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: Obs. pollen abortion	Heterozygotes: Ovule abortion	Source of information
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 a_2 - bt region.

Data on recombination in plants heterozygous for In 5a and In 2b have been reported by Morgan (1950). For In 5a, A_2 (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.

$$\frac{A_2 \quad Bm \downarrow Bt \quad pr \quad In \downarrow}{a_2 \quad bm \quad bt \quad Pr \quad +} \quad \times \quad a_2 \; bm \; bt \; pr \; + \quad \frac{\overset{0.0}{\downarrow} \qquad\qquad\qquad\qquad \overset{.67}{\downarrow}}{a_2 \quad bm \quad bt \quad pr}$$

1 2 3 4 1 2 3 4

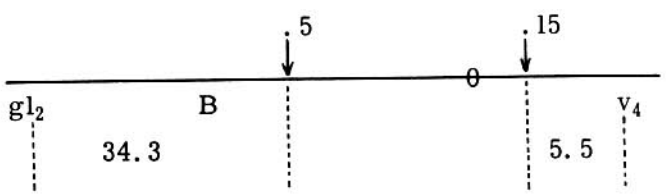
The data are summarized as follows:

499	A_2 Bm Bt pr	non-c.o.	
540	a_2 bm bt —*	non-c.o.	
20	A_2 bm bt Pr	c.o. in 1	a_2 - bt = 4.5%
29	a_2 Bm Bt —*	c.o. in 1	bt - pr = 0.4%
2	A_2 Bm Bt Pr	c.o. in 3	

* Pr vs. pr not classifiable

There was complete linkage between bm and bt, but the normal value is less than 1%. The two crossovers in region 3 were grown and found to carry the inversion. They were double crossovers in regions 3 and 4 shown in the map. No single crossovers within the inversion were recovered. Recombination in the bt-pr region within the inversion was only 0.4%, whereas the normal value is 30 to 35% (Rhoades 1941). The data suggest a reduction in crossing over in the a_2-bt region, but since recombination between A_2 and bt in the ♀ in normal lines was reported by Rhoades to be 6% in a low line and 17% in a high line, no conclusion should be drawn as to the effect of the inversion on crossing over in that region.

In the study of recombination in In 2b, glossy seedling (gl_2), plant color (B) and virescent seedling (v_4) were used (Morgan 1950). The inversion points and the recombination values between the inversion and the marker genes are shown in the chromosome map below:



The recombination between gl_2 and v_4 was 36.0% as compared with 37 in normal stocks, very nearly the same, in spite of the observed 20.1% of ovule abortion that probably resulted from the inviability of single exchanges in the inversion loop. One of the reasons given is that the inversion includes regions adjacent to the centromere in which crossing over is normally low. This is probably true, but the observed 20.1% of ovule abortion must have been from crossing over within the inversion. Short pericentric inversions which include the heterochromatic regions adjacent to the centromere would not be expected to show an easily detected reduction in crossing over, but abortion from crossing over in the heterochromatic portion should be correspondingly low.

Drosophila. The effect on genetic crossing over and on egg hatch were studied in two pericentric inversion heterozygotes in D. melanogaster (Alexander, 1952). The breakage positions in the chromosome II linkage map were as shown in Figure 15.

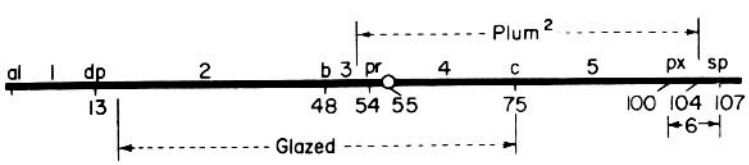


FIG. 15. Cytological positions of the Plum2 and Glazed pericentric inversions in Drosophila melanogaster. (Modified from Alexander, 1952, Univ. Texas Publ. 5204, p. 220). Later examination showed that Glazed probably had been induced in an In (2L) Cy paracentric inversion with one break between al and dp and one between dp and b (Grell, 1962).

