THE EFFECTS OF INVERSIONS ON CROSSING OVER IN DROSOPHILA ROBUSTA

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FACTORS which affect the amount of genetic recombination in a species are of great importance in determining its evolutionary pattern. Natural selection, operating on the genetic system, in many cases tends to strike a balance between too much recombination, which may break up adaptive complexes of genes, and too little, which leads to specialization and evolutionary rigidity. The process of crossing over, as one of the primary effectors of recombination, occupies a position of central importance primarily because the intensity of its effect may, in an evolutionary sense, be more easily modified genetically than that of segregation or syngamy.

Extensive information has accumulated which shows that the most significant effect of an inversion of a chromosome segment is to reduce or effectively prevent recombination between sections of chromosomes. In organisms where the conditions for the persistence of inversions in natural populations are fulfilled, the gene complexes so isolated from recombination may react as units to natural selection, or may be held in equilibrium by coadaptation, so that the species displays the phenomenon of balanced polymorphism.

In most discussions of the effects of inversions on the recombination system, emphasis has been placed on their effects in greatly reducing recombination within the limits of the inverted segment itself (e.g., STURTEVANT and BEADLE 1936). On the other hand, considerable information exists that inversion heterozygosity not only affects crossing over outside the limits of the inversion in the same chromosome, but also under some circumstances profoundly influences crossing over in the other chromosomes (SCHULTZ and REDFIELD, in MORGAN, BRIDGES and SCHULTZ 1930; SCHULTZ and REDFIELD 1951; STEINBERG 1936, 1937; STEINBERG and FRASER 1944; KOMAI and TAKAKU 1940, 1942). These facts have important implications for any thorough understanding of the dynamics of inversions in natural populations.

The present study represents an attempt to discover the major outlines of the effects of inversions on crossing over in *Drosophila robusta* STURTEVANT. This species has been the object of considerable study of natural populations (CARSON and STALKER 1947; STALKER and CARSON 1947; LEVITAN 1951). These populations show a large amount of natural chromosomal polymorphism due to the presence of inverted sections. The inversions have become established in nature under the influence of natural selection. It is, therefore, of considerable interest to compare their effects on crossing over with those in other species, and to reach a better understanding, even if only in a general way, of the recombination pattern imposed upon the species by their presence.

MATERIALS AND METHODS

Drosophila robusta has a metaphase chromosome group which consists of three pairs of V-shaped chromosomes in addition to a pair of dots. These entities may be easily recognized in the salivary gland nuclei inasmuch as the two arms of the V-shaped chromosomes are commonly connected through the chromocenter, which is small and diffuse in this species. These chromosomes, together with the different gene arrangements used in this study are dia-

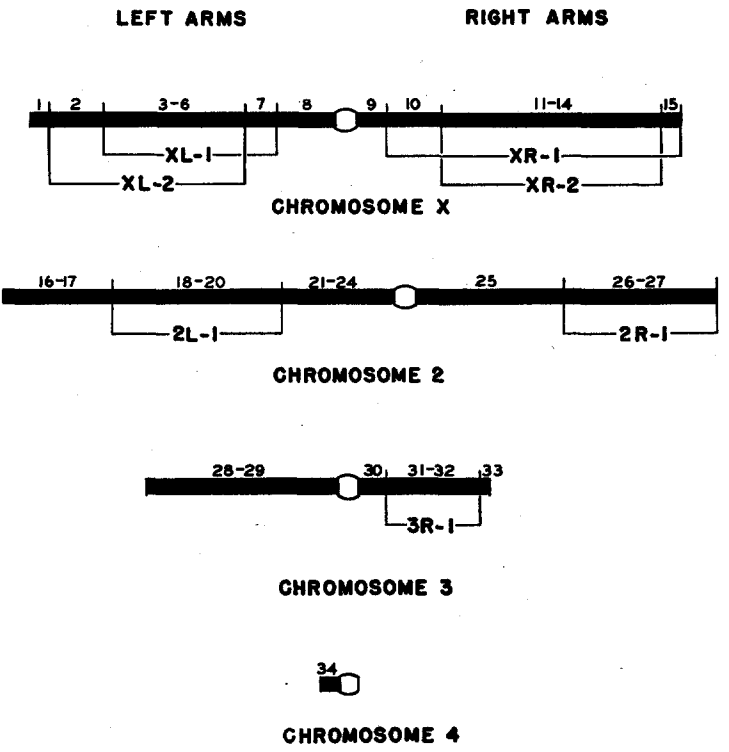

FIGURE 1.—Diagrams of the relative length of the euchromatic sections of the salivary gland chromosomes of *D. robusta*. The numbers above the dark lines, which represent the standard arrangement of the euchromatin, refer to the map regions (see CARSON and STALKER 1947). The inverted sections studied in this paper are bracketed; the light areas represent the heterochromatic bases of the chromosome arms and the centromeres.

grammed in figure 1. More detailed descriptions of the gene arrangements of this species, including the less common ones not used in this study, may be found in CARSON and STALKER (1947). The arbitrary "standard" gene sequences of *D. robusta* are designated XL, XR, 2L, 2R, 3L and 3R and it will be noted in figure 1 that there are alternative arrangements, due to inverted segments, in all chromosome arms except the left arm of chromosome 3. The small 4th chromosome has not been considered in this study.

