INTERCHROMOSOMAL EFFECTS ON SOMATIC RECOMBINATION IN DROSOPHILA MELANOGASTER[1]

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RECOMBINATION in somatic cells of Drosophila is influenced by various genetic and environmental factors that also affect meiotic recombination. Thus, the amount of somatic crossing over in a given chromosome is controlled by the genetic contents of the chromosomal set (WEAVER 1960) and by the presence of various genes (STERN 1936; KAPLAN 1953). It may also be affected by changes in temperature (STERN and RENTSCHLER 1936; KAPLAN 1953; BROSSEAU 1957) and by irradiations (RONEN 1962; ABBADESSA and BURDICK 1963).

The purpose of the present paper is (1) to show that heterologous inversions, whose role in determining meiotic recombination has drawn much attention, also affect somatic crossing over. These interchromosomal effects are similar to those known from meiotic crossing over; (2) to demonstrate the employment of spontaneous and X-ray induced somatic recombination in studying the mechanism of the interchromosomal effects.

MATERIALS AND METHODS

Strains: Two isogenic strains were produced by employing the *FM6*, Curly *(Cy)* and Dichaete *(D)* balancer chromosomes. Of the two strains, one carried yellow, white-apricot ($y\ w^a$) X chromosomes, and the other carried singed *(sn)* X chromosomes. The autosomes were coisogenic in the two strains. Females from the *sn* strain were crossed to a single male carrying *In(2L+R)Cy* and *In(3LR)DcxF* on chromosomes 2 and 3, respectively. Their *sn/Y; Cy/+; D/+* sons were then mated with homozygous $y\ w^a$ females. In this cross, some 20 pairs were kept for 24 hours in each culture bottle.

All F_1 daughters were heterozygous for $y\ w^a$ and *sn*, in the *trans* configuration. In addition, they carried one of the following autosomal combinations: *Cy/+*; +/+, +/+; *D/+*, *Cy/+*; *D/+* and +/+; +/+.

In the second experiment, all the second chromosomes that did not carry the *Cy* inversions were marked by the recessive gene vestigial. This marker was introduced to facilitate mounting and inspection of nonDichaete flies. During the work it was felt, however, that *vg* flies were by no means easier to mount, and this marker was not therefore employed in the third experiment.

Irradiation: In the third experiment, larvae were collected when climbing on the walls of the culture bottles, just before pupation. Irradiation took place in small glass tubes. The source of X rays was a General Electric Maximar 250-III machine, operated at 200 kv and 15 ma, through a 1 mm copper + 1 mm aluminum filter. The total dose, administered at the rate of 180r per min, was 1170r. The larvae were subsequently allowed to pupate in fresh, ordinary

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culture bottles. Unirradiated controls were given the same treatment with the exception of exposure to X rays.

Mounting the Flies: Two- to three-day old F_1 females were collected and mounted on slides (BROSSEAU 1957). In the first two experiments, ten flies were mounted on each slide, in a drop of Euparal. Cover slips and copper weights were employed and the slides were allowed to stand for 2 to 3 days before being examined. To facilitate statistical analysis, equal numbers of flies of each genotype were examined.

In the third experiment, each female was mounted on a separate slide and the cover glass was gently pressed with a needle before adding the weight. All slides were code-numbered, in order to avoid possible bias in scoring of spots.

The slides were examined with 90× magnification using transmitted light. Yellow or singed single spots, as well as yellow-singed twin spots occurring on the thorax and the abdomen of each fly (the thorax only, in the third experiment) were scored. The number of bristles in each spot, and their location on the tergite, were recorded. To check the accuracy of the scoring technique, as well as the effect of aging on the mounts, the microscopic examination was repeated after several weeks. An excellent agreement was found between the two studies.

