THE EFFECT OF HETEROZYGOUS AUTOSOMAL INVERSIONS ON
INDUCED EXCHANGES BETWEEN THE ATTACHED-X
AND Y CHROMOSOMES IN *DROSOPHILA*
MELANOGASTER FEMALES¹

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SINCE the original observation of STURTEVANT (1919) a large body of data has accumulated substantiating the contention that, in *Drosophila melanogaster* females, inversion heterozygosity in one part of the genome increases the frequency of recombination in the remainder of the genome. This effect has been demonstrated for spontaneous meiotic crossing over (see, for instance, SCHULTZ and REDFIELD 1951; RAMEL 1962; SUZUKI 1963), and for spontaneous somatic crossing over (BROWN and WELSHONS 1955; RONEN 1964). COOPER (1946) studied the effects of autosomal inversions on the frequency of spontaneous exchanges between attached-X and Y chromosomes and reported that heterologous structural heterozygosity had no effect on the rate of detachment of the attached-X. The purpose of this paper is to present experimental evidence of a significant increase of *induced* exchanges between the attached-X and Y chromosomes when heterozygous autosomal inversions are present in the genome.

MATERIALS AND METHODS

The autosomal inversions used were *Ins(2L+2R) Cy*, *cn*² marked by the dominant wing mutant Curly (*Cy*) and the recessive eye mutant cinnabar (*cn*), and *In(3LR) Cx, D* marked by the dominant wing and bristle mutant Dichaete (*D*). All females had an attached-X chromosome (reversed metacentric) homozygous for the mutant γ (yellow body and bristles), and the *B^SY γ ⁺* of BROSSÉAU (1958). This Y has attached to its long arm an X chromosome segment bearing the dominant mutant *B^S* (Bar eyes of Stone) and to its short arm a segment bearing γ ⁺ (wild-type allele of yellow); both duplications are distal to the fertility factors.

Virgin sisters of the constitution $\gamma/B^SY\gamma^+$, with normal autosomes or heterozygous for one or both of the inversions listed above, were collected for a period of two days. On the third day they were mated to XY, $\gamma^2 su-w^a w^a Y^S$. Y^L/Y males (γ^2 is recessive to γ^+ but is dominant over and distinguishable from γ ; *w^a*-apricot eyes; *su*-suppressor) subcultured for three 3-day broods, then discarded. Matings consisted of six to eight females with four to five males placed in quarter-pint milk bottles. In another experiment, females were treated in the manner just described with the exception that they were exposed for 6 minutes to an X-ray dose of about 3000r prior to mating.

Both experiments yield regular progeny consisting of phenotypically γ females and *su-w^aw^aB^S* males. Exchanges between the Y and the attached-X result in detachments of the latter chromo-

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some; these are recovered as phenotypically $+$ or γB^s males and $+$ or $\gamma^2 B^s$ females. In addition, among exceptional offspring, males and females occur which have received a Y *fragment*; these fragments, representing Y chromosomes which have lost one or the other terminal markers, can result from true translocations of the Y, gross deletions and single hit deletions of the markers. Fragment-bearing males are phenotypically $\gamma^2 su-w^a w^a B^s$ or $su-w^a w^a$; females are γB^s or $+$ (note that this is the only case of phenotypic overlap: half of the detachment-bearing females are also $+$). In these experiments, Y-fragment exceptions also include the reciprocal types of the true Y translocations described above, i.e., females in which an attached-X or autosomal telomere was exchanged for one of the Y markers. Finally, nondisjunctional progeny consist of B^s females and $\gamma^2 su-w^a w^a$ males. A more detailed description and documentation of the chromosomal types just listed can be found in LUCCHESI (1965).