In order to define the position and extent of the inversions used, camera

lucida tracings of a large number of heterozygotes were made from salivary gland chromosome smears; chromosomes were selected which showed the minimum amount of distortion. The relative lengths of each section of the chromosome were then measured with a map-measure and the average length of each section calculated in terms of its percent of the total chromosome length. The proportionate lengths of each chromosome arm of the set have been calculated relative to the length of the arms of the second chromosome. This was done by making tracings of XL, for example, along with tracings of 2L, 2R or both from the same nucleus. The resulting proportions are carefully reproduced in figure 1, and give a reasonably accurate picture of the positions of the various inversions and the relative sizes of the euchromatic sections of the chromosomes as observed in the salivary gland cells.

The following shorthand method of writing the structural karyotype of an individual female of *D. robusta* has been adopted for use in this paper. The conditions in each chromosome are written as three sets of symbols, from left to right, following the order X, 2 and 3. The capital letter " S " is used to refer to the standard arrangement in each case and numerals are used to refer to the various alternative gene orders in that particular arm. Thus, the formula for an individual female homozygous for all standard arrangements would be written:

X	2	3R
SS	SS	S
SS	SS	S

In the symbols for chromosomes X and 2, the S's at the left in each case refer to the gene arrangements in the left arms, the centromeres are inferred as being in the center, so that the S's on the right refer to the conditions in the right arms. In chromosome 3, the left arm is omitted in the formula, inasmuch as in none of the structural karyotypes used in this study was it involved in an inversion. Following this scheme, any combination may be simply symbolized and visualized. For example, an individual heterozygous for the four inversions XL/XL-1, XR/XR, 2L/2L-1, 2R/2R-1, 3R/3R-1 may be written as follows:

X	2	3
SS	S1	S
1S	1S	1

This formula gives at a glance the further information that in this case the arrangements in the second chromosome bear a " repulsion " relationship to one another rather than " coupling," which would be written:

2
SS
11

Most of the strains of *D. robusta* used in this study were derived from single wild-caught females. The gene arrangements present for each arm of each

chromosome were in most cases determined by making acetocarmine smears of the salivary glands of offspring of the original wild female. In a number of cases, it was necessary to select for certain arrangements in a stock so that a desired structural karyotype could be obtained. In this selective procedure, twelve or fifteen virgin females were taken at random from a stock and mated individually to single males from the same stock. Salivary gland chromosome smears of the offspring of each of these pair matings were then examined and the pairs transmitting the desired combinations were selected and new stocks established from these. Several repetitions of this process were sometimes necessary to obtain the desired combinations.

By making appropriate matings between individuals from such stocks, F_1 females of a large variety of inversion karyotypes could be obtained. Crossing over in this study was for the most part detected cytologically in salivary gland chromosome smears of the offspring of such selected females, which were mated to males homozygous for gene arrangement. For example, from a cross such as the following:

$$\begin{array}{ccc} \text{female} & & \text{male} \\ \dfrac{\begin{matrix}2\\S1\end{matrix}}{1S} & \times & \dfrac{\begin{matrix}2\\SS\end{matrix}}{SS} \end{array}$$

the non-crossovers from chromosome 2 would be:

$$\frac{S1}{SS} \quad \text{and} \quad \frac{1S}{SS}$$

Individuals which had received a gamete from the female parent in which crossing over had occurred in the central region of the chromosome between the two inversions would be:

$$\frac{SS}{SS} \quad \text{and} \quad \frac{11}{SS}$$

Because of the favorableness of the chromosomes of this species both heterozygotes and homozygotes can be quickly and accurately determined. In practice, a single gland from each larva was used, and ten such glands smeared in two rows of five each under a single 22×40-mm coverslip. The rapidity of this method has made cytological detection of crossovers possible at a reasonably quantitative level.

In all of the tests here reported, data on the offspring of single females were recorded. The female was transferred to a new vial every two days and the larvae to be smeared were selected at random as they matured in the older vials. The data obtained on each day were kept separately. In general, smears were made from larvae which hatched from eggs oviposited over the first ten days of active egg-laying by young females. The above precautions were taken to minimize possible effects of the age of the female on crossing over. Under these conditions there was no pronounced or consistent age effect and the

temporal results have therefore been combined in the tables. All experiments were carried out at 25 ± 1°C.

OBSERVATIONS

Crossing over in the central region of chromosome 2 under various conditions of structural homo- and heterozygosity in chromosomes X and 3. In these experiments, the second chromosome configuration S 1/1 S was selected as a test object and crossing over in the section of the chromosome between the