RESULTS

The results of the first experiment are summarized in Table 1. In this small sample, no thoracic spots of any kind were found in any of the four groups of flies. The total frequency of abdominal spots (single and twin spots combined) is higher in the flies carrying either one of the two autosomal inversions, than in the structurally normal flies. It is still higher in the flies carrying both inversions. However, the differences do not seem to be significant in an analysis of variance (Table 2). Yet, the difference between the +/+; +/+ and the *Cy*/+; *D*/+ flies is significant ($\chi^2_1 = 5.25, .02 < P < .05$).

TABLE 1

The frequency of abdominal spots in normal and inversion-carrying females (first experiment)

Genotype (autosomes)	Total number of flies	Spots							
		All spots		*y*		*sn*		*y*+*sn*	
		frequency	number	frequency	number	frequency	number	frequency	number
+/+; +/+	40	0.62	25	0.15	6	0.20	8	0.28	11
Cy/+; +/+	40	0.82	33	0.18	7	0.40	16	0.25	10
+/+; *D*/+	40	0.85	34	0.18	7	0.48	19	0.20	8
Cy/+; *D*/+	40	1.10	44	0.30	12	0.45	18	0.35	14

TABLE 2

The analysis of variance for all types of spots (first experiment)

Source	Degrees of freedom	S.S.	M.S.	V.R.
Genotypes	3	45.5	15.17	1.44†
Cultures	12	126.5	10.54	
Total	15	172.0		

† Not significant at 0.05 level.

In the second experiment attention was concentrated only on flies with the normal and the *Cy*/+; *D*/+ genotypes. Here, a sample of 25 flies of each genotype was taken for examination from each of eight culture bottles. The results are shown in Table 3. Only few thoracic spots were found. Their distribution in the two genotypes indicates a very high sensitivity to the presence of the *Cy* and *D* inversions. Statistical analysis of the difference is difficult, however: nine spots were found in the inversion-carrying females, as compared to one spot in the normal group. The abdominal spots are also more frequent in the inversion-carrying flies than in the normal ones, the difference being more than 50 percent. This difference is significant, as indicated by a t-test for paired samples ($t_7 = 2.0$, $.025 < P < .05$). There seems to be no correlation, either positive or negative, in spot-frequencies between the normal and inversion-carrying individuals of the same cultures ($r = 0.14$, $P = 0.38$). This may be indicative of the relatively minor role of microecological conditions in determining the frequency of mutant spots.

In the third experiment, a total of 439 flies was studied, and the total number of spots was 619. The results are summarized in Table 4.

TABLE 3

The frequency of thoracic and abdominal spots in +/+; +/+ and Cy/+; D/+ females (second experiment)

Culture	Total number of flies	+/+; +/+				*Cy*/+; *D*/+			
		All spots	*y*	*sn*	*y*+*sn*	All spots	*y*	*sn*	*y*+*sn*
Abdominal spots									
	50	10	3	4	3	17	6	10	1
	50	18	7	7	4	15	5	6	4
	50	16	7	5	4	16	3	7	6
	50	11	1	4	6	18	2	13	3
	50	7	1	3	3	22	8	8	6
	50	8	0	4	4	17	2	7	8
	50	9	1	5	3	11	0	5	6
	50	6	1	4	1	14	3	7	4
All spots	400	85	21	36	28	130	29	63	38
Thoracic spots									
All spots	400	1	1	0	0	9	6	0	3

TABLE 4

Spot frequencies in the four groups (third experiment)

Group	Total number of flies	Total frequency	Spots			
			Total no.	*y*	*sn*	*y*+*sn*
NC	100	0.66	66	19	28	19
RC	94	0.98	92	28	41	23
NT	147	1.90	280	82	109	89
RT	98	1.85	181	47	84	50

All three types of spots are more frequent in the unirradiated, inversion-carrying flies (RC) than in their structurally normal sisters (NC). This, however, does not hold for the irradiated flies: here, the spot-frequencies in the structurally normal flies (NT) and in the inversion-carrying ones (RT) are about the same.

