MODIFICATION OF RECOMBINATION FREQUENCY IN DROSOPHILA. II. THE POLYGENIC CONTROL OF CROSSING OVER

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IN a previous paper, CHINNICI (1971) reported that the frequency of genetic recombination (crossing over) between the sex linked genes scute (*sc*) and crossveinless (*cv*) in *Drosophila melanogaster* was modified by selection practiced at the levels of the family and the individual. A low line and a high line were established. In the low line, the amount of crossing over between *sc–cv* was reduced from an original value of 15.4% to 8.5% after 33 generations of selection, while in the high line, the value increased from 15.4% to 22.1%. The response in each line was specific to the *sc–cv* region, for the amount of crossing over in adjacent regions did not differ between the two lines. Examination of polytene chromosomes eliminated the possibility that these changes were due to inversions, deletions, or duplications detectable at the light microscope level (see CHINNICI 1970, 1971 for complete details).

The slow, gradual nature of the response to selection in each line indicated polygenic control of recombination frequency for the *sc–cv* region. The nature of the polygenic system was studied by employing the technique of chromosome substitution. Information on the following features of the system were obtained: (1) the regional specificity of the modification effect; (2) the distribution of modifier activity among the various chromosomes (chromosome *4* excluded); (3) the presence or absence of interchromosomal interactions; and (4) the dominance relationships within the system. The description and results of these chromosome substitution experiments are presented in this paper.

MATERIALS AND METHODS

Various stocks of *Drosophila melanogaster* were used in the experiment. A Complete Multiple Inversion stock (CMI) was generously supplied by Dr. T. R. F. WRIGHT at the University of Virginia. The CMI stock contains multiply-inverted *X (FM6)*, second *(SM5)* and third *(TM3)* chromosomes, and non-inverted second *(Sp bw^D)* and third *(Sb)* chromosomes, so that a CMI female has the karyotype *FM6/FM6; SM5/Sp bw^D; TM3/Sb*. All second and third chromosomes are recessive lethals (see SEIGER 1966; LINDSLEY and GRELL 1968, for complete descriptions of these chromosomes). An isogenic wild-type stock was prepared from the Swedish-C strain using the CMI method.

Three additional stocks were prepared each containing a different set of homologous morphological mutant genes. One of these stocks contained 7 *X*-chromosome mutants, another carried 5 second chromosome mutants, and the other had 7 third chromosome mutants. Table 1 lists and

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TABLE 1

List describing mutants used in this experiment

X-chromosome:

Name of Mutant	Locus	Description
sc scute	1-00.0	scutellar bristles missing
w white	1-01.5	eyes pure white in color
bi bifid	1-06.9	wing veins fused at base
cv crossveinless	1-13.7	posterior crossvein on wings missing
sn3 singed-3	1-21.0	bristles gnarled, thickened
m- miniature	1-36.1	wings miniaturized
B Bar	1-57.0	eyes reduced in shape to a slit or bar; dominant

Second chromosome:

al aristaless	2-00.0	aristae much reduced in size
dp dumpy	2-13.0	wings short and wide
b black	2-48.5	body black in color
cn cinnabar	2-57.5	eyes cinnabar in color
sca scabrous	2-66.7	eyes roughened in texture

Third chromosome:

ve veinlet	3-00.2	wing veins end abruptly
h hairy	3-26.5	body has extra hairs
th thread	3-43.2	aristae reduced to thin stalks
ss spineless	3-58.5	bristles much reduced in size
e^s ebony-sooty	3-70.7	body sooty black in color
ro roughoid	3-91.1	eyes roughened in color

See LINDSLEY and GRELL (1968) for more complete descriptions.

briefly describes the mutant genes present in these stocks. Each of these three stocks was inbred by brother-sister mating for 15 generations prior to their use in the experiments described below.