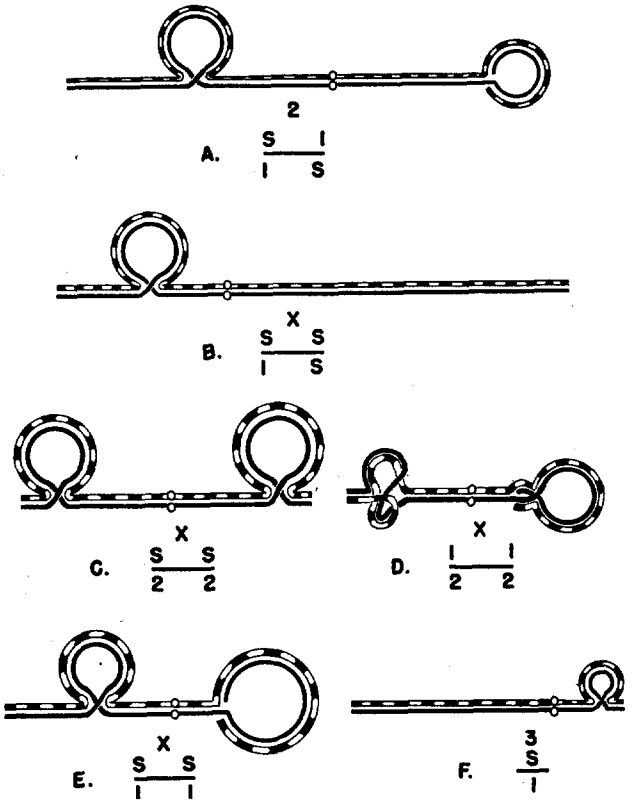

FIGURE 2.—Diagrams of the synaptic configurations, as observed in the salivary glands, formed by the various structural heterozygotes considered in tables 1, 2 and 3. The symbols below the figures are explained in the text.

inversions was studied under varying conditions of gene sequence in chromosomes X and 3. The cytological method for the detection of crossing over permits the observation of any crossovers which occur between the inversions in the second chromosome configuration S 1/1 S, that is, in regions 21–25 (see chromosome 2, figure 1). These regions together make up 37.6% of the total euchromatic length of the chromosome. This, of course, represents a minimum figure for the length of chromosome available for detected crossovers, inasmuch as it does not include the heterochromatin at the bases of the two arms. A dia-

gram of the double loop configuration formed by S 1/1 S, as seen in the salivary gland cell, is given in figure 2, A. The heterozygous configurations formed by the inversions in chromosomes X and 3, which have been used in various combinations with the above, are diagrammed in figure 2, B–F.

Table 1 shows the results obtained from a study of crossing over in the test configuration of chromosome 2 when there are (a) no inversion loops in X

TABLE 1

Crossing over in the central part (regions 21–25) of the doubly heterozygous chromosome 2, S 1/1 S, under varying conditions of homozygosity and single heterozygosity in chromosomes X and 3.

Expt. No.	Conditions in chromosome		No. of females	No. of F_1 larvae smeared	Percent of recombination
	X	3			
1	$\frac{11}{11}$	$\frac{S}{S}$	24	467	0.0
2		$\frac{S}{1}$	3	304	0.0
3	$\frac{22}{22}$	$\frac{1}{1}$	1	100	0.0
4		$\frac{S}{1}$	3	225	0.4
5A & B	$\frac{SS}{SS}$	$\frac{S}{S}$	(5A) 1	166	0.6
			(5B*) 4	589	4.2
6	$\frac{S1}{S1}$	$\frac{S}{S}$	2	55	0.0
7	$\frac{S1}{11}$	$\frac{S}{S}$	3	250	0.0
8		$\frac{S}{1}$	2	200	0.5

* Females in this experiment carried the inversions in the second chromosome in the coupling combination, SS/11.

and 3 (experiments 1, 3, 5 and 6) and (b) when X and 3 are singly heterozygous, either separately (experiments 2, 4 and 7) or coincidentally (experiment 8). Under these conditions, it is clear that crossing over in the test region of chromosome 2 is a very rare event. Except for the results in experiment 5B, which will be interpreted later, there are only 3 recombinations, or 0.2%, in 1767 F_1 larvae smeared.

When the X chromosome is structurally heterozygous in both arms, crossing

over in the test configuration is strikingly increased (table 2). Within each of the experiments reported in this table, the data are homogeneous (i.e., the p values are all above .05) except for experiment 13. In the latter case, χ^2 for the group of 12 females is 20.17, which, with 11 degrees of freedom, gives a p value of between .05 and .04. In view of the number of such tests made, no particular significance has been attached to this possible heterogeneity.

TABLE 2

Crossing over in the central part (regions 21–25) of the doubly heterozygous chromosome 2, S1/1S, when chromosome X is also doubly heterozygous.

Expt. No.	Conditions in chromosome		No. of females	No. of F_1 larvae smeared	Percent of recombination
	X	3			
9A & B	SS / 11	S / S	(9A) 3	300	3.3
			(9B*) 5	400	5.3
			total 8	700	4.4
10		S / 1	3	300	9.0
11		1 / 1	2	200	6.5
12	11 / 22	S / S	4	332	19.3
13		S / 1	12	1150	34.3
14		1 / 1	4	400	7.5
15*	SS / 22	S / S	3	300	12.0
16		S / 1	6	473	33.8

* Females in this experiment carried the inversions in the second chromosome in the coupling combination, SS/11.

Inspection of the data reveals that the boosting effect of certain X chromosome double heterozygotes is stronger than others. Thus in experiments 9 and 10 (table 2), we observe that the X configuration SS/11 exerts a boosting effect on crossing over in chromosome 2 (compare with experiments 7 and 8, table 1). This effect, however, is considerably less than that of the X chromosome configurations which are heterozygous for XL–2: XR–2 (experiments 12–13; 15–16).

Conditions in the third chromosome also exert an important effect. In the first place, females which are heterozygous S/1 in the third chromosome (experiments 10, 13 and 16) show more crossing over than when chromosome 3 is homozygous S/S. These differences are significant: for experiment 9 vs. experiment 10, χ^2 is 8.03, p less than .01; for experiment 15 vs. experiment 16, χ^2 is 46.04, p negligible. Despite the suspicion of heterogeneity within experiment 13, there is no question that the results differ widely from those obtained in experiment 12.

