

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.	<u>in plants:</u> embryo sac and pollen abortion. <u>in Drosophila:</u> zygote abortion.
paracentric	bridge + acentric fragment c.o. chromatids have Df + Dp.	<u>in plants:</u> pollen abortion, but embryo sac abortion low or absent* <u>in Drosophila:</u> 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 cross-overs.

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 σ and ϕ . In animals, such a comparison in σ and ϕ 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 σ and hence no zygotic sterility is expected in crosses of normal ϕ x σ 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.

Another example is furnished by the X-chromosome 3-point data (Table 11) from three different crosses of $y^2 w^a spl/+++ \text{♀}$ x $y^2 su-w^a w^a spl \text{♂}$ reported by Redfield (1957). Cross #1 had no inversions, #2, had inversions only in the ♂ parent used in the backcross, and #3 had inversions only in the ♀ but in both arms of chromosomes 2 and 3. Crosses #1 and #2 are essentially checks since no effect of the inversions in the ♂ parent is expected.

Table 11. Three-point backcross data for the X-chromosome when inversions were present in both arms of chromosomes 2 and 3. Included also are results from crosses in which no inversions were present or only in the male. (From Redfield 1957, Tables 2, 3, 4, pages 716, 719, 723, Genetics 42).

C.O. type in X	(1) no inversions in ♂ or ♀ obs. no.	(2) inversions only in ♂ in 2, 3 obs. no.	(3) inversions only in ♀ Cy 2L, 2R, Payne C3L, 3R obs. no.
0	4808 + 4762	4393 + 4090	2733 + 2602
1	42 + 35	48 + 47	137 + 136
2	31 + 26	33 + 37	85 + 66
1, 2	0 + 0	0 + 0	1 + 0
N (totals)	9704	8648	5760
C.O. values			
$y^2 - w^a$	0.79 \pm 0.09	1.10 \pm 0.11	4.76 \pm 0.28
$w^a - spl$	0.59 \pm 0.08	0.81 \pm 0.10	2.64 \pm 0.21

Note the several-fold increases in crossing over in the distal y^2-w^a-spl end of the X when the second and third chromosomes were heterozygous for inversions in both arms. Also one double crossover was recovered in the very short region. This is a rare double crossover that has never been found in mothers lacking inversions. As Redfield stated it, "the X chromosomes are definitely more free to exchange very small segments when heterozygous inversions are present in II and III." Also markers closer together would be needed if all crossovers in such experiments were to be detected.

Various explanations of these interchromosomal effects on crossing over have been offered. For example, Mather (1936) suggested that the total amount of crossing over in the genome may be relatively constant. Hence a decrease in one or more chromosomes would be balanced by an increase in crossing over in the others or in distant non-inverted regions of the same chromosome. Another was that at least part of the increase might be the result of selective elimination in unhatched eggs of chromatids with the lower numbers of crossovers; this having been brought about by pairing of non-homologous chromosomes in complex structural heterozygotes (Cooper, Zimmering and Krivshenko 1955). In the experiments by Redfield (1957), egg mortality was too low to account for the observed increases. A lesser effect was not precluded. None of these seems likely to furnish an explanation for the observed differential effects, or for the greater tendency to produce types of higher multiples not observed in the absence of inversions.

A more accurate appraisal of the effects and interrelations might be obtained if the amount of cytological crossing over in the presence of the inversions were known.

The inclusion of inversions in two of the chromosomes when searching for rare crossovers in the other chromosome is standard experimental procedure in Drosophila.

Green (1959) used this method in a study of crossing over between members of an allelic series.

Genetics of paracentric inversions in maize and in other plant species

In maize, paracentric inversions are characterized by pollen abortion, the degree presumably dependent on the amount of crossing over within the inversion. Their behavior in selfs and in crosses may be illustrated by In 2c in chromosome 2. The offspring from the cross of low sterile (heterozygous inversion) ♀ x normal included 872 partially sterile: 895 normal; a 1:1 ratio, although individual cultures ranged from 39 to 68% of partially sterile plants. The cross of normal ♀ x low sterile or low sterile, self-pollinated, also produced a 1:1 ratio of the two classes. The normal progeny from self-pollination are expected to be of two types, either standard normal or homozygous for the inversion. To distinguish them, the normals were selfed and also test-crossed on a standard normal stock. Normals whose test cross progeny were all partially sterile were identified as homozygotes for the inversion. This is the usual procedure for pericentric as well as paracentric inversions.

