. about 45% of the eggs should carry a single crossover. Hence the high egg hatch could not be from a lack of crossing over.

In the second method, crosses were made between stocks with different inversions involving the same chromosome. There are three possible major kinds of F_1's based on relative break

* If the chromatid tie operates, considering the nuclei at opposite ends of the quartet, one of the terminal nuclei from a 2-strand double and also from each of the two 3-strand doubles would have a double crossover; whereas both would have no-X from one a 4-strand double. The following is expected:

proportion	type of double	among the offspring	ratio
2	2-strand double	1 double c.o.	3
4	3-strand double	2 double c.o.	to
2	4-strand double	2 patroclinous males	2

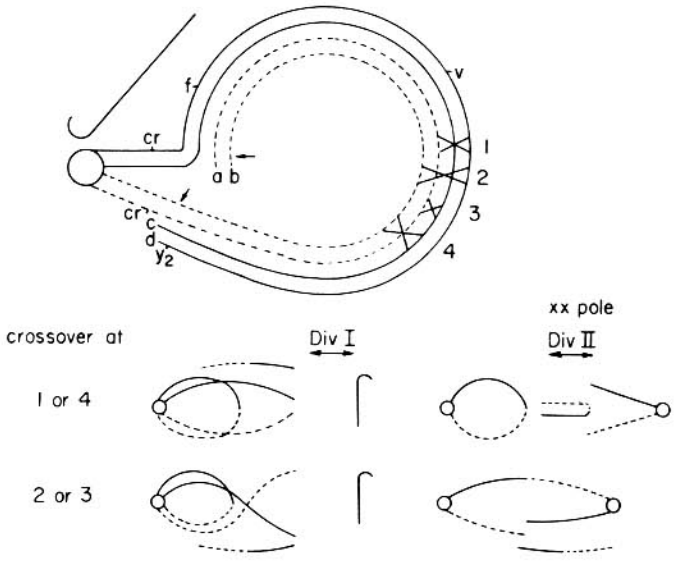
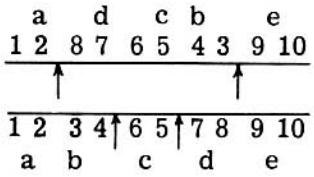


FIG. 13. Attached -X chromosome in Drosophila heterozygous for InY-4. Above. Pairing of the inverted and non-inverted sections. Below. On the left, chromatids at anaphase of Division I from single exchanges at 1 or 4; or at 2 or 3; on the right, for the XX pole, chromatids at anaphase of Division II, a ring chromosome in the upper, a dicentric in the lower figure. (Modified from Sturtevant and Beadle, 1936, Fig. 5, p. 584, Genetics 21).

positions in the two inversions that were crossed, i.e. included (one inverted segment entirely included in the longer one), overlapping (one inverted segment overlapping the other inverted segment), and independent (each involving segments that neither overlap nor are included). The first two were used in these experiments. As will be shown below, certain crossovers produce chromatids that carry a deficiency, a duplication, or both. Genetic markers were used and the crosses planned to recover the products of crossing over occurring in different regions. In some crosses the deficiency types produced by crossing over were viable and recognizable by the gene combinations; from which the frequency of crossing over in certain segments could be measured. In certain crosses where a long deficiency was expected, its survival was obtained by crossing with a translocation heterozygote which would form the proper duplication-carrying gamete to cover the deficiency. One might think that triploids would be of use for this purpose, but Sturtevant (1931) reported they are not.

The results expected from crossing over in the various regions in the included and overlapping types of F_1's will now be described.

1. a. Included. One inversion entirely included within a longer one:

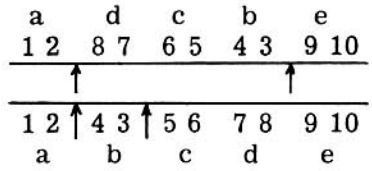


A crossover in the common inverted region, c, results in one chromatid with a deficiency for b plus a duplication for d, or in one with a deficiency for d plus a duplication for b. If the centromere is in c or in a or e, both crossover chroma-

tids are monocentric. If the centromere is in b or d, one crossover chromatid is dicentric, the other acentric.

