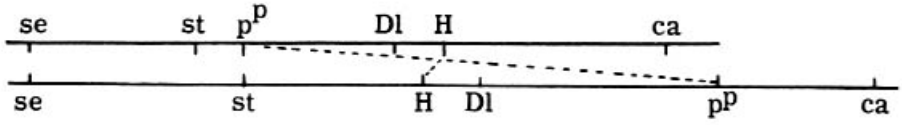
CHAPTER 3 INVERSIONS

In the early data on linkage in Drosophila there were great variations which were not explainable until Muller (1916) found that certain stocks carried a crossover reducer. Their effect was noticeable only when they were heterozygous*, but when heterozygous, they practially eliminated genetic crossovers in portions of the linkage map. Also, in individuals homozygous for them, crossing over was similar to or even exceeded that in the normal (Muller 1916, Sturtevant 1917, 1919). For a summary see Sturtevant (1931). These crossover reducers or suppressors were designated by the letter C followed by information as to the chromosome and the arm in which they were located, for example, C 2 R has a crossover reducer in chromosome 2 in the right arm, and C 3 L C 3 R, has one in the left arm and one in the right arm of 3. The ClB stock, extensively used in mutation experiments, has such a reducer in the X chromosome associated with a lethal (l) and has Bar (B) as a dominant marker gene.

The first clue as to the nature of these crossover modifiers was established by comparative genetic studies of Drosophila simulans and D. melanogaster. Three mutant genes in chromosome 3 of D. simulans were identical with three similar mutants in D. melanogaster (F_1's of the interspecific crosses were sterile but had the same mutant phenotype) but they were in a different order in D. simulans, i.e. $\frac{41.5 \quad 39.0 \quad 76.5}{\text{scarlet deltoid peach}}$, whereas in D. melanogaster the order and map positions were $\frac{41.5 \quad 44.5 \quad 63.5}{\text{scarlet peach delta}}$ Sturtevant 1921). Two possible explanations were offered: one, a shift of the peach-carrying segment in melanogaster to the end of a normal chromosome; the other, that a section of the normal chromosome had become inverted possibly by an accident at the time of crossing over. He suggested that in a chromosome pair heterozygous for such an inversion, synapsis might be expected to be "abnormal or absent" and show no crossing over; whereas in a pair homozygous for the change crossing over should be normal. By 1926, Sturtevant and Plunkett could compare the orders of seven linked genes in the two species, as shown in the following maps:



These show that the section from p to H in melanogaster and in simulans are in different orders in relation to the other genes.

now $In(3R)C$ ~~C 3 B~~. The final genetic proof came in melanogaster from studies of flies homozygous for By introducing recessives into C 3 B by crossing over and then crossing with the original C 3 B that had the normal alleles; Sturtevant (1926) found the gene order was st sr ro e^S, not the normal st sr e^S ro. Both sets of experiments, therefore, indicated that the dominant C "genes" were inversions.

* Bridges and Brehme (1944) list one crossover reducer that was recessive, c (1) a, but the stock was lost.

Types of inversions

As shown in Figure 9 there are two main types of inversions based on the positions of the two break points in relation to the centromere.

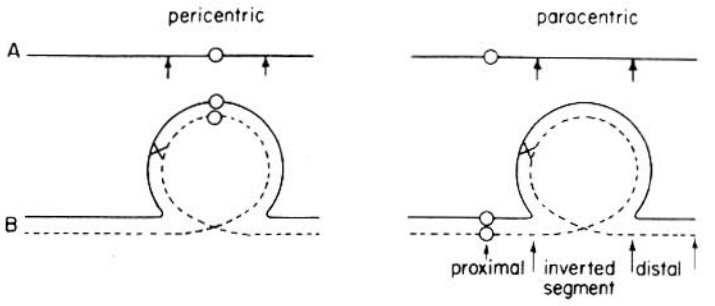


FIG. 9. The two types of inversions.
A. The inverted segment is between the arrows indicating the two break points. In the pericentric type on the left, the breaks are around the centromere. In the paracentric type on the right, both breaks are in one arm of the chromosome.
B. Typical "reverse loop" pairing of homologous parts in a chromosome pair, one member of which has an inversion. In the paracentric configuration, the proximal segment is the region between the inversion and the centromere. It is referred to as the interstitial segment in the text. (Rhoades, 1955, Fig. 9, p. 153, Corn and Corn Improvement, G. F. Sprague, Ed., Academic Press, N. Y.)