The meaning of the three types of spots: In all four groups of flies, the frequency of twin spots is higher than that of the single, yellow spots. This is anticipated on the basis of STERN's (1936) interpretation of the mechanism of somatic recombination. However, the frequency of singed spots is even higher than that of the twin spots, which is in defiance of that expectation. The occurrences of yellow spots, twin spots and singed spots were explained by STERN (1936, Figures 3, 5, 7) as the results of crossing-over events between *y* and *sn*, between *sn* and the centromere and in both regions (double crossing over), respectively. Obviously, one cannot expect here the frequency of double crossing over to be as high as twice the frequency of one class of singles.

Similar deviations from the expectation on the basis of STERN's (1936) model were observed by KAPLAN (1953) and BROSSEAU (1957). HANNAH (1953) demonstrated that mutant spots in the otherwise normal tissue are subject to selection as well as to some degree of nonautonomy. This could account for the above discrepancy, but it also raises the possibility that all three types of somatic spots are the result of one class of recombination events (i.e., proximal to *sn*). This possibility must be examined before one can proceed in evaluating data from *y/sn* somatic recombination.

Proximal recombination is expected to give rise to twin spots. However, the appearance of a twin spot requires that *both products* of a reciprocal recombination event be represented in the integument of the mature fly. This requirement is antagonized by various factors, including (1) irradiation damage, (2) nonautonomy of the mutant genotype in the normal environment, (3) differences in selective values and (4) divergent differentiational history of the (recombinant) daughter cells.

To test the possibility that the three classes of spots represent differences on the level of cell lineage, rather than on the chromosomal level, the following test was made. The ratio of yellow to singed spots was compared with that of yellow to singed bristles in the twin spots (Tables 5 to 7). Similar ratios are expected when the three types of spots reflect the chances of the recombination products to survive factors (3) and (4), mentioned in the preceding section. Conversely, if single spots reflect different events on the chromosomal level, no similarity in ratios is expected. As the same yellow-singed ratio is found for both spots and bristles, it follows that the different classes of spots may be regarded as resulting from differences in post-recombinational cell histories. On the basis of this conclusion, the combined frequencies of all three types of spots were used for further analyses of the interchromosomal and irradiation effects.

Tests for randomness: In many flies, more than one spot can be found. Therefore, the possibility of nonrandomness (clustering) has to be eliminated. Clustering may result from mainly two sources: biologically, a single crossover-cell

TABLE 5

Frequency of yellow and singed bristles in twin spots

Group	Total twin spots	Bristles		
		Total bristles	yellow	singed
NC	19	77	36	41
RC	23	99	37	62
NT	89	476	190	286
RT	50	267	102	165

TABLE 6

Frequency of yellow and singed single spots

Group	All single spots			One-bristle spots		
	Total spots	yellow	singed	Total spots	yellow	singed
NC	47	19	28	36	15	21
RC	69	28	41	55	24	31
NT	191	82	109	97	43	54
RT	131	47	84	62	24	38

TABLE 7

Ratio of yellow:singed spots and bristles

Group	1 Bristles in twin spots	2 Spots (all spots)	3 Spots (one bristle)	P For difference between 1 and 2
NC	0.47	0.40	0.42	0.30–0.50
RC	0.37	0.41	0.44	0.50–0.70
NT	0.40	0.43	0.44	0.30–0.50
RT	0.38	0.36	0.39	0.70–0.80
P (homogeneity between groups)	0.50–0.70	~0.70	~0.90	

may give rise to two separated patches of mutant hypodermis, thus producing two spots (either single or twin spots). In addition, a fly in which one spot has been found is exposed to a longer, and more thorough examination (while making records of the size, type and location of the spot). This may result in detecting more spots which otherwise would have escaped detection (owing to their small size, etc.).

A random distribution of spots among the flies should fit a Poisson distribution. Conversely, any significant deviation from the Poisson should be interpreted as nonrandomness in the distribution of spots among flies. The actual distribution of spots is shown in Table 8. In none of the four groups is there any indication of deviation from randomness.