RESULTS

The first experiment was performed to extend COOPER's data (1946) on the effect of autosomal inversions on spontaneous exchanges between attached-X and Y chromosomes. In addition, an estimate of the frequency of premeiotic events leading to the recovery of clusters of exchanges was sought, since these would constitute "noise" in the induced recombination experiments. The results are presented in Table 1. The detachment females listed were phenotypically $\gamma^2 B^s$; since the complementary class could not be distinguished from a class of fragment-bearing females, it was not considered in the table. There were only two obvious clusters in the entire experiment (one consisted of 32 $su-w^a w^a$ males among 669 offspring produced by $Cy/+; D/+$ mothers; the other consisted of 5 $\gamma^2 B^s$ and 9 $+$ females, 6 $\gamma^2 B^s$ and 3 $+$ males among 824 offspring from control females); progenies from the two bottles which produced these clusters are not included in the numbers listed. The presence of $Cy/+$ or $Cy/+; D/+$ causes no significant increase in the frequency of detachments. There is a significant increase in the presence of $D/+$ among male offspring. This may be explained by the occurrence of a few clusters too small to be recognized. The data confirm COOPER's conclusion that the presence of heterozygous autosomal inversions does not affect the rate of spontaneous exchange between attached-X and Y chromosomes.

The results of crosses involving irradiated females are presented in Tables 2 and 3. The latter contains the total number of fragments recovered in males and one class of fragments, marked with B^s , recovered in females (those bearing the other type of fragment, marked with γ^+ , overlapped phenotypically with one class of detachment females and, therefore, are not included in the table).

TABLE 1

Frequency of spontaneous detachments in the progeny of y attached-X/ B^s $Y\gamma^+$ females

Autosomal constitution of female parents	Detachment males	Total males	Percent $\times 10^{-3}$	Detachment females	Total females	Percent $\times 10^{-3}$	Clusters
$+/+; +/+$	4	12,718	0.31	6	13,336	0.45	1
$+/+; D/+$	11	6,248	1.76	4	7,784	0.51	.
$Cy/+; +/+$	5	12,237	0.41	0	12,559	...	.
$Cy/+; D/+$	1	3,468	0.29	4	3,550	1.13	1

TABLE 2

Frequency of X-ray induced detachments in the progeny of y attached-X/B^S Y_Y⁺ females

Brood	Autosomal constitution of female parents	Detachment males	Total males	Percent	Detachment females	Total females	Percent
I	+/+;+/+	56	2888	1.94	21	2878	0.73
	+/+;D/+	36	1420	2.54	18	1350	1.33
	Cy/+;+/+	81	3148	2.57	42	2860	1.47**
	Cy/+;D/+	30	1006	2.98*	10	829	1.21
II	+/+;+/+	42	3579	1.17	15	3741	0.40
	+/+;D/+	40	1983	2.02†	15	2110	0.71
	Cy/+;+/+	105	4862	2.16**	35	4561	0.77*
	Cy/+;D/+	40	1481	2.70**	14	1348	1.04†
III	+/+;+/+	13	1789	0.73	..		...
	+/+;D/+	22	1094	2.01**	..		...
	Cy/+;+/+	41	2816	1.46†	(Numbers too small)		
	Cy/+;D/+	47	1202	3.91**	..		...

* Differs significantly from the control frequency in the same brood at the 5% level of confidence.

† At the 2% level of confidence.

** At the 1% level of confidence.

The qualifications made in the preceding section, regarding detachment females, also apply to the set of data presented in Table 2. Fifteen comparisons can be made between the progeny of inversion-bearing females and of control females if the three broods are considered separately. Of these, five exhibit no significant differences, two are significant at the 5% level, three at the 2% level, and five at the 1% level of confidence. These results warrant the conclusions that (1) the presence of an inverted major autosome increases the frequency with which induced exchanges between the attached-X and Y chromosomes are

TABLE 3

Recovery of X-ray induced fragments in the progeny of y attached-X/B^S Y_Y⁺

Brood	Autosomal constitution of female parents	Fragment males		Total males	Fragment females		Total females
		B ^S marker	γ ⁺ marker		B ^S marker	Total females	
I	+/+;+/+	22	43	2888	7	2878	
	+/+;D/+	20	39	1420	1	1350	
	Cy/+;+/+	28	77	3148	6	2860	
	Cy/+;D/+	19	27	1006	2	829	
II	+/+;+/+	24	48	3579	3	3741	
	+/+;D/+	14	31	1983	2	2110	
	Cy/+;+/+	56	84	4862	1	4561	
	Cy/+;D/+	9	24	1481	4	1348	
III	+/+;+/+	2	10	1789	1	1879	
	+/+;D/+	3	6	1094	1	1420	
	Cy/+;+/+	11	25	2816	1	3382	
	Cy/+;D/+	7	14	1202	4	1465	

recovered (it is not possible to distinguish between the action of $Cy/+$ and $D/+$ in this respect); (2) this increase is magnified when both autosomes are structurally heterozygous; (3) the effect is manifested in all three broods but is increasingly marked in successive broods.