The pericentric inversions shown in Figure 15 represent the two major types, one symmetrical the other asymmetrical with respect to the centromere. Glazed shifted the centromere position very little, but the Plum inversion changed the chromosome into a j-shaped one. Plum also produced a new arrangement of heterochromatic and euchromatic segments. For Glazed, the long inversion with break points symmetrical to the centromere, no double crossovers or singles within the inversion were recovered. For regions outside the inversion, recombination was greatly reduced, more in the adjacent region than in regions farther away. For $Plum^2$, the long inversion confined mainly to one arm, no singles but a few doubles within the inversion were recovered (slightly less than 1%). In the arm which was mostly outside the inversion there was some reduction in the distal regions but a considerable increase in the genetically short heterochromatic region 3 adjacent to the break. There is no apparent explanation for this increase. Reduction of crossing over in regions adjacent to the breakpoints might have been expected as a result of variability in the pairing.

Counts on egg hatch were made to measure the frequency of deficiency + duplication carrying gametes produced by single crossovers and certain doubles within the inversion. The cross of normal ♀ x heterozygous inversion ♂ was used as the control for comparison with heterozygous inversion ♀ x normal ♂ . For Glazed, the controls showed 93.1 per cent of egg hatch, and the reciprocals 86.4%, a difference of 6.7 per cent. For $Plum^2$ the corresponding values for egg hatch were 94.7 and 84.8 per cent, a difference of 9.9 per cent. The difference should represent the frequency of Df-Dp chromosomes produced by crossovers within the inversion. The percentages are not as great as might have been expected from the degree of reduction in the recombination values. The presence of the In(2L)Cy paracentric inversion in the Glazed stock (Grell 1962) could have reduced recombination without an increase in sterility. Alexander's conclusion that pericentric inversions may not be under as great a handicap from lowered fertility as previously supposed may still apply to the results for the $Plum^2$ inversion. Variable pairing and asynapsis could be the explanation.

Methods of locating the break points

The inversion break points may be determined cytologically by observing the position of the inversion loop in individuals heterozygous for the inversion: at pachytene of meiosis in corn, in salivary gland chromosomes in Drosophila. In maize, camera lucida drawings can be made of the configurations and then measured. If the two regions in which the break points occurred differ in chromomere density or if one break occurred in a knob, the nucleolar organizer region or in the satellite, it may be possible to determine the precise points of breakage by cytological analysis of the inversion homozygote.

Study of the banding pattern in the salivary gland chromosomes of Drosophila may be used to locate the break points accurately, unless one or both breaks are in heterochromatic regions.

Information as to the locations of the break points in the genetic map may be obtained in several ways. A direct test of the amount of recombination between the genetic markers and spore abortion can be used in plants. In paracentric inversions usually only pollen abortion can be used to identify the plants carrying the inversion. In pericentric inversions, pollen or ovule abortion may be used. If the test includes several genetic markers some of which are in the inverted region, the positions of the inversion breaks can be determined. A gene that shows considerable crossing over with the partial sterility must be outside the inversion. The portion of the linkage map in which recombination is greatly reduced is likely to be within or near the inversion.

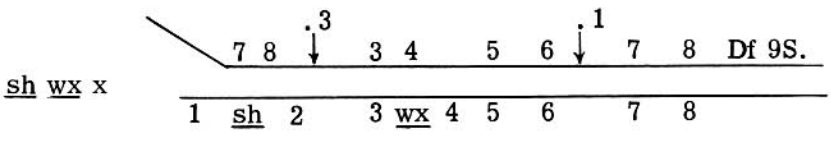
In Drosophila, females heterozygous for a pericentric inversion might be identified by the reduced egg hatch, but this would be too laborious for a linkage test.

Evidence as to the positions of the break points may be obtained by noting which combinations of genes from the normal chromosome are picked up by the inverted chromosome by double crossing over; and which alleles from the inverted chromosome are picked up by the normal one by crossing over. For example, the linkage data from the C 3 inversion in Table 8, in which the females were C3 _____ / st sr e^s ro ca, show close linkage between sr e^s ro ca. Considering the recombinants with the lowest frequency, the one with a non-inverted chromosome and st sr e^s had picked up the $+^{ro}$ and $+^{ca}$ alleles from the inverted chromosome, and the one with an inverted chromosome had picked up e^s and ro from the normal chromosome. Hence these genes are in the inverted segment. If ca had been outside the inverted segment the st sr e^s crossover would have picked up ca.