It is thus clear that double structural heterozygosity in the X chromosome greatly increases crossing over in chromosome 2 and that this effect is intensified by heterozygosity in the right arm of chromosome 3.

A second and very important fact is revealed by a comparison of experiment 11 with experiment 12 and 14 with 15 (table 2). In each of these pairs of experiments, the inversion karyotypes are structurally the same; they differ only in the nature of the 3rd chromosome homozygote (either 1/1 or S/S). Nevertheless, in both cases the amount of crossing over is significantly less in homozygous 1/1 individuals. For experiments 11 and 12, χ^2 is 16.49, which, with one degree of freedom gives a negligible p value; for experiments 14 and 15, χ^2 is 4.05, giving a p value of .05–.02. These, then, represent instances where females with karyotypes which are the same as far as the presence of inversion loops goes, show significant differences in crossing over. Such differences must be due to genetic differences apart from the presence or absence of inversion loops.

There is further evidence which supports the above suggestion of a genic effect. It will be noted (table 1) that experiment 5A differs from 5B, although the karyotypes are the same. This case is not entirely satisfactory inasmuch as the females in experiment 5B carried the test inversions in coupling, rather than in the usual repulsion. *A priori,* one would not expect any differential effect on crossing over between coupling and repulsion inversion figures, and the similarity of the results in experiments 9A and 9B (table 2) supports this conclusion. It is therefore suggestive that the differences between 5A and 5B are due to genic differences. The females used in experiments 5 and 6, unlike the rest, were wild females collected in nature and thus represent a rather more heterogeneous sample than the inbred laboratory strains used in the other experiments.

In experiments of this kind, where selection and backcrossing must be used in order to obtain the desired inversion karyotypes, it has not been possible to control in a systematic way the genetic background of the females tested. Nonetheless, in a number of cases the females for the experiments were selected from among a segregating group of sibs. Thus, for example, females in the following experiments were sibs: 14 and 16; 9B and 15, 9A, 10, 12 and some of the females in 1, 3, 4 and 11. In each of these experiments females with identical structural karyotypes showed no significant differences, while the interkaryotype differences were large. This suggests a prominent effect of the structural karyotype.

The above results may be summed up as follows. When chromosome 2 has

an inversion in each arm, and crossing over in the central section is observed, it is found that recombination is very low unless there is a considerable degree of structural heterozygosity in the other chromosomes. Double heterozygosity in chromosome X raises crossing over in chromosome 2 to the point where an affect of heterozygosity in the right arm of chromosome 3 also can be observed. In addition to these apparently direct interchromosomal effects of structural heterozygosity, clear differences in crossing over between individuals of similar inversion karyotypes indicate that there is also a genic effect.

Crossing over in the X chromosome. Crossing over in the X chromosome under varying conditions of structural hetero- and homozygosity has been studied with the use of two sex-linked recessive genes, echinus eye (*e*) and scarlet eye (*st*). This stock was kindly supplied by DR. A. H. STURTEVANT. Salivary gland chromosome smears showed the X chromosome of this strain to be homozygous XL–1 : XR–1.

The location of echinus and scarlet. A number of experiments were done in an attempt to determine the positions of *e* and *st* in the X chromosome with respect to the positions of the relatively inverted segments. From these experiments, the best estimate of the positions of these genes which can be given is that echinus is located in the left arm distal to region 8 and that scarlet is located in the right arm distal to region 9 (see figure 1).

The above conclusion was reached as follows. In females homozygous XL–1 : XR–1 and simultaneously *e st*/++ (experiment 17, table 3), the two genes segregate at random. This indicates that they are either in separate arms or are at least 50 units apart in the same arm. The females in this experiment, and also in experiment 18, were unavoidably heterozygous for gene arrangement 2L–3 (see CARSON and STALKER 1947); in view of experiments to be reported later, however, this probably has no appreciable effect on crossing over in an X chromosome homozygous for gene arrangement.

Echinus and scarlet also segregate at random when the X chromosome is *e st*/++ and simultaneously XR/XR–1 (experiment 18, table 3). It will be noted that this inversion covers a large percentage of the euchromatin in the right arm and is terminal (figure 1 and figure 2, E, part to the right of the centromere). Six echinus and six scarlet crossover males were tested for gene arrangement by crossing them individually to females homozygous for gene arrangement. Each of the scarlet individuals was XL–1 : XR–1 and each of the echinus individuals was XL–1 : XR, that is, all were crossovers between *e* and the XR–1 inversion. These individuals are considered to arise as simple single crossovers. From these results, it will be seen that *e* cannot be located within the XR–1 inversion and, further, must be located to the left of *st*.

Similar results are obtained when the X chromosome is *e st*/++ and simultaneously XL/XL–1 (experiments 7 and 19, table 3); under these conditions *e* and *st* again segregate at random. Again, six crossover males of each type from experiment 19 were tested and as all six scarlet individuals were also crossovers between XL–1 and *st*, the latter gene cannot be located within XL–1. Because echinus must be to the left of scarlet, the latter cannot be

TABLE 3

Crossing over between e (echinus) and st (scarlet) in the X chromosome under varying conditions of structural heterozygosity.