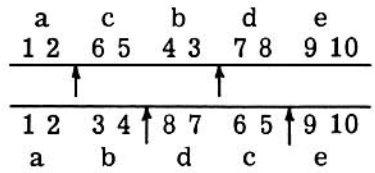
A crossover in b or in d results in one chromatid with a deficiency for a and duplication for e; the other chromatid with a duplication for a and a deficiency for e. Whether both crossover chromatids from a given crossover are monocentric or one dicentric, the other acentric again depends on the position of the centromere.

1. b. Included, but the two inversions have one break at the same locus, the other at different loci:



Crossing over in the common region b results in one chromatid with a duplication for c and d and no deficiency, and one with a deficiency for c and d and no duplication. This is one method of producing a duplication with no deficiency. The centromere must not be in c or d, if it is, the chromosome will be dicentric. Crossing over in region c results in one crossover chromatid with a duplication for a and a deficiency for e, and one with a deficiency for a and a duplication for e.

2. Overlapping. One inverted segment overlaps the other:



Crossing over in b produces one chromatid with a duplication for a plus a deficiency for d and e, and one with a deficiency for a and a duplication for d and e.

Crossing over in d results in one crossover chromatid with a duplication for a and b plus a deficiency for e and one chromatid with a deficiency for a and b plus a duplication for e. Crossing over in c, the common region, produces one crossover chromatid with a duplication for b and d, with no deficiency; and one crossover chromatid with a deficiency for b and d and no duplication. Here is another combination from which a duplication with no deficiency may be obtained (the centromere must not be in b or d).

From the data obtained from experiments using overlapping or included inversions, they concluded that single crossing over in heterozygotes for long inversions "approaches the frequency characteristic of the same segments normally arranged." In the shorter inversions; sc-7, dl-49, and probably C1-B which gives so few doubles, cytological crossing over seems to be decreased. The crossover frequency for these could not be accurately measured but Beadle and Sturtevant's (1935) data for In sc-6 suggested the amount occurring is one-half to two thirds of the normal value for the segment. All experiments dealt with inversions in the X chromosome.

The experiments proved that single crossing over did occur. Their assumption of the bridge acting as a tie to orient the crossovers toward the two inner cells

of the linear quartet leaving the normal chromatids to pass to the end nuclei would explain the failure to recover the crossovers. Cytological evidence for such an orientation of the dicentrics was reported in Sciara by Carson (1946). It was accompanied by lack of egg mortality also.

Matters stood thus until Novitski (1952) reported the results of extensive experiments with inversion heterozygotes in which one or both X chromosomes were bi-armed either by addition of the long arm of Y or the short arm of Y. Those results show that the directed orientation of both crossover chromatids from 4-strand doubles "holds only when the chromosomes involved are essentially telocentric, or, in some cases, when one, but only one, of the two chromosomes carries a small short arm not involved in the bridges." In some cases where both X chromosomes had an additional short arm (as the short arm of the Y chromosome) and in all cases where one was short, the other long (the long arm of the Y chromosome), the observed frequency of patroclinous males was about half that expected. When both X chromosomes had an additional long arm, patroclinous males were eliminated almost completely. This latter appears to apply also to flies heterozygous for paracentric inversions in chromosomes II and III, since egg hatch counts indicate a low mortality value which probably corresponds only to the frequency of cells with two bridges or with a bridge at anaphase II. That is, only these result in deficient eggs.

To summarize:

1. In cells with two sister bridges, directed orientation of both bridges occurs consistently only when both members of the pair are telocentric (acrocentric in Drosophila terminology). If one member is not telocentric but has an additional short arm, directed orientation is reduced; if the additional arm of both chromosomes is longer, there is very little directed orientation.

2. The directed orientation of crossover chromatids in single bridge configurations occurs in Drosophila in both telocentric and bi-armed chromosomes.