Table 2 lists the chromosome substitution crosses set up, and the type of data obtainable from each cross. These crosses were set up employing a mating scheme essentially similar to that used by SEIGER (1966), but more complex. Due to the logistical problems of rearing and scoring the flies, these crosses were set up in three time groups, using low line flies from generations 36 (Group I), 38 (Group II), and 40 (Group III) and high line flies from generations 37 (Group I), 39 (Group II) and 41 (Group III) for each respective grouping. The crosses performed within each group are listed in Table 2. The mating scheme involved in Group III is presented in Figure 1. The other mating setups are similar to that for Group III and are listed in CHINNICI (1970). Generally, 19 or more single pair matings of each type listed in Table 2 were set up in 8 dram shell vials on standard medium. The female parents were 48 ± 6 hours old when initially mated. Each set of parents was transferred three times into fresh vials at 3 day intervals so that a total of four three-day laying periods were allowed. The parents were then discarded and the progeny were scored so that the crossover values for each female parent could be ascertained for the particular mutant-carrying chromosomes.

RESULTS

The raw data from each single pair mating in each cross outlined in Table 2

TABLE 2

Outline of the chromosome substitution crosses performed

Cross Number	Karyotype	Selected Chromosome(s) Tested	Recombination Observed in Chromosome	Data Obtained
1	$\frac{E}{M}\frac{+}{+}\frac{+}{+} \times \frac{M}{Y}\frac{+}{+}\frac{+}{+}$	X	X	Specificity Distribution
2	$\frac{M}{+}\frac{E}{+}\frac{+}{+} \times \frac{M}{Y}\frac{+}{+}\frac{+}{+}$	2	X	Specificity Distribution
3	$\frac{M}{+}\frac{+}{+}\frac{E}{+} \times \frac{M}{Y}\frac{+}{+}\frac{+}{+}$	3	X	Specificity Distribution
4	$\frac{E}{M}\frac{E}{+}\frac{E}{+} \times \frac{M}{Y}\frac{+}{+}\frac{+}{+}$	X,2,3	X	Interaction
5	$\frac{M}{+}\frac{+}{+}\frac{E}{E} \times \frac{M}{Y}\frac{+}{+}\frac{+}{+}$	3,3	X	Dominance
6	$\frac{+}{+}\frac{E}{M}\frac{+}{+} \times \frac{+}{Y}\frac{M}{M}\frac{+}{+}$	2	2	Specificity
7	$\frac{+}{+}\frac{M}{+}\frac{E}{+} \times \frac{+}{Y}\frac{M}{M}\frac{+}{+}$	3	2	Specificity
8	$\frac{+}{+}\frac{E}{+}\frac{M}{+} \times \frac{+}{Y}\frac{+}{+}\frac{M}{M}$	2	3	Specificity
9	$\frac{+}{+}\frac{+}{+}\frac{E}{M} \times \frac{+}{Y}\frac{+}{+}\frac{M}{M}$	3	3	Specificity

E = chromosomes derived from the experimental high or low recombinant lines; M = chromosomes carrying multiple mutant genes listed in Table 1.

are recorded in CHINNICI (1970). Mean crossover values and 95% confidence limits for each cross were obtained from these raw data after they had been transformed into angular values (MATHER 1951). These values, along with data on family size are presented in Tables 3 ,4, 5, 6, and 7. These mean crossover

TABLE 3

Results of chromosome substitution tests: Effect of experimental chromosomes from the low recombinant line on crossing over in the X chromosome

Cross: Number from Table 2	Treatment (Experimental chromosomes tested)	Number of families	Progeny $\bar{x} \pm SE$	Mean percent recombination					
				Region of *X* chromosome					
				sc–w	*w–bi*	*bi–cv*	*cv–sn*^{3}	*sn*^{3}*–m*	*m–B*
—	Low control	30	243±44	3.6	8.8	10.1	8.6	15.5	18.8
1	Low *X*	35	203±44	1.8†	5.6†	8.9*	9.1	15.5	19.0
2	Low *2*	27	237±42	1.0†	6.1†	8.6†	9.5	16.2	19.5
3	Low *3*	33	229±31	1.2†	5.3†	7.6†	7.8	15.3	20.5
4	Low *X23*	25	202±37	0.3†	3.5†	6.6†	8.1	16.5	19.4

* $.01 < P < .025$ by analysis of variance comparison with control.