Cytological determinations of the frequencies of bridges at divisions I and II of meiosis, and observations on pollen and ovule abortion have been reported for four paracentric inversions in maize. The data are summarized in Table 12.

Table 12. Summary of break points, cytological observations and spore abortion values for paracentric inversion heterozygotes in maize.

	Break positions	Total cells	Cytology Ana. I			%	Pollen abortion		Ovule abortion
			1 Bridge	Frag. only	2 Bridges		Predicted	Observed	
In 4a (McClintock 1938, Morgan 1950)	4L.35,.88	536	222	14	17	47.2	25.2	28.2	4.0
In 2c (Russell & B.)	2L.1,.6	86	50	2	0	60.0	30.2	16.5	normal
In 3a (Rhoades & D.)	3L.4,.9+†	1645	614	87	19	43.8	22.5	18.6*	12
In 8 (McClintock 1933)	S.1,.9+†	281	25		1**	8.9			

† near the end of the arm

* average of published figures

** one in over 1400 figures

The predicted pollen abortion values are based on the assumption that deficiency for the segment distal to the inversion resulting from the loss of the acentric fragment causes spore abortion. A predicted value somewhat higher than observed might be expected since the fragment may cover the deficiency in part of the spores. This was not the case. The observed values ranged from 16.5 to 28.2% and agree reasonably well with predicted values except for In 2c. A re-check of the cytological information is needed for In 2c.

Ovule abortion in the In 2c and In 4a heterozygotes was no greater than in normal plants. This can be accounted for if the bridges produced by crossing over acted as a chromatid tie to orient the crossover chromatids away from the end cells, as proposed by Beadle and Sturtevant (1935) in *Drosophila*. An exception to this in corn is In 3a in which there was 12% ovule abortion, only about a third less than the degree of pollen abortion. This will be discussed again after considering the genetic tests that deal with this problem.

Partial sterility of the pollen may be used in linkage tests in corn to identify plants carrying the inversion. A four-point backcross test for In 2c as reported by Russell and Burnham (1950) was made as follows: a liguleless (lg), glossy (gl₂), virescent (v₄), chocolate pericarp (Ch) stock was crossed with the inversion stock and the partially sterile F_1 plants backcrossed to lg gl₂ v₄ ch with normal chromosomes. The plants in the progeny were classified for pollen sterility and for the genetic characters. The recombination values with partial sterility in the 5-point test were: lg - 43.4, gl₂ - 48.2, v₄ - 12.0 and Ch - 42.8. The recombination values between these marker genes were: lg 27.7 gl₂ 47.0 v₄ 38.0 Ch, the distances in normal stocks being 19, 52, and 42 respectively. If any of the genes had been in the inverted segment, the recombination value with sterility should have been very low since only doubles would have been recovered. Since there was only a slight reduction in recombination between v₄ and Ch which are both in the long arm, these genes must be outside the inversion.

Data on recombination within a paracentric inversion in the long arm of chromosome 3 (3L.4 - .94) in maize have been reported by Rhoades and Dempsey (1953). A liguleless (lg₂) colorless aleurone (a₁) stock was crossed with the inversion stock (Lg₂A₁) and the partially sterile F_1 's backcrossed as pollen parents to lg₂a₁; i. e.

$$\begin{array}{c} \text{In} \\ \text{N } \underline{\text{lg}_2\text{a}_1} \times \begin{array}{c} \downarrow \text{Lg} \quad \text{A}_1 \quad \downarrow \\ \text{lg}_2 \quad \text{a}_1 \\ 1 \quad 2 \quad 3 \end{array} \end{array}$$

By classifying the progeny for pollen sterility as well as for the two marker genes, crossovers in the three regions could be recognized by their phenotypes. As in *Drosophila*, only the doubles were recovered, i. e. 1-2, 1-3, and 2-3. They obtained the following data on crossing over between lg₂ and a₁ when the F_1 was used as the σ parent and in another cross when it was used as the φ parent.