Spot size: In Table 9, the data are gathered according to the size of spots. It can be seen that there is no difference between the distributions of the numbers

TABLE 8

Distribution of flies according to number of spots

Group		Total number of flies	Number of spots per fly							P
			0	1	2	3	4	5	6	
NC	observed	100	47	41	11	1	0	0	0	0.30–0.50
	expected		51.7	34.1	11.3	3.0	*	*	*	
RC	observed	94	38	33	13	7	3	0	0	0.50–0.70
	expected		35.4	34.6	16.9	5.5	1.7	*	*	
NT	observed	147	20	47	31	32	12	3	2	0.50–0.70
	expected		21.9	41.7	39.7	25.1	12.0	4.6	2.0	
RT	observed	98	13	27	34	12	10	2	0	0.20–0.30
	expected		15.4	28.6	26.4	13.8	7.5	6.3	*	

* The expected value is smaller than 1.

of bristles per spot in the normal and *Cy*, *D* flies. On the other hand, there are on the average more bristles per spot in the irradiated, than in the nonirradiated flies. This may be accounted for by the loss of cells damaged by the irradiation to the point where they were not able to divide any more, and which was compensated for by surviving recombinant cells. The latter will thus have a chance to grow more than in the untreated flies. This possibility is supported by the work of HADORN (1953), WADDINGTON (1953), PANTELOURIS and WADDINGTON (1955) and HADORN and CHEN (1956).

Sensitivity of the various tergites: No difference was found in the present experiment between the frequencies of spots on the different tergites. This is in accordance with BROSSEAU's (1957) findings, and in disagreement with STERN's (1936) data. As was already indicated by the former author, this might be the result of the methods used: while in STERN's experiment the flies were illuminated from above for examination, in the present experiments (as in BROSSEAU's) transmitted light was used, giving better resolution in the posterior segments.

TABLE 9

Spot sizes in the four groups of flies

Group	yellow spots			singed spots			Twin spots		
	number	m*	SE	number	m*	SE	number	m*	SE
NC†	40	1.73	.24	64	1.44	.15	47	4.42	.35
RC†	57	1.63	.17	104	1.29	.12	61	4.26	.27
NT	82	1.77	.13	109	1.96	.13	89	5.35	.29
RT	47	2.15	.22	84	2.40	.22	50	5.34	.13

* Average number of bristles per spot.
† Including flies from second experiment.

Interchromosomal effects and induced somatic recombination: There is a fair agreement among the frequencies of spontaneous somatic recombination in the three experiments (0.62, 0.42 and 0.66 spots per normal fly). Also the increase in spot frequency, observed in the presence of the *Cy* and *D* inversions, is consistent (177, 153 and 148 percent, respectively). In the third experiment there is no difference in total recombination beween the two irradiated classes, NT and RT (1.90 and 1.85 spots per fly, respectively). They do differ, however, in the frequency of *induced* recombination. The latter frequency can be calculated by deducting the spontaneous frequencies for normal and inversion-carrying flies (NC and RC) from the total frequencies in groups NT and RT. Thus, out of an average of 1.90 spots per fly in the normal, irradiated flies, 0.66 may be considered spontaneous, and 1.24 are induced (almost threefold increase due to irradiation). In the *Cy D* flies, however, the spot frequency is less than doubled by irradiation (the average total frequency of 1.85 being comprised of 0.98 spontaneous and 0.87 induced spots per fly).

DISCUSSION

Somatic crossing over in *D. melanogaster* is known to be influenced by the presence, in the genome, of various oligo- and polygenes (STERN 1936; KAPLAN 1953; WEAVER 1960). In the latter aspect, it resembles meiotic crossing over (LAWRENCE 1963). BROWN and WELSHONS (1955) studied somatic crossing over in attached-X chromosomes. They did not find in the presence of the Curly inversion any increase in recombination frequency over that found in flies with structurally normal autosomes. It is therefore of major interest, that heterozygosity for autosomal inversions increases free-X chromosome crossing over in mitotic cells, as it does in the oocyte.