In the pericentric type, the two breaks are in different arms whereas in the paracentric type they are in the same arm (Muller 1940). As a memory aid, when thinking of the pericentric type, think of the perimeter of a circle, the breaks are "around" the centromere. The breaks in the pericentric type may be symmetrical or asymmetrical with respect to the centromere. As shown in Figure 9 B, the configurations in the two types of inversion heterozygotes are similar. Note, however, that the single crossover within the paracentric inversion will result in a chromosome with two centromeres (dicentric) and a chromosome with no centromere (acentric), whereas the two chromosomes produced by the single crossover in the pericentric inversion will each have one centromere (Figure 9). The chromosomes are shown as single-stranded for ease of seeing the results of crossing over. The crossover chromosomes in both cases have a duplication and a deficiency and thus account at least in part for the decrease in genetic recombination.

The first cytological information on chromosome pairing at meiosis in inversion heterozygotes was obtained by McClintock (1931, 1933) in maize. In plants heterozygous for a long inversion (In 2a, S .6-L .6), * she found that most sporocytes showed a "reverse loop" type of synapsis which brings homologous parts together, although there was considerable asynapasis in some figures (Figure 10).

In a short inversion including most of the short arm of chromosome 8, present in several of the maize genetic stocks, non-homologous association at pachytene to form a rod was frequent, also there was asynapsis in the short arm in many figures. At diakinesis this bivalent frequently was V-shaped, the members being held together only in the long arm.

* The inversion points are determined from camera lucida drawings of pachytene configurations showing the inversion loop. The designations S .6L .6 mean that one break was in the short arm at a point .6 of the distance from the centromere to the end of the short arm, the other break in the long arm. In 2a means the inversion is in chromosome 2, the a indicates it is the first one found in that chromosome.

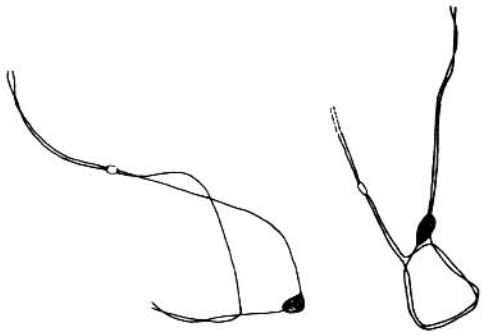


FIG. 10. Pachytene configurations observed in a maize plant heterozygous for In 2c. The "reverse loop" configuration shown on the right was common, but in occasional figures there was considerable asynapsis, as shown in the figure on the left. (Russell and Burnham, 1950, Fig. 2, 3, p. 99, Scientific Agric. 30, Agricultural Institute of Canada).

In one strain of corn, the arm ratio for chromosome 4 was 1:2, not the usual 1:1.6. In plants carrying both these chromosomes, the two centromeres were at different positions. Inversion-type synapsis was observed in only four sporocytes of over 400 examined. These indicated that a short inversion including unequal segments on each side of the centromere had become established in one strain of corn. Hence at pachytene in the heterozygote there was non-homologous association in the inverted region in most of the sporocytes (McClintock 1931, 1933).

Salivary gland cells in eight stocks heterozygous for different inversions in the X chromosome of D. melanogaster were studied in detail to determine the breakage points and the frequency of complete synapsis (Hoover 1938). The inversions included from 922 to 150 bands (total for X = 1024). Based on 100 observations for each, 67 to 86 per cent were synapsed completely, i.e. homologous parts associated over the entire length. Asynapsis in most of the remainder was in the inverted segment with fully half extending beyond. In one, a region was inverted and transposed to a different position. Even in this one, synapsis was complete in most cells. If this indicates what occurs at meiosis in Drosophila, considerable cytological crossing over should occur in inversion heterozygotes.