	parentals		<u>Lg₂ a₁</u>	recombinants	
	<u>Lg₂ A₁</u>	<u>lg₂a₁</u>		<u>lg₂ A₁</u>	% recombinants
F_1 as σ	1410	1215	6	8	0.5
F_1 as φ	3015	2482	24	84	1.9

Crossing over in the σ between the two gene markers was 0.5% whereas in normal stocks it was 28%. Note the marked inequality of the recombinant classes when the F_1 was used as the φ parent. Fifty one of the 84 lg₂ A₁ plants were tested genetically and cytologically. Sixteen had received a complete chromosome 3 from the φ parent and therefore constituted one class of expected double crossovers. The other 35 had a deficient-duplicate chromosome. All were deficient for the very short segment distal to the inversion and duplicate or triplicate for different portions of other non-inverted regions. Nineteen had the inverted, 16 the non-inverted order. At pachytene one chromosome was shorter than the other but often paired in a loop. Plants in the lg₂ A₁ class were crossed reciprocally with a₁. Part of them segregated in 1:1 ratios and therefore had the complete chromosome, either normal or inverted. Others had a Dp - Df chromosome. From one of these there were no A₁ kernels through the pollen and only 3.2% through the φ . Another had 1.5 and 35.9% respectively. There was no transmission of the Dp-Df chromosome through the σ .

The expected constitutions of these Dp-Df chromosomes immediately following the breakage of the first anaphase bridge are shown in Fig. 14.

Plants that were Dp-Df with the normal sequence had a low frequency of bridges while those with the inverted sequence had a high frequency of bridges. There was some evidence that the breakage in the dicentric occurred more frequently in regions proximal

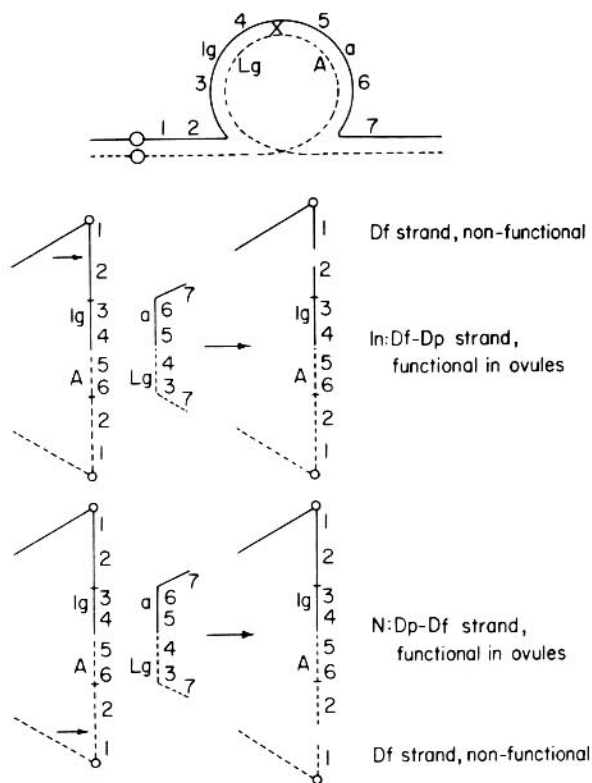


FIG. 14. Above. The genetic constitution of a plant heterozygous for a paracentric inversion in the long arm of chromosome 3.

Below. The constitution of the dicentric bridge and the acentric fragment at anaphase I resulting from the crossover shown in the upper diagram between the Lg_3 and A_1 loci. The strands that result from the breakage at two positions in the bridge are shown on the right. The constitution of the functional Dp - Df strands depends on the position of the break in the bridge. When the strands replicate, they are fused at the broken ends, and a bridge appears at anaphase of the first post-meiotic mitosis (modified, from Rhoades and Dempsey, 1935, Fig. 19, p. 412, Amer. Jour. Bot. 40).

backcrossed twice to normal. Plants with pollen abortion still have a similar degree of ovule abortion (Marshall, Fuad unpub., Kasha 1961). Hence, in barley in the one paracentric inversion for which there is information, the bridge at anaphase I is not effective as a chromatid tie mechanism for bringing about nearly normal seed set.