† $P < .01$ by analysis of variance comparison with control.

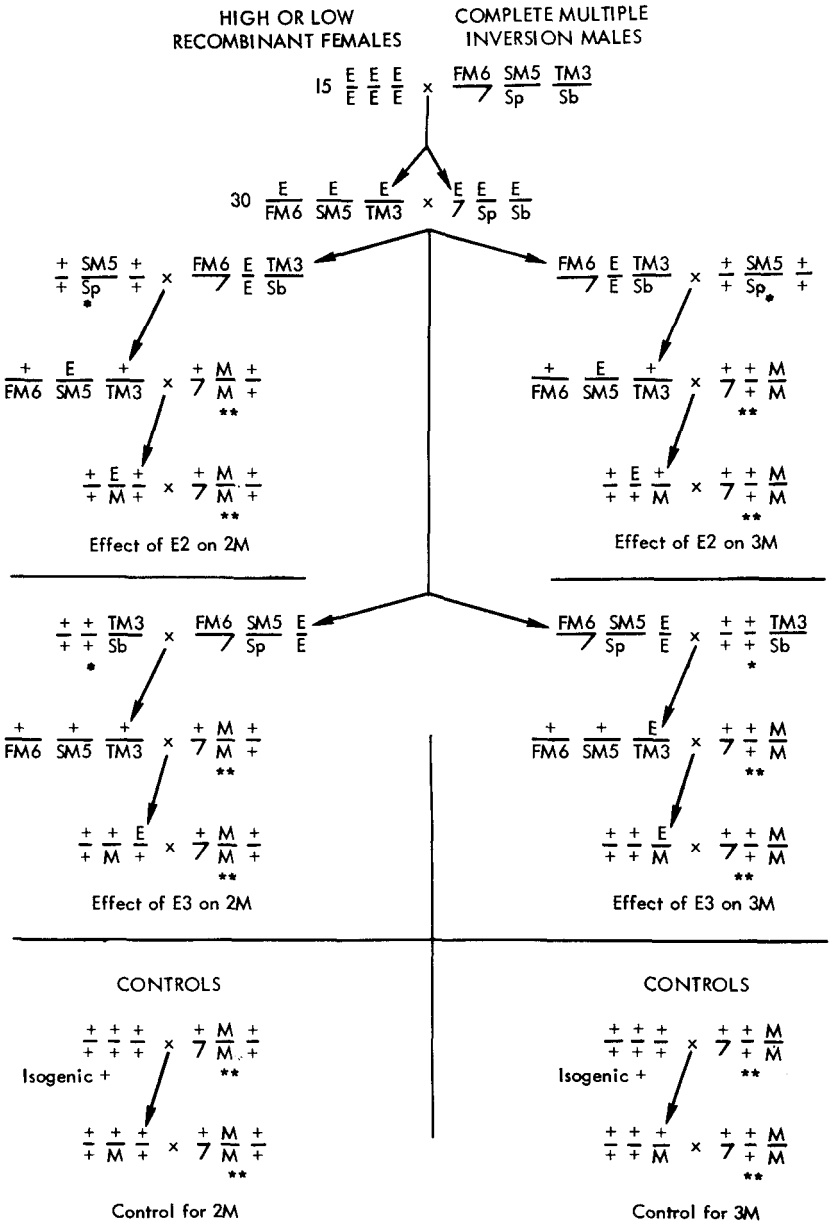


FIGURE 1.—The mating scheme used in Phase III of the chromosome substitution experiments described in the text. * = Stocks of flies of these karyotypes were synthesized earlier by selecting the appropriate F_1 and F_2 progeny of CMI × isogenic wild-type crosses