Expt. No.	Structural karyotype of tested female: X	2	3R	Total counted	Percent crossovers between *e* and *st*	Karyotypes of recombined X chromosomes	Percent recombination in central region of chromosome 2 (from tables 1 & 2)
17	$\frac{1++1}{1\,e\,st\,1}$	$\frac{1S}{3S}$	$\frac{S}{S}$	5638	49.5	*e*: $\frac{1\,1}{}$ *st*: $\frac{1\,1}{}$	....
1	$\frac{1++1}{1++1}$	$\frac{S\,1}{1S}$	$\frac{S}{S}$	....	....	....	0.0
18	$\frac{1++S}{1\,e\,st\,1}$	$\frac{1S}{3S}$	$\frac{S}{S}$	2111	48.8	*e*: $\frac{1S}{}$ *st*: $\frac{1\,1}{}$	....
7	$\frac{S++1}{1\,e\,st\,1}$	$\frac{S\,1}{1S}$	$\frac{S}{S}$	1155	48.1	(Not tested)	0.0
19	$\frac{S++1}{1\,e\,st\,1}$	$\frac{1S}{1S}$	$\frac{S}{S}$	319	48.6	*e*: $\frac{1\,1}{}$ *st*: $\frac{S\,1}{}$	....
20	$\frac{S++1}{1\,e\,st\,1}$	Variable*	$\frac{S}{S}$	4166	47.2	(Not tested)	....
12	$\frac{1\,e\,st\,1}{2++2}$	$\frac{S\,1}{1S}$	$\frac{S}{S}$	1458	0.00	(None obtained)	19.3
13	$\frac{1\,e\,st\,1}{2++2}$	$\frac{S\,1}{1S}$	$\frac{S}{1}$	1010	0.29	*st*: 3 individuals; abnormal; sterile	34.3
21	$\frac{1\,e\,st\,1}{2++2}$	$\frac{1S}{1S}$	$\frac{S}{1}$	2272	0.00	(None obtained)	....
9A	$\frac{S++S}{1\,e\,st\,1}$	$\frac{S\,1}{1S}$	$\frac{S}{S}$	3512	0.03	*st*: $\frac{1\,1}{}$	3.3
22	$\frac{S++S}{1\,e\,st\,1}$	Variable†	$\frac{S}{S}$	13260	0.02	*e*: $\frac{S\,S}{}$ *e*: $\frac{1\,1}{}$	....
10	$\frac{S++S}{1\,e\,st\,1}$	$\frac{S\,1}{1S}$	$\frac{S}{1}$	1301	0.77	*e*: $\frac{1\,1}{}$ *st*: $\frac{S\,S}{}$	9.0

* $\frac{1S}{1S}$, $\frac{S\,1}{1S}$ or $\frac{S\,S}{1S}$.

† $\frac{S\,S}{1S}$ or $\frac{S\,S}{S\,1}$.

located to the left of the distal break of XL–1 (regions 1 and 2), because a crossover between *e* and *st* in this position could not also be a crossover between *st* and XL–1.

In the experiments with the doubly heterozygous X chromosome configuration S (++) S/1 (*e st*) 1 (experiments 9A, 22 and 10, table 3) there are very few crossovers between *e* and *st* (14 out of 18,073, or 0.08%). Nine of these individuals were tested for gene arrangement in the X; four were *e*: 1 1, three were *st*: S S; one was *e*: S S; one was *st*: 1 1. This result is crucial inasmuch as none of these chromosomes is also a recombination between the inversions. This means that, whether they were formed by single or by double crossing over, in no case did a single crossover occur in the central part of the chromosome (regions 8–9) between the inversions. If this event had occurred, the sequences S 1 or 1 S would have been recovered. Thus, *st* cannot be located in the center section of the chromosome, because on this assumption, the only way that the three *st*: S S and the four *e*: 1 1 chromatids could form would be by double crossing over in this center section. This would require the occurrence of double crossing over seven times in a section where no singles have been detected. By elimination, therefore, *st* must be located in the right arm, within the XR–1 inversion (regions 10–15). Likewise, if *e* were in the center section, the recovery of the *e*: S S and *st*: 1 1 chromosomes could only be explained by double crossing over in the center section, effectively removing *e* to S S. It is therefore concluded that *e* is located in the left arm of the X, to the left of region 8.

On the above basis, the crossover individuals tested from experiments 9A, 22 and 10 (table 3) would be explained as follows: The *e*: 1 1 and *st*: S S individuals arose through double crossing over within the large inversion loop in the right arm. The *e*: S S and *st*: 1 1 individuals could have arisen either through a single crossover between *e* and XL–1, if *e* is distal to the latter, or through double crossing over within inversion XL–1, if *e* is located there. The latter hypothesis is considered the more likely, due to the very great rarity of crossovers between *e* and XL–1.

Concurrent crossing over in chromosomes X and 2. As has been shown above, crossing over in the central region of the doubly heterozygous chromosome 2 is very low when the X chromosome is structurally homozygous or singly heterozygous. The information given by experiment 7 (table 3) shows that under these conditions crossovers are abundant in the X chromosome, and no difference is detectable when chromosome 2 is homozygous (experiment 19) or singly heterozygous (experiments 17 and 18). When, however, chromosome X is doubly heterozygous, either 1 1/2 2 or S S/1 1, we have seen previously that crossing over in the central region of chromosome 2 is greatly increased. It may now be seen that this increase is correlated with a very pronounced blocking of crossing over in chromosome X by the presence of an inversion in each arm. This effect is strikingly emphasized by the fact that from the doubly heterozygous X chromosomes studied, not a single case of crossing over in the central region of the chromosome has been observed

(experiments 12, 21, 9A, 22 and 10, table 3). The three *st* crossovers in experiment 13, table 3 represent possible exceptions; as they were sterile, they could not be tested for gene arrangement.