Bridges plus fragments at meiosis have been reported in many other species of flowering plants. Smith (1935) reported observations on an inversion in *Trillium* in which fragments were included in one of the daughter nuclei. Also the fragment with no bridge at division I, followed by a bridge with no fragment at division II was observed.

In the larger cells of the embryo-sac divisions in *Lilium testaceum* the inversion bridge at anaphase I did not always break but often caused the spindle to be bent, and thus changed the orientation of the two telophase groups (Darlington and La Cour 1941).

As we have seen, non-random orientation of the bridge-forming crossover chroma-

to one centromere. Ovule abortion was too high to be accounted for by the observed frequencies of 4-strand doubles and anaphase II bridges. Had the chromatid tie been completely effective in orientating the crossover chromatids, Dp-Df chromatids should not have been recovered. Their survival shows that it did fail part of the time and that the deficiency for the segment distal to the inversion was only semi-lethal in the ovules. Its average transmission was 25%. They noted that the bridge at anaphase I did not persist to the end of the division but broke early; and concluded that in maize the bridge is not always effective in orienting non-crossover chromatids toward the end cells of the megaspore quartet. It appears that the orientation is effective in In 4a and In 2c, since seed set was essentially normal. It is probable that megaspores receiving a crossover Dp-Df chromatid from those inversions would have been lethal and the degree of ovule sterility higher. If the chromatid tie were ineffective and the Dp-Df usually capable of surviving, normal seed set would be expected. Subsequent tests of In 2c show that the Dp-Df chromosome is not recovered (Burnham 1962). More studies are needed on paracentric inversions which differ in length and in the position of the inverted segment. These should include genetic tests combined with cytological studies of the offspring to determine the frequency of survival of Dp-Df chromatids produced by crossing over.

In barley, Das (1951) reported that a paracentric inversion heterozygote showed about 30% ovule abortion. This inversion has been crossed to a normal stock and

tids may occur in any species in which meiosis produces a linear quartet of cells. This is the pattern of development of oogenesis in *Drosophila*, of megasporogenesis in many seed plants, of ascospore formation in most ascomycetes, and basidiospore formation in basidiomycetes. In these two groups of fungi, it should lead to non-random orientation of abortive spores, the end ones being more frequently normal, and might be a method of recognizing paracentric inversions.

In most seed plants, the microspores are not in a linear quartet, and observations on randomness of orientation cannot be made. In *Alpina calcarata*, they are reported to be in a linear quartet, also in some species of *Asclepiadaceae* (Gager 1902) and in *Halophila ovata* (Kausik and Rao, 1942). Inversions in these species would be of some interest.

Genetics of pericentric inversions in *Zea* and *Drosophila*

As noted earlier, in this type of inversion, single crossovers and 3-strand and 4-strand doubles within the inversion give rise to monocentric chromatids that carry a deficiency and a duplication, referred to as Dp-Df chromosomes (Fig. 11). The positions of breakage for four pericentric inversions and the available information on spore abortion are summarized in Table 13.

Table 13. Summary of information on pericentric inversions in maize.

	Break position	Heterozygotes		Source of information
		Obs. pollen abortion	Ovule abortion	
In 2a	S.7,L.8	44.6	50	Anderson 1941
In 2b	S.5,L.15	22.3	20.1	Morgan 1950
In 5a	S0.0,L.67	34.9*	12.5	Morgan 1950 Burnham 1950
In 2a/2b		44.6	---	Morgan 1950
In 9	S.7,L.1	25	--	Li 1950

* Higher c.o. in the ♂

With one exception, the percentages of pollen and ovule abortion are similar. The excess of pollen abortion in In 5a may be a result of higher crossing over in the male which has been reported by Rhoades (1941) for the a₂ - bt region.

Data on recombination in plants heterozygous for In 5a and In 2b have been reported by Morgan (1950). For In 5a, A₂ (colored aleurone), bm, (brown midrib), bt (brittle endosperm), and Pr-pr (purple vs. red aleurone) were used; with bt and pr in the inverted segment.