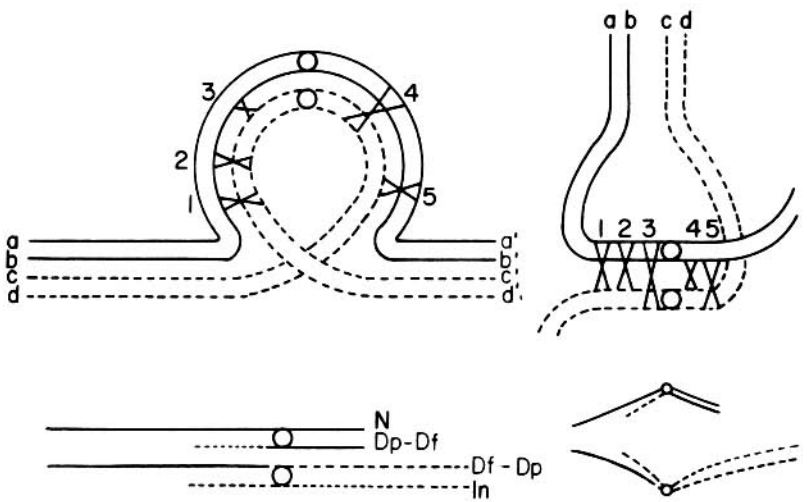


FIG. 11. Upper. Meiotic pachytene configurations in an individual heterozygous for a pericentric inversion. The numbers indicate the positions at which various crossovers are assumed to occur, the results of which are listed in Table 4.
Lower. The chromatids resulting from a single crossover at position 3 are shown on the lower left below the pachytene figures, and the anaphase I configuration on the lower right.

Expected results of crossing over in inversion heterozygotes

As noted earlier, for both types of inversion heterozygotes, the two resulting chromatids have a duplication and a deficiency and are complementary to each other. Also for the paracentric type, one is dicentric the other acentric. As we shall see shortly, the dicentric one forms a bridge at anaphase I.

Pericentric inversions

The kinds of chromatids expected from single and multiple crossovers in a pericentric inversion heterozygote may be derived by following either of the diagrams in Figure 11 in which the chromosomes are shown as double-stranded.

The chromatids expected following a single crossover within the inversion and the anaphase I configuration are shown also in Figure 11. Note that each of the four chromatids, including the Dp - Df ones, has one centromere. The kinds of chromatids from double crossovers as well as singles are listed in Table 4.

Table 4. Kinds of chromatids produced by various crossovers within the inversion in a pericentric inversion heterozygote. The numbers in column 2 refer to those in Figure 11. c.o. = crossover, N. = normal, In. = inversion.

c.o. event in the inverted region	Position of c.o.	Constitution of the 4 chromatids after c.o. dup. + defic. chromatids		Chromatids with full complement		Remarks	
single c.o.	any point	dup.+ def.,	def. + dup.,	N.,	In.	one centromere in each	
doubles, 2-strand	1,2	---	---	2N.,	2In.	"	"
" 3-strand	1,3 or 1,4	dup. + def.,	def. + dup.,	N,	In	"	"
" 4-strand	1,5	2 dup. + def.	2 def. + dup.	--	--	"	"

Note that each duplication + deficient chromatid includes the segment involved in the inversion. In one of the crossover chromatids the segment distal to the inversion break in one arm is duplicated, and the segment distal to the break in the other arm is deficient. The other crossover chromatid is the reverse to this. Since each chromatid has a single centromere, no bridges are expected at either meiotic division. In higher plants, both pollen and ovule abortion are expected as a result of crossing over in the inverted segment.

A short pericentric inversion in a chromosome that has a short arm will produce pairs of chromatids of unequal length as a result of crossovers within the inversion.

Paracentric inversions

The different kinds of chromatids expected from single and multiple crossovers within a paracentric inversion and from crossing over in the segment between the centromere and the inversion (an interstitial segment) and the configurations at anaphase I and II are shown in Figure 12.

The kinds of chromatids resulting from crossing over at various positions in the inversion heterozygote are listed in Table 5. They may be derived by following either of the pachytene pairing diagrams in Figure 12.