As yet, no full agreement has been reached as to the mechanism of these interchromosomal effects. Some students of crossing over in Drosophila postulate a primarily physiological effect exerted by the inversions (STEINBERG and FRASER 1944; LEVINE and LEVINE 1954; RAMEL 1962). SUZUKI (1963) favors a combined effect where mechanical interference with chromosome orientation is followed by a physiologically mediated increase in crossing over. Others agree that interchromosomal effects have an essentially mechanical explanation.

According to OKSALA (1958) there is, in the structurally normal cell, a marked degree of nonhomologous pairing of chromosomes. Structural heterozygosity in a given pair of chromosomes renders them less likely (owing to loop formation) to engage in illegitimate pairing. The chances thus increase for other pairs to undergo true (legitimate) pairing and recombination. On the other hand, SCHULTZ (in SCHULTZ and REDFIELD 1951) suggested that "a chromosome that is allowed to pair without disturbance would show a minimum amount of crossing over." Heterozygous bivalents, having difficulties in pairing may *interfere* with other bivalents' pairing, thus increasing the amount of crossing over in them. This idea is supported by THOMPSON (1963), who has found that heterozygosity for inversions increases centric repulsion in heterologous bivalents. The

question being asked is, therefore, the following: Is homologous pairing impaired or enhanced in the presence of heterologous inversions? The finding, in the present study, that X rays induce recombination less effectively in the presence of heterologous inversions, may help answer this question.

In the irradiated cell, one or more chromatids may be hit and broken by an ionizing particle. A viable exchange between chromatids (whether it constitutes true or pseudo-crossing over) is more likely to take place when the two chromatids are intimately paired, than when they are in repulsion. Regardless of whether induced recombination follows a one-hit or two-hit curve, closely synapsed chromosomes have a better chance of being both hit by one ionization. Exchange-type restitution is also facilitated by synapsis.

The strong irradiation effect observed in the normal flies can, therefore, be accounted for by fully synapsed homologues. On the other hand, it might be assumed that unsynapsed *Cy* and *D* chromosomes interfere (by competition) with full pairing in the X chromosomes, thus reducing their ability to undergo radiation-induced recombination.

As mentioned earlier, both spontaneous and induced somatic recombination events in the X chromosome are not randomly distributed. While our data can only locate them proximally to *sn*, there is other evidence (KAPLAN 1953) restricting somatic recombination mostly to the region that lies to the right of M(1)0, namely, close to the heterochromatic block or even within its limits. Recently it was demonstrated by WALEN (1964) that the occurrence of recombination in the proximal end of the chromosome is positively correlated with the amount of heterochromatin present there. The presence of heterologous inversions thus seems to interfere with centric pairing in somatic cells.

Finally, PARKER (1948) could not find any interchromosomal effects on irradiation-induced recombination in spermatogonia. RAMEL (1962) showed that spontaneous gonial recombination was also unaffected by the presence of heterologous inversions. While those two works are consistent with each other, they raise the possibility that the forces governing crossing over are not the same everywhere even within the same tissue.

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SUMMARY

Somatic crossing over was studied in *y/sn* females. The frequency of single and twin spots per fly was higher in the presence of the autosomal inversions Curly and Dichaete, than in a control group where the autosomes were structurally and genetically homozygous. The spot-frequency was even higher in females which had been irradiated as third-instar larvae with 1170r of X rays. The data indicate that both spontaneous and induced recombination are restricted to the proximal part of the chromosome. In the irradiated group, there were fewer induced spots in the inversion-carrying females than in the normal ones. It is suggested that centric pairing in the X chromosome, and therefore its chance to

undergo induced somatic recombination, are impaired in the presence of the autosomal inversions.