The rare crossovers in the X chromosome appear primarily in experiments where the second chromosome was doubly heterozygous and the right arm of the third chromosome was likewise heterozygous. This is indicated by a comparison of the results in experiment 13 with those in experiments 12 and 21, table 3 and the comparison of experiment 10 (10 double crossovers in 1301) with experiments 9A and 22 (4 crossovers, at least one of which was a double, in 16,772). This suggests that such crossovers are "forced out" by the residual structural heterozygosity. The complex configuration in the X formed by the double heterozygote 1 1/2 2 (see figure 2, D: overlapping inversions in the left arm and included inversions in the right) appears to be more resistant to such forcing. The only crossovers which were obtained from it were weak, abnormal, sterile females. This suggests, as does the fact that they were scarlet, that they arose by a crossover within the included portion of the figure in the right arm of the X, an event which would result in duplication-deficiency. This is further supported by the fact that among 51 F_1 larvae smeared from a wild female of the constitution:

X	2	3
S 1	S S	S
1 2	1 S	S

there were no crossovers between the inversions in the X, but one larva carried a chromosome which was duplication region 10 and deficiency region 15 in the right arm. This is the expected result following an exchange within the included portion of this right arm figure.

The facts presented above suggest that reductions or suppressions of crossing over in the X chromosome may bear some relationship to the degree of complexity of the configuration involved. Thus, the X configuration 1 1/2 2 has a stronger boosting effect on crossing over in chromosome 2 than does the simpler configuration S S/1 1. Correlatively, the former appears to permit fewer crossovers in the X itself.

Despite the above considerations, however, several facts suggest that these interrelationships are again not wholly structural. In the first place, the structural heterozygote S S/2 2, which is simpler than the others studied (see figure 2, C) and leaves a long central area, amounting to about 29% of the euchromatin, available for crossing over, has the same effect on the doubly heterozygous chromosome 2 as does the complex figure 1 1/2 2. As these differ strikingly in this respect from the heterozygote S S/1 1, one is led to consider that some peculiar property of the combination XL–2: XR–2 is involved rather than the purely structural inversion figures formed. XL–2 and XR–2 heretofore have been suspected of showing unusual behavior. A highly significant association exists between these two arrangements in natural populations (CARSON and STALKER 1949) and it has been suggested that XR–2

acts as a " protector " of XL–2, inasmuch as the latter has only in several rare instances been observed to occur in natural populations apart from XR–2 in the same chromosome.

In the laboratory, no certain instance of the separation of the XL–2 : XR–2 complex by crossing over has been observed. This has been studied genetically, in heterozygotes with 1 (*e st*) 1 and cytologically in heterozygotes with S S. The latter was done by NELSON (1951) who examined 414 F_1 larvae of females which were heterozygous S S/2 2 without finding a single recombination. Unfortunately, the difficulty of extracting *e* and *st* from their association with XL–1 : XR–1 has hampered the usefulness of these genes in the study of crossing over in various X chromosome heterozygotes.

The analysis of the interchromosomal effects of inversions is probably affected in some measure by the following considerations. When a V-shaped chromosome is heterozygous for an inversion in each arm, there is likely to be a considerable number of double crossovers which are made up of singles within each inversion loop. Half of these will lead to the death of the zygote (or to X0 males, in the case of the X chromosome) rather than selective elimination of the crossover strands. If these events were to occur in the X chromosome, they would be expected to result in an increase of recovered crossovers in chromosome 2, not because more crossovers occurred but because more non-crossovers died.

From the data presented in this paper, it is not possible to estimate the magnitude of this effect. Certain of the results given in table 2, however, have some bearing on this question and make it seem unlikely that this effect is a prominent one. It will be noted in figures 1 and 2 that the X chromosome double heterozygotes S S/1 1 and S S/2 2 form loops which are rather similar in size. XR–1 is the largest, covering 46.3% of the euchromatin; the figures for the others are: XL–1, 27.1%; XL–2, 30.8%; XR–2, 34.6%. When the results of experiments 10 and 16 (table 2) are compared, it appears to be highly unlikely that the very large difference in recombination frequency observed in the second chromosome could be due to the more frequent occurrence of the required types of double crossovers in the X configuration S S/2 2 rather than in S S/1 1. The results in experiments 9 and 15 (table 2) may be similarly compared. Under any circumstances, moreover, the *effective* frequency of recombination in chromosome 2, in a population sense, would be increased.

The major effects of the inversions of *D. robusta* on crossing over, as revealed in the above experiments, may be outlined as follows.

1. There is an apparent intrachromosomal suppression of crossing over in the central section of a chromosome which has an inversion in each arm. This suppression is absolute in the X chromosome cases studied but is less pronounced in the case of the second chromosome.

2. There is interchromosomal intensification of crossing over in certain chromosome sections when a certain level of structural heterozygosity is reached. The double heterozygotes in chromosome 2 and X interact on each

other. Central intrachromosomal suppression in chromosome 2 is weakened by the interchromosomal boosting effects of double heterozygotes in the X. Double heterozygotes in chromosome 2, however, appear to be less effective in intensifying crossing over in chromosome X.

3. Structural heterozygosity in chromosome 3 intensifies the above interchromosomal effects.

4. Certain of the X chromosome double heterozygotes, especially those involving XL–2 : XR–2, have a stronger interchromosomal effect than others.