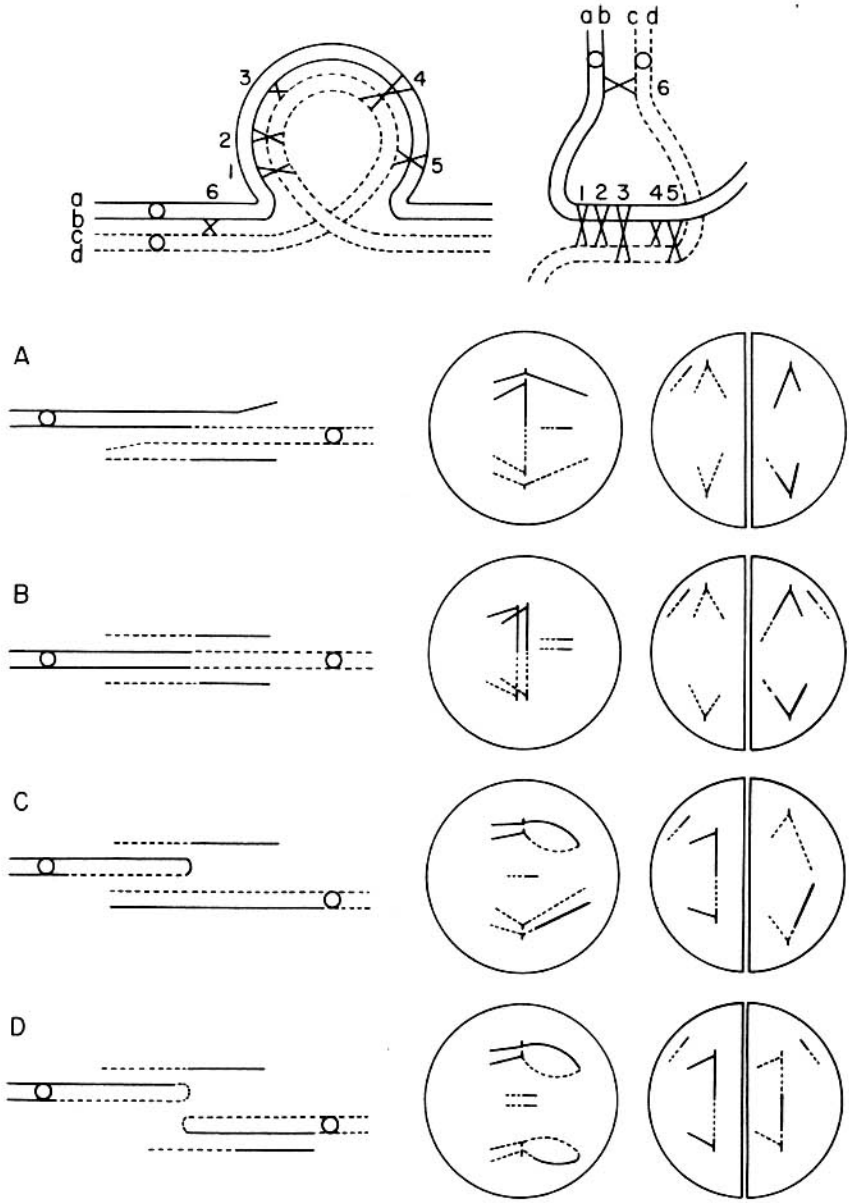


FIG. 12. Upper. Meiotic pachytene configurations in an individual heterozygous for a paracentric inversion. The numbers indicate the positions at which various crossovers are assumed to occur, the results of which are listed in Table 5.

Lower. A, B, C, D are representative types of chromatids and anaphase configurations resulting from crossing over. The chromatids are shown on the left, followed on the right by the expected anaphase I and II configurations. The crossover chromatids are made up of parts of the two homologues, depending on the positions of the crossovers. The ones diagrammed are schematic and do not necessarily correspond to the stated crossover points.

A. A single crossover within the inversion, e.g., at 3 or 4, results in a dicentric and an acentric chromatid and a bridge plus fragment at anaphase I.

B. A 4-strand double crossover within the inversion, e.g., at 3 and 4, results in two dicentric and two acentric chromatids and two bridges plus two fragments at anaphase I.

C. A crossover in the interstitial segment at 6, and certain ones within the inversion, e.g., a single at 4, results in a dicentric with sister centromeres and an acentric chromatid. At anaphase I there is a fragment but no bridge, but at anaphase II there is a bridge.