DISCUSSION

The significant increase in induced recombination obtained with heterologous rearrangements, in regions where, for all practical purposes, no spontaneous meiotic crossing over occurs, may be the result of: (1) a physiological effect of the inversions on heterochromatin such that the latter's susceptibility to X-ray breakage is increased; (2) a mechanical disturbance of pairing between the attached-X and Y chromosomes by the presence of the inversions such that radiation-induced breaks which would normally heal now give rise to exchanges; (3) a basic mechanism of interchromosomal effects yet to be elucidated.

If heterozygosity for autosomal inversions causes heterochromatin to be more susceptible to X-ray breakage, then one might expect the frequency of Y-translocations to increase as the rate of induced exchanges between the attached-X and Y increases with greater structural complexity in the autosomes. Inspection of Table 4, where the frequency of detachments and of fragments recovered in the male progeny of normal and inversion-bearing females are listed, shows no difference in these two types of events among the four progeny classes in the first brood; in the second and third broods, as structural heterozygosity increases the ratios also increase, denoting a departure from the expected direction. Although more fragments are recovered in the presence of autosomal inversions than in their absence, the observation that detachments increase proportionately more than fragments seems to weaken the validity of an explanation based on increased susceptibility of heterochromatin to X-ray breakage.

TABLE 4

Relative frequency of induced detachments and Y fragments in the male progeny of y attached-X/B^s Y⁺

Brood	Autosomal constitution of female parents	Percent detachments	Percent fragments	Ratio: detachment/fragments
I	$+/+;+/+$	1.94	2.25	0.86
	$+/+;D/+$	2.54	4.15	0.61
	$Cy/+;+/+$	2.57	3.34	0.77
	$Cy/+;D/+$	2.98	4.57	0.65
II	$+/+;+/+$	1.17	2.01	0.58
	$+/+;D/+$	2.02	2.27	0.89
	$Cy/+;+/+$	2.16	2.88	0.75
	$Cy/+;D/+$	2.70	2.23	1.21
III	$+/+;+/+$	0.73	0.67	1.09
	$+/+;D/+$	2.01	0.82	2.45
	$Cy/+;+/+$	1.46	1.28	1.14
	$Cy/+;D/+$	3.91	1.75	2.23

An alternative explanation would make use of the model proposed by SCHULTZ and REDFIELD (1951) to account for interchromosomal effects on crossing over and segregation. These authors suggested that in structurally heterozygous chromosomes, some degree of asynapsis may occur; this may lead to nonhomologous associations, exerting a stress on those regions which are homologously paired, thereby increasing the assumed breakage-and-fusion process of crossing over. The results presented in this paper can be explained by assuming that pairing between the attached-X and Y chromosomes is disturbed by the presence of autosomal inversions in such a fashion that radiation-induced breaks which would normally heal now give rise to exchanges.

The possibility that neither the above nor any of the various other published models are sufficient to explain all of the experimental data on interchromosomal effects must be entertained, in light of new evidence recently reported by WILLIAMSON and HINTON (1965). Briefly, these authors observed that three autosomal translocations which, as heterozygotes, caused either a decrease, no change or an increase in X chromosome recombination (HINTON 1965), when homozygous significantly decreased the map length of the X. Knowledge of the mechanism(s) which mediate the wide spectrum of spontaneous and induced, meiotic and mitotic interchromosomal effects needs, perhaps, to follow new insights on the molecular organization of the chromosomes and on the process of crossing over itself.

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SUMMARY

The presence of an inverted major autosome increases the frequency with which X-ray induced exchanges between the attached-X and Y chromosomes are recovered. This increase, magnified when both autosomes are structurally heterozygous, is manifested in three 3-day progeny broods but is increasingly marked in successive broods. These experimental results are considered in terms of possible physiological and mechanical explanations.

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