5. The amount of crossing over in a given section is not wholly dependent on structural conditions in either the same chromosome or in other chromosomes. Thus a genic influence may affect in a minor way the amount of crossing over as conditioned by the structural heterozygosity.

As a means of visualizing the results, the following crude scheme is suggested; it is used for exposition only, and is not to be taken as having an explanatory value. Assume that a nucleus about to undergo meiosis has a certain total quota of crossovers available to it. The size of this quota may or may not be fixed; it may be affected by structural conditions or genic factors or both, and it may differ from individual to individual. These crossovers may be visualized as forming in those sections of the chromosomes where the least resistance to crossover formation is met. Thus in an individual of the formula:

X	2	3
1 1	S 1	S
1 1	1 S	S

we know that crossing over is largely suppressed between the inversions in chromosome 2; we would then visualize increased crossing over in less resistant sections, possibly in those chromosome arms homozygous for gene arrangement. In a highly heterozygous individual, such as:

X	2	3
1 1	S 1	S
2 2	1 S	1

the crossovers meet strong interference due to inter-inversion suppressions and possibly intra- and para-inversion suppression. We may visualize crossing over as occurring, maximally, perhaps, in the one free homozygous arm (3L); the remainder of the quota, however, must be distributed in sections where crossing over meets with more resistance. It is suggested that these sections differ in their degree of resistance so that, for instance, the central section of the second chromosome, a region of relatively low resistance, now acquires more crossovers than it would when the inversions are absent in the other chromosomes.

DISCUSSION

The essential effects of heterozygous inversions on the frequency and pattern of crossing over fall into three interrelated categories and may be set forth as follows:

1. *Suppression of crossovers within an inverted section.* The definitive work of STURTEVANT and BEADLE (1936) on *D. melanogaster* shows that effective genetic recombination between relatively inverted chromosome segments is negligible, with the possible exception of very long inversions, in which a significant number of 2-strand double exchanges could occur. This blocking of recombination, however, is not due primarily to the suppression of exchanges initially, but rather to the selective elimination of most types of crossover strands from the definitive egg nucleus (see also CARSON 1946). Quite apart from this mechanical elimination of the products of crossing over, however, it is clear that inversions, especially short ones, do interfere to some extent with the actual formation of crossovers within the inverted segment. Thus from individuals heterozygous for the relatively short X-chromosome inversions delta-49 and scute-7, STURTEVANT and BEADLE recovered fewer 2-strand double exchanges than expected. This was not true for longer inversions such as scute-8. The critical length for such effects has not been determined and, indeed, other unspecified effects on crossing over may exist as properties of individual inversions. There is thus no reason to believe that the suppressive effect of an inversion on crossovers within it must necessarily be directly proportional to the length of that inversion.

2. *Suppression of crossing over distal or proximal to the inversion in the same chromosome.* STURTEVANT and BEADLE (1936) found that certain X chromosome inversions in *D. melanogaster* suppressed crossovers adjacent to them; these effects were generally greater distally (between the inversion and the free end of the chromosome) than proximally. Similar effects were found by DOBZHANSKY and EPLING (1948) for certain 3rd chromosome inversions in *D. pseudoobscura,* although in some cases proximal suppression was also strong. In the above cases, the inversions studied have one break point close to the distal end. That these cases of strong distal suppression may be mediated, at least in part, by this fact is indicated by the findings of KOMAI and TAKAKU (1940, 1942) in *D. virilis.* Although there is strong suppression proximally when two independent inversions are present in the rod-shaped X chromosome of this species, the distal end was affected only at a point close to the inversion, and crossing over was actually increased in the long free distal end. When an inversion is present in each arm of the V-shaped chromosomes X or 2 of *D. robusta,* crossovers appear to be strongly suppressed in the region between the inversions. Such suppression appears to be closely comparable to that found in the V-shaped autosomes of *D. melanogaster* when an inversion is present in each arm (e.g., Curly inversions in chromosome 2; Payne inversions in chromosome 3). A similar case has been described in the V-shaped X chromosome of species hybrids between *D. pseudoobscura* and *D. persimilis* (LANCEFIELD 1929; MACKNIGHT 1937).

3. *Intensification of crossing over in heterologous chromosomes.* Inversion heterozygosity in one chromosome, under many if not all conditions, increases crossing over in the other chromosomes. The effects are not confined to regions which are homozygous for gene arrangement; thus if other heterozygous inversions are present, the frequency of recoverable double crossovers

is increased under these conditions. These phenomena have been subjected to intensive study in *D. melanogaster* (SCHULTZ and REDFIELD in MORGAN, BRIDGES and SCHULTZ 1930; STEINBERG 1936, 1937; STEINBERG and FRASER 1944; SCHULTZ and REDFIELD 1951) and have been studied in *D. virilis* (KOMAI and TAKAKU 1940, 1942), *D. pseudoobscura* (LEVINE and DICKINSON 1952) and in hybrids between *D. pseudoobscura* and *D. persimilis* (MACKNIGHT 1937).