D. A crossover in the interstitial segment at 6, and a double at 3 and 4 produce a dicentric at each pole and two acentric fragments at anaphase I, and a bridge in each of the two cells of the dyad at anaphase II. At anaphase I, there are two fragments but no bridge. (Modified from McClintock, 1938, Fig. 3, p. 10, Mo. Res. Bull. 290).

Table 5. Kinds of chromatids produced by various crossovers in a paracentric inversion heterozygote. The configurations expected at anaphase I and II are included. All dicentrics have non-sister centromeres unless indicated otherwise.

c.o. in inversion	Constitutions of the strands after c.o. dup. + defic. chromatids	chromatids with full complement		anaphase div. 1	anaphase div. 2
A. No crossover in 6					
any single	dicent.dup.+def.; acent.def.+dup.	N	In	bridge+frag.	no bridge
1,2=2 str.double	--- ---	2N	2In	no bridge	"
1,3=3 " "	dicent.dup.+def.; acent.def.+dup.	N	In*	bridge+frag.	"
1,4=3 " "	" ; "	N*	In	" "	"
1,5=4 " "	2 " ; 2 "	--	--	2bridges+2frag.	"
B. Crossover in 6 and single at					
1=2 str.	dicent.dup.+def.; acent.def.+dup.	N	In	bridge+frag.	no bridge
3=3- "	" dup.+def.(sister cent.); acent.def.+dup.	N	In	frag.,no bridge	bridge in 1 cell
4=3- "	" " " " ; "	N	In	"	"
5=4- "	dicent.dup.+def.; acent. "	N	In	bridge+frag.	no bridge
double at**					
1,2	--- ; ---	2N	2In	no bridge	no bridge
1,3	dicent.dup.+def.; acent.def.+dup.	N	In*	bridge+frag.	"
1,4	dicent.dup.+def.(sister cent.); acent.def.+dup.	N*	In	frag.,no bridge	bridge in 1 cell
1,5	2 dicent.dup.+def.; 2 acent.def.+dup.	--	--	2bridges+2frag.	no bridge
3,4	2 dicent.dup.+def.(sister cent.); 2 acent.def.+dup.	--	--	2frag.,no bridge	bridge in both cells

* These chromatids have a double crossover between the inversion breaks. Two of the chromatids from 2-strand doubles also have a double crossover.

** Only part of the possible combinations are shown.

As shown in Table 5, the configurations seen at anaphase I and II depend upon what crossovers have occurred within the inversion and in the interstitial segment between the proximal inversion break and the centromere.

When the two centromeres in the dup. + def. dicentric chromatid belong to non-sister chromatids, there is a bridge at anaphase I, but when the two centromeres belong to sister chromatids the bridge is in one cell of the dyad at anaphase II (Figure 12). This latter is the result of certain crossovers which have occurred simultaneously in the interstitial segment (region 6 in Figure 12) and within the inversion (Table 5). In either case the acentric fragment is seen on or near the metaphase I plate.

Cytological behavior of bridges and fragments

The frequencies of the various configurations at anaphase I and anaphase II and an analysis of the cytological behavior of bridges and fragments were reported by McClintock (1938) for a paracentric inversion heterozygote in maize. The breaks were in the long arm of chromosome 4 (In 4a L. 35 and .88), and thus included about half the length of that arm.

Her cytological observations on first and second meiotic division anaphases are summarized in Table 6 A and B respectively. Each class in Table 6 B is assumed to have come from the class at anaphase I directly above it in the same table.

Table 6. A. First meiotic anaphase configurations in a paracentric inversion heterozygote;
B. Second anaphase configurations, two sister cells as a unit. (From McClintock, 1938, Tables 1 and 3, Mo. Res. Bull. 290).