Still another method of locating break points in relation to genetic loci makes use of viable deficiency-duplications from the inversion. For example, Li (1950) reported tests of this type in maize using a pericentric inversion in chromosome 9 with breaks at S. 7 and L. 1.

Single crossovers and 3-strand doubles within this inversion heterozygote produce monocentric chromatids that are either:

(1) deficient for the distal .3 of the short arm and duplicated for the distal .9 of the long arm, or (2) duplicated for the distal .3 of the short arm and deficient for the distal .9 of the long arm. The cross $\frac{\text{Sh In}}{\text{Sh N}}$ x sh N produced 77 sh seeds in a total of 3180 (2.4% sh). Cytological examination of plants from the sh seeds confirmed the presence of the type with a deficiency in the short arm and a duplication for the long arm. Hence the gene sh must be in the distal .3 of the short arm. The cross sh wx x $\frac{\text{Df 9S Wx}}{\text{sh N wx}}$ was used to measure the amount of crossing over between wx and the inversion break in that arm. The observed value was 9.3%. This cross might be diagrammed as follows:



The frequency of sh Wx measures the genetic length of region 3 between wx and the inversion break point in the short arm. It may be somewhat lower than normal because of reduced pairing.

In another method, crosses between overlapping inversions are crossed with recessive markers for tests of pseudodominance. The locus of any recessive which appears in F_1 is probably between the breakpoints of the two inversions.

Frequencies of inversions in *Drosophila*

Many of the inversions in Drosophila occurred naturally. Many more were induced by X-rays and other radiations. A summary of those listed by Bridges and Brehme (1944) is in Table 14.

Table 14. Inversions in Drosophila melanogaster, a tabulation of those listed in Bridges and Brehme (1944).

	Chrom. 1	Chrom. 2	Chrom. 3	
paracentric	57*	12	39	
pericentric	-	11	3	
one paracentric in each arm		3	1	
TOTALS	57	26	43	= 126

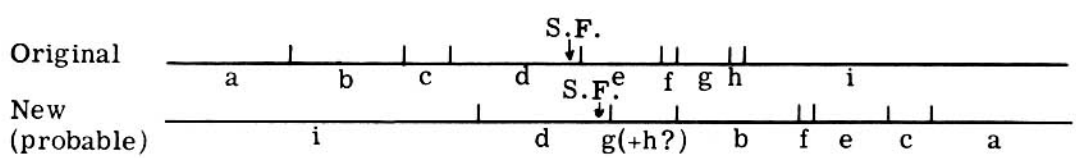
* a few may be pericentric

Many of the inversions in Drosophila, including Cl-B, are lethal when homozygous. Three of the eight inversions studied in detail in salivary gland chromosomes by Hoover (1938) and possibly two more were associated with deficiencies for one or more bands at one or both breakage points. One, 268-13, was a tandem inversion with one breakage point in-common and a deficiency at each break point:

A B C D E F A X C B X E D X F

Those with visible deficiencies might be expected to be lethal when homozygous, but of those with no obvious deficiency, roughly half when homozygous were either lethal, nearly lethal, female-sterile, or lower in viability than normal. The homozygotes for certain inversions were originally viable but later became lethal. The maintenance from the beginning as heterozygotes furnished the opportunity for carrying along a lethal mutation that occurred later.

Occasional inversions produced by X-rays have other rearrangements in the same chromosomes. Two of these, one involving eight breaks, the other seven, were analyzed by Bridges. As described by Bridges and Brehme (1944), the original breaks and the new arrangements for one of them, -- In (2 L R) dp, are as shown in the second diagram below:



From the heterozygote there were no recovered crossovers between locus 15 and 65, probably between at least dp and po in the linkage map.

Inversions in wild populations

In wild populations of Drosophila, practically all the inversions that have been found are paracentric. As pointed out by Sturtevant and Beadle (1936), this is probably because they are not handicapped by as much sterility as are the pericentric inversions. This results from the fact that the anaphase I bridges resulting from crossing over in paracentric inversions orient the crossovers away from the functional eggs. Since there is no crossing over in the ♂, there should be no selection against either type as the male.

Inversions are more frequent than chromosomal interchanges in wild populations of Drosophila, probably for the same reason, namely that interchanges are usually accompanied by lower fertility.