3. Cytological crossing over does occur within the inversions in inversion heterozygotes in Drosophila, often with frequencies approaching those in normal chromosomes, especially in the longer inversions. In the short inversions, cytological crossing over may be reduced by a third to a half. The decreased genetic crossing over is the result in part of failure to recover crossovers which do occur. Hence in experiments on crossing over where inversions are used to modify the conditions, results should be interpreted in terms of differences in cytological crossing over in the experiments being compared and not merely in terms of differences in genetic recombination. Inversions do not "eliminate crossing over" as is so often stated. Even though the writer undoubtedly knows that he means "genetic crossing over", the reader may not, and must be aware of the distinction.

SUMMARY OF DIFFERENCES IN BEHAVIOR OF THE PRODUCTS OF CROSSING OVER. The differences in chromosome configurations at anaphase in the two types of inversions and their effects on fertility in plants (maize and barley) and in Drosophila are shown in Table 10.

Table 10. Summary of the cytogenetic behavior of products of crossing over in para- and pericentric inversions in higher plants and animals (diploids).

Type of inversion	Cytological results of C.O. in inverted region	Effects on fertility
pericentric	no bridge, c.o. chromatids have Df + Dp.	in plants: embryo sac and pollen abortion.
		in Drosophila: zygote abortion.
paracentric	bridge + acentric fragment c.o. chromatids have Df + Dp.	in plants: pollen abortion, but embryo sac abortion low or absent*
		in Drosophila: zygote abortion low or absent.*

As shown in Table 10, the major difference in fertility is the zygote abortion in animals and the gametophyte abortion in plants. In plants pollen abortion is expected to result from crossing over in both types of inversions. In short inversions, it should be a relatively accurate measure of the amount, but in long pericentric ones, some separate measure of the amount of multiple crossing over is needed. In the paracentric ones, the frequency of double bridges furnishes a measure of at least part of the multiple crossovers.

In pericentric inversions no bridges are expected and in plants the degree of ovule abortion should be similar to the degree of pollen abortion unless crossing over is different in ♂ and ♀. In animals, such a comparison in ♂ and ♀ can be made only by determining egg hatch or litter size in reciprocal crosses between inversion heterozygotes and normals. In Drosophila there is no crossing over in the ♂ and hence no zygotic sterility is expected in crosses of normal ♀ x ♂ inversion heterozygote.

Interchromosomal effects on crossing over

Interchromosomal effects on crossing over have been reported in Drosophila melanogaster by Morgan, Bridges and Schultz (1933), Komai and Takahu (1942), Steinberg and Fraser (1944), Schultz and Redfield (1951), Redfield (1955, 1957), and Levine and Levine (1955). That is, if cytological crossing over in two chromosomes is decreased by the presence of heterozygous inversions, crossing over is increased in the other long chromosome. For example, in an experiment in which the X chromosome was marked at 7 loci, and the other chromosomes were heterozygous for inversions, the total crossing over in the X was roughly twice that in the absence of the inversions (Morgan, Redfield and Morgan 1943). Carson (1953) found in D. robusta that in the presence of heterozygous inversions, increases in crossing over could be demonstrated not only in other chromosomes that lacked inversions but also in the same chromosome in regions that had similar arrangements, i.e. in all pairing segments. The inversions differed in the intensity of their effect. Levitan (1958) in a study of recombination in regions between linked inversions in D. robusta found that in the X chromosome the frequency of crossing over was independent of inversion heterozygosity in 2, but was influenced by the presence of inversions in 3. Inversions in the X as well as in 3 influenced the frequency of crossing over in regions between second chromosome inversions.

*In species in which the products of meiosis form a linear quartet, the inversion bridge may orient the crossover chromatids so they do not pass to the end cells of the linear quartet, i.e. the bridge acts as a chromatid tie. In many cases it is almost completely effective, but in barley and in some inversions in corn it is either not effective or is less effective. In Drosophila, it is effective in 4-strand doubles only for the telocentric X chromosome, not for bi-armed chromosomes. It is effective in single crossovers in telocentric and in bi-armed chromosomes, probably also in 3-strand doubles.