A.	no bridge	Single bridge: fragment free	Single bridge: fragment attached	two bridges two free fragments	no bridge one free fragment	total
	Obs. No. 283	131	91	17	14	536
	Obs. %	41.4		3.1	2.6	

B.	No bridge No frag.	frag. in cyt. of one cell	No bridge: frag. in spindle of one cell	two frag. in cyt.	bridge in one cell, frag. in cyt. in one cell	total
	Obs. No. 205	102	64	5	12	388
	Obs. %	42.7		1.3	3.0	

As shown in Table 6 A, the single bridge occurred in 41.4% of the anaphase I cells, the double bridge in 3.1 per cent. A single bridge results when single crossovers or 3-strand doubles occur within the inverted segment. A double bridge results when a 4-strand double occurs in that segment. The cells which had one or two bridges at anaphase I showed no bridge at anaphase II. The cells which had a free fragment but no bridge at anaphase I had a bridge at anaphase II. Note that the percentages at anaphase II are in close agreement with the percentages at anaphase I that are directly above them in Table 6. These bear out the assumed behavior at the two divisions following the various kinds of crossing over.

As shown in Table 6 A, the fragment which occurs along with a single bridge is not always free. In many of the figures with a single bridge, the fragment was not seen. Some figures clearly indicated it was attached near the end of the normal chromatid supposedly as a result of crossing over.

The free fragment at anaphase I shows up in the cytoplasm of one cell at anaphase II. The missing or attached fragment at anaphase I shows up in the spindle of one of the anaphase II cells. Apparently the attached fragment had been carried into the telophase I nucleus and into the division II spindle whereas the free fragment was not. The two free fragments associated with two bridges at anaphase I showed up as two fragments in the cytoplasm at anaphase II. The meiocyte with no bridge but a fragment at anaphase I is expected when there is a dicentric with sister centromeres at the first division. The bridge does not appear until anaphase II and then in one cell of the dyad. The pollen mother cell with two fragments and no bridge at division I is expected when there are two dicentrics each with sister centromeres at division I. The bridges appear at anaphase II, one bridge in each member of the dyad (Figure 12). This type is dependent on a single crossover in region 6 and a certain 4-strand double in the inversion. It was rare, but was observed. At telophase II the acentric fragment may form a micronucleus.

The behavior at anaphase of the bridge produced by a dicentric chromatid is variable. As the centromeres move to opposite poles, the bridge may break at any point between them and leave no evidence of the bridge. Or the strand may become very much attenuated without breaking, and finally may be cut by the cell plate. When this happens the thin strands may extend toward the cell plate and retain this position into the next division. When there is a bridge at anaphase II, again it may not break before telophase when the cell plate cuts the strand. This extended thread persists in the early stages of the spore quartet and indicates which nuclei have a broken chromosome 4. There were 95 spores with a fragment and a broken chromosome 4, and 97 with a fragment and a normal chromosome 4, indicating the free fragment associated with the anaphase I bridge passes at random to the four spores in the quartet.

The two broken ends resulting from the separation of a double bridge at anaphase I usually do not fuse. Had they fused, a bridge in each cell of the dyad at anaphase II would be expected. Evidence as to the behavior of chromatids that had broken during the meiotic divisions was furnished by McClintock's observations that a bridge appeared at anaphase of the first division of the microspore nucleus. Her summary of representative counts at anaphase of the first microspore division in slides with differing percentages of binucleate spores are in Table 7.

Table 7. Data on the frequency of spores at anaphase with a bridge; and also the proportion with a fragment in (1) those with no bridge and (2) those with a single chromosome involved in a bridge. (McClintock, 1938, Table 8, p. 33, Mo. Res. Bull. 290).

	Spores with no bridge				Spores with bridge				Total spores
% binucleated spores	No fragment	Fragment in cytoplasm	Fragment in spindle	% fragment	No fragment	Fragment in cytoplasm	Fragment in spindle	% fragment	% with bridge
0 - 49.9	290	10	0	3.3	5	2	7	64.3	4.5
50 - 79.9	214	10	7	7.3	24	8	5	35.1	13.8
80 - 95	88	3	0	3.3	63	14	3	21.2	46.8
Total	592	23	7	4.8	92	24	15	29.8	17.4

Among the spores at anaphase, the frequency of those with a bridge was much higher in slides with the higher percentages of binucleate spores (spores that had completed this division). This is expected if normal spores divide earlier than those with a deficiency, and is the probable explanation for the differences shown in the last column of Table 7. She noted that of the spores that divided early and had a broken chromosome 4, about half also had a fragment which probably compensated for the deficiency.

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).