The results obtained with *D. robusta* are in close general agreement with previous findings. Because of the fact, however, that the test regions, such as the central portion of chromosome 2, are under the influence of suppressions by inversions in the same chromosome, a considerable degree of inversion heterozygosity is necessary for the observation of a strong interchromosomal effect. The results are nonetheless striking, and the test region showed an approximately 30-fold increase under some conditions. Insufficient genetic markers are available in *D. robusta* for the testing of chromosome regions in the way that is possible in *D. melanogaster,* and control crossover frequencies and patterns in structural homozygotes are not available. Such control frequencies, however, would have little meaning for most natural populations of this species, in which a structural homozygote is a rarity (CARSON and STALKER 1947, 1949). Conventional chromosome maps for species which show extensive inversion heterozygosity in natural populations are useful primarily as an artificial frame of reference and do not reflect the dynamics of recombination as it occurs under natural conditions.

The fact that certain X chromosome heterozygotes in *D. robusta* have a stronger boosting effect than others correlates well with the findings of STEINBERG and FRASER (1944) who found similar differences among a number of X chromosome inversions in *D. melanogaster.* SCHULTZ and REDFIELD (1951) have confirmed and extended these observations. The latter authors, furthermore, found differential effects of the X chromosome homozygotes scute-8/scute-8 and +/+ on crossing over in the 3rd chromosome; this recalls the observation that 3R/3R and 3R–1/3R–1 have similar differential effects in *D. robusta.* As STEINBERG and FRASER have pointed out, factors other than the length of the inversion, its position relative to the chromosome ends and probably also the number of crossovers permitted within and adjacent to it, are involved in the interchromosomal effects.

Not only do individual inversion heterozygotes and their homozygotes thus vary in their intensification effects elsewhere in the chromosome set, but also different regions of the affected chromosomes react differentially to the intensification stimulus. This has been repeatedly demonstrated for *D. melanogaster* and has been confirmed in *D. virilis.* In these cases the chromosome regions most sensitive to the boosting effects lie near the centromere and distally in the chromosome arm. These are regions where, in the absence of structural heterozygosity, crossing over appears to be relatively low.

The data reviewed above indicate that crossovers tend to be suppressed within and adjacent to heterozygous inversions and that this is directly correlated with an increase of crossing over elsewhere. This interplay of intra-

chromosomal suppression and interchromosomal intensification of crossing over has suggested to a number of authors (e.g., MATHER 1936) that, by and large, inversions do not drastically affect the total frequency of exchanges in a nucleus; suppression in one region is accompanied by an increase in another. One may recall the remark of MULLER, who likened this phenomenon to the reaction of a balloon; if it is pressed in at one point, it bulges out at another. In most cases, this appears to be at least approximately true.

These considerations have important implications for natural populations. Natural selection for co-adapted inversion heterozygotes in a species may operate with chromosome sections greater than that involved in an inversion if recombination outside its limits, or between adjacent inversions, is strongly suppressed. The case of the XL–2: XR–2 chromosome described in this paper is an extreme example of this; no certain recombinants between the left and right arm have been found in natural populations or in laboratory experiments, even where the situation has been forced by strong inversion heterozygosity in other chromosomes. The whole chromosome thus appears to function as a non-recombining unit.

Co-adaptation of inversion heterozygotes is a flexible system which permits the species to maintain adaptations to a number of different ecological niches (DOBZHANSKY 1950). This is accomplished, however, with the sacrifice of a certain amount of recombination. Although recombination through crossing over may occur freely between homologous chromosomes of like gene sequence, two alternative arrangements are effectively isolated from one another. As inversion heterozygotes accumulate in a species, its hereditary material becomes more and more a mosaic of such isolations. Heterozygous inversions, however, effectively shunt crossovers into other sections of the chromosome set, apparently primarily those which are not involved in inversions. Such a result would keep available for the species the possibility of evolutionary adjustment through a highly active recombination of genes in at least part of the chromosome set. The species may thus exploit the advantages of adaptive chromosomal polymorphism and at the same time retain the more conventional advantage of intense recombination. This powerful side effect of the inversion system would seem to be of special importance in the higher Diptera which, because of the elimination of crossing over in males, are highly dependent on crossing over in females for the release of concealed variability.

SUMMARY

1. The influence of naturally-occurring inversions on crossing over in *Drosophila robusta* has been investigated for a series of selected structural heterozygotes. Both cytological and genetic methods for the detection of crossing over have been employed.

2. When an inversion is present in each arm of a V-shaped chromosome, there is a strong intrachromosomal suppression of crossing over in the portion of the chromosome between the inversions. This suppression is absolute in the X chromosome cases studied but is less pronounced in the second chromosome.

3. Crossing over is intensified in certain chromosome sections when a relatively high degree of structural heterozygosity is reached. Thus double heterozygosity in chromosome X is correlated with a significant increase of crossing over in the section between the two second chromosome inversions. The doubly heterozygous condition in chromosome 2, however, appears to be much less effective in intensifying crossing over in the X chromosome.

4. Structural heterozygosity in the right arm of chromosome 3 intensifies the above interchromosomal effects.

5. Certain X chromosome double heterozygotes, especially those involving XL–2 : XR–2, have a stronger boosting effect than others.

6. The amount of crossing over in a given section is not wholly dependent on structural conditions in either the same chromosome or in other chromosomes. A genic influence may thus affect in a minor way the pattern and intensity of crossing over as conditioned by the structural heterozygosity.

7. The interplay of intrachromosomal suppression and interchromosomal intensification of crossing over indicates that inversions do not greatly reduce the total frequency of exchanges. On the other hand, crossovers appear to be shunted principally into sections of the chromosome set which are not involved in inversions. The suggestion is made that in this way the species may 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.

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