The few pericentric inversions that have been found in the wild were either so short that very little crossing over occurred (Carson and Stalker 1947); or if long they were associated with one or more paracentric inversions (Miller, 1939). There is evidence, however, from comparisons of the metaphase and salivary chromosome morphology of various species of Drosophila that pericentric inversions may have been important in their evolution (Wharton 1943). Several species differ in the number of euchromatic arms. The chromosome configuration of D. repleta with five pairs of rod-shaped chromosomes, five long euchromatic arms, and a pair of dot chromosomes is the probable ancestral type. A rod-shaped chromosome with one euchromatic arm may become V-shaped with both arms euchromatic as the result of a pericentric inversion. D. montana with four pairs of rod-shaped and one pair of V-shaped chromosomes, six euchromatic arms is an example of this. Such a change in two rod-shaped chromosomes would produce D. polychaeta with two pairs of V-shaped chromosomes and seven long euchromatic arms (Wharton 1943). Although successive translocations at the proper positions will accomplish the same result, this appears to be less probable. Changes in arrangement of eu- and heterochromatin may be the result of inversion or translocation.

Sturtevant and Novitski (1941) have pointed out that pericentric inversions followed by chromosome breakage at the centromere, then rearrangement, and fusion might be interpreted later as having resulted from translocation if the intermediate step were not known. However, a portion of the translocated arm would be inverted. The rearrangement of arms that they described might be considered as a form of interchange.

Inversions offer one possibility of a change which will finally be sterile with its original parents, as pointed out by Sturtevant (1938). For example, a paracentric inversion may arise (no sterility as a result of the chromatid-tie mechanism), followed by a second one only a little different from the first (see #6 and #7 in Fig. 50 of Patterson and Stone 1952):

(1) A o B C D E F G H (2) A o B C G F E D H (3) A o B C G E F D H

In the hybrid (3) x (1) crossing over in the EF segment produces monocentric chromatids that are deficient. Crossing over in the D and G segments produces dicentric chromatids. Some of the doubles produce deficient chromatids also. Thus the hybrid between 3 and the original stock, #1, is partially sterile.

Sturtevant (1931) reported genetic linkage tests with a series of 16 new crossover reducers involving the autosomes, most of which were from wild stocks of D. melanogaster. Those affecting chromosome 3 were most frequent. Many that proved to be "allelic", that is similar, were found in widely different geographical locations, indicating that the same inversion had probably arisen independently. Also, there seemed to be a correlation between the occurrence of an inversion in the two arms of the same chromosome, since six of the 7 stocks known to have an inversion in the left arm of chromosome 2 or 3 also had an inversion in the right arm, also paracentric.

Later, wild populations of other species of Drosophila were studied to determine the number of different inversions and other aberrations present, their frequencies, how they fluctuated, and the reasons for the changes. In surveys of the inversions in the chromosomes of wild strains of D. pseudoobscura, the third chromosome was the most variable with a total of 17 different arrangements. There were five for the second, two in the fourth, and five in the X chromosome (Sturtevant and Dobzhansky 1936, Dobzhansky and Sturtevant 1938, and Dobzhansky and Epling 1944). All the gene arrangements found in chromosome 3 could have arisen by the inversion of segments of this chromosome. Nearly all were related to each other as overlapping inversions. Overlapping inversions are of special interest, since they are the only ones which permit inferences regarding the

phylogeny of the resulting gene arrangements as pointed out by Sturtevant and Dobzhansky (1936). One gene sequence arbitrarily designated as the "standard" has a wide range and is the only sequence found in both the A and B races. The Pike's Peak and Arrowhead strains of race A differ from the standard by one inversion each. One of these overlaps the other as shown in the following diagrams:

```
(1) Standard        65                  70                  76
                    63 64  ABCD 66 67 68 69  ABCD  71 72 73 74 75  ABC  77 78 79 80 81
                           ↑                ↑ A                 ↑    ↑ A
                           P                                    P
```

The results may be easier to see in the following simplified diagrams:

```
(1) Standard   1  2  3  4  5  6  7  8  9  10  11  12  13
                  ↑     ↑A        ↑     ↑A
                  P               P

(2) A          1  2  3  4 | 9  8  7  6  5 | 10  11  12  13

(3) P          1  2 | 7  6  5  4  3 | 8  9  10  11  12  13
```

Here the phylogenetic sequence is either 1 ⟶ 2, 1 ⟶ 3 or the reverse of these: but not 1 ⟶ 2 ⟶ 3.

A subsequent overlapping inversion occurring in one of these inversion stocks, for example #3 will produce a new type #4. If the new type, #3 and #1 were found in a species, #3 might have originated from 1 or vice versa, 4 from 3 or vice versa, but 4 from 1 or 1 from 4 are very unlikely events, i.e. 1 ⟶ 3 ⟶ 4 or 4 ⟶ 3 ⟶ 1 or 1 ⟵ 3 ⟶ 4 are the more likely phylogenetic sequences. The direction of the sequence cannot be determined.

All the inversions they found in race A and those in race B could have been derived either directly or indirectly from the standard line. For 21 gene arrangements a phylogenetic tree was constructed, 11 of which are shown in Figure 16.

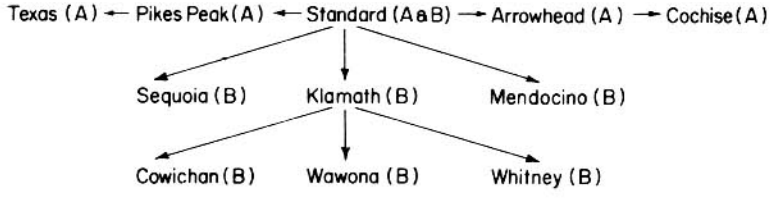


FIG. 16. The phylogeny of eleven gene arrangements in the third chromosome of Drosophila persimilis (from Patterson and Stone, 1952, in turn from Dobzhansky, courtesy of Genetics).

Any two gene arrangements connected by a line differ by one inversion. One could have arisen from the other. For example, Klamath differs from standard by one inversion. Cowichan differs from Klamath by one inversion, but is related to standard only through the intermediate step, Klamath. In the tree shown in their figure, all connecting links but one were found in wild populations.

Pavan (1946) found 17 different gene arrangements in different strains of D. nebulosa, and showed how they could arise through recombination of 8 different inversions.

Dobzhansky (1943) has stated that the seasonal fluctuations in relative frequencies

of the different gene arrangments would suggest that they affect the adaptive capacity of individuals carrying them. Extensive series of experiments were run using population cages and crosses between strains with different inversions. In the first experiments, inverions that had originated in the same area were used, but the initial proportions introduced were varied. The results showed that in the larval stage under optimal conditions the heterozygotes and homozygotes occurred in proportions close to those expected from the Hardy-Weinberg formula, with a slight excess of heterozygotes that was significant. Among the adult flies there was a considerable excess of the heterozygotes. The final equilibrium for a given combination of inversions, reached after several months, was similar for the different initial mixtures; but different for different combinations of inversions (cf. also Wright and Dobzhansky 1946).

Later experiments included combinations between inversions from widely separated areas as well as between those from the same area (Dobzhansky 1950). The experiments showed that only in the latter were the heterozygotes consistently favored. Dobzhansky concluded that there is natural selection within a population for combinations which give heterosis with each other. To restate it, the observed heterosis in F_1's carrying two chromosomes from the same population is the result of an interaction of gene combinations that have become mutually adjusted by natural selection, i.e. co-adapted. When the chromosomes for the experimental populations came from widely separated areas, often there was no heterosis, and in one instance the heterozygotes were inferior to the homozygotes. Frequently an equilibrium was not reached but one arrangement displaced the other. The interpretation of these results was that in the wild there had been no opportunity for natural selection to coadapt the two chromosome arrangements.

A natural population in which two chromosomes are in stable equilibrium is said to be polymorphic. As Wallace (1954) has stated it, this "is virtual proof of the adaptive superiority of heterozygous individuals," and goes on to state that "The studies on coadaptation lead to three conclusions: (1) The selective forces acting upon an inversion within a population are determined, at least in part, by factors other than the breakage points that determine the gene arrangement characteristic of that inversion. (2) The genetic materials carried by chromosomes with the same gene arrangement vary from locality to locality. (3) Interactions between blocks of genes, quite aside from the mere suppression of deleterious recessive mutations, affect the adaptive values of structural heterozygotes". Wallace also pointed out that the length of the region within which little or no recombination occurs differs for different combinations of inversions. For example, Santa Cruz (SC)/ Chiricahua (CH) affects a region that includes about 18% of the chromosome, while SC / Cuernavaca (CU) affects 56% of the length of the chromosome, and Cu/ Olympic (OL) affects about 80%.

The frequencies of the different inversions in D. pseudoobscura found in 1939-1942 in the San Jacinto Mountains near Los Angeles, California were compared with those found in the same area in 1952 to 1956, a period calculated to have included at least 100 generations, (Epling and Lower 1957). The inversions were chiefly in the acrocentric, i.e. nearly telocentric 3 and in the right arm of the V-shaped X. The principal arrangements fluctuated in a fairly regular way. New inversions increased in certain cases. The same points of breakage were found in arrangements present in widely separated areas that were climatically different. They concluded that similar pairs of simultaneous breaks have occurred independently.

Studies of populations of D. willistoni have been reported by Cunha and Dobzhansky (1954) and Dobzhansky (1957). This species is one of the most common of the genus in a "territory extending from the West Indies to LaPlata and from the Atlantic to the eastern slope of the Andes." Forty seven kinds of inversions have been found, up to an average of 9.4 heterozygous inversions per individual in a breeding population and up to 16 in a

single individual. Hybrids between them and a strain selected as having the standard gene arrangements in all its chromosomes had configurations so complex they were difficult to analyze. The results of the entire survey led to the conclusion that "the amount of adaptive polymorphism in a population tends to be proportional to the diversity of environmental opportunities which this population exploits".

Extensive surveys of the inversions in wild populations of other species of Drosophila have been reported in the series of "Studies of the Genetics of Drosophila" (Patterson et al. 1940 to 1954), and Dobzhansky et al. (1950).

As to the origin and maintenance of inversions in natural populations, the first step is the occurrence of the inversion. If any of the heterozygotes from the various natural crosses happen also to be heterozygous for genes located within the inversion which give one of them an advantage over both homozygotes that inversion is likely to persist and increase to a state of equilibrium in the population. A new inversion in the same chromosome may occur. If a cross brings the two together, a much longer region may be included in the heterozygosity maintenance system. There is a chance of their becoming coadapted. However, if a third inversion in this chromosome occurs, and its break positions are such as to permit recombinations of genes between the three chromosomal types, there will be a breakdown of the coadaptation between the original and the second inversion. The newest arrangement is not likely to become established. As Wallace has stated it (in a talk on this topic), three arrangements related serially by overlapping inversions can pass genes from one to another as in a bucket brigade and break down the previously established fixed gene complex. In populations of D. pseudoobscura, Wallace was able to show that three such arrangements were rarely, if ever, common in the same population. This does not prevent the third type from becoming established in new areas at the periphery of the geographic area occupied by the first two coadapted arrangements (Wallace 1959). He has applied a similar analysis to certain combinations of interchange heterozygotes in Oenothera.

Levitan (1961) has shown that in wild populations of D. robusta two chromosome combinations that were complementary (XL.XR) and (XL-1.XR-2) were in excess in one population and that (XL.XR-2) and (XL-1.XR) were in excess in another. In one environment the ancestral type was favored, in another the recombinant type.

The inter-chromosomal effects of inversions described earlier may influence the behavior also. Wallace (1954) has pointed out that data reported by Dobzhansky and Epling (1948) show that an SR arrangement which has 3 inversions in the X chromosome coexists with the Pikes Peak arrangement (chromosome 3) in local populations less frequently than it should by chance (r=-.593, P=.01-.05). This can be explained if the presence of SR brings about an increase of crossing over in other chromosomes. An increase in the frequency of double crossovers within inversions present in other chromosomes would be likely to bring about a breakdown of the mutual adaptation. Hence there would be natural selection against the SR chromosome which brought it about.

Carson (1953) has suggested that this "interplay of intrachromosomal suppression and interchromosomal intensification of crossing over" furnishes a way for the species to "exploit the advantages of adaptive chromosomal polymorphism and at the same time retain evolutionary plasticity through a high degree of recombination in structurally homozygous chromosome sections". Epling, et al. (1957) have used this principle to explain the rhythm of fluctuations of inversion heterozygosity in D. pseudoobscura.