LITERATURE CITED

ABBADESSA, R., and A. B. BURDICK, 1963 The effect of X-irradiation on somatic crossing over in *Drosophila melanogaster*. Genetics **48**: 1345–1356.

BROSSEAU, G. E., 1957 The environmental modification of somatic crossing over in *Drosophila melanogaster* with special reference to developmental phase. J. Exptl. Zool. **136**: 567–593.

BROWN, S. W., and W. WELSHONS, 1955 Maternal aging and somatic crossing over of attached-X chromosomes. Proc. Natl. Acad. Sci. U.S. **41**: 209–215.

HADORN, E., 1953 Regulation and differentiation within field-districts in imaginal discs of Drosophila. J. Embryol. Exptl. Morph. **1**: 213–216.

HADORN, E., and P. S. CHEN, 1956 Die Feldorganisation der Spermatheken-Anlage bei *Drosophila melanogaster*. Rev. Suisse Zool. **63**: 268–277.

HANNAH, A. M., 1953 Non-autonomy of yellow in gynandromorphs of *Drosophila melanogaster*. J. Exptl. Zool. **123**: 523–560.

KAPLAN, W. D., 1953 The influence of Minutes upon somatic crossing-over in *Drosophila melanogaster*. Genetics **38**: 630–651.

LAWRENCE, C. W., 1963 The control of crossing over in the X-chromosome of *Drosophila melanogaster*. Heredity **18**: 27–46.

LEVINE, R. P., and E. E. LEVINE, 1954 The genotypic control of crossing-over in *Drosophila pseudoobscura*. Genetics **39**: 677–691.

OKSALA, T., 1958 Chromosome pairing, crossing-over and segregation in meiosis in *Drosophila melanogaster* females. Cold Spring Harbor Symp. Quant. Biol. **23**: 197–210.

PANTELOURIS, E. M., and C. H. WADDINGTON, 1955 Regulation capacities of the wing-and haltere-discs of wild type and bithorax Drosophila. Roux' Arch. Entw. Mech. Organ. **147**: 539–546.

PARKER, D. R., 1948 Observations on crossing over induced by X-rays in the males of Drosophila. Genetics **33**: 304–310.

RAMEL, C., 1962 Interchromosomal effects of inversions in *Drosophila melanogaster*. I. Crossing over. Hereditas **48**: 1–58.

RONEN, A., 1962 Induced somatic recombination in the third chromosome of *Drosophila melanogaster*. Dros. Inform. Service **36**: 112–113.

SCHULTZ, J., and H. REDFIELD, 1951 Interchromosomal effects on crossing over in Drosophila. Cold Spring Harbor Symp. Quant. Biol. **16**: 175–197.

STEINBERG, A. G., and F. C. FRASER, 1944 Studies on the effect of X-chromosome inversions on crossing over in the third chromosome of *Drosophila melanogaster*. Genetics **29**: 83–103.

STERN, C., 1936 Somatic crossing over and segregation in *Drosophila melanogaster*. Genetics **21**: 625–730.

STERN, C., and V. RENTSCHLER, 1936 The effect of temperature on the frequency of somatic crossing over in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **22**: 451–453.

SUZUKI, D. T., 1963 Interchromosomal effects on crossing over in *Drosophila melanogaster*. II. A reexamination of X chromosome inversion effects. Genetics **48**: 1605–1617.

THOMPSON, P. E., 1963 Centric pairing and crossing over in *Drosophila melanogaster*. Genetics **48**: 697–701.

WADDINGTON, C. H., 1953 The interactions of some morphogenetic genes in *Drosophila melanogaster*. J. Genet. **51**: 243–258.

WALEN, K. H., 1964 Somatic crossing over in relationship to heterochromatin in *Drosophila melanogaster*. Genetics **49**: 905–923.

WEAVER, E. C., 1960 Somatic crossing over and its genetic control in Drosophila. Genetics **45**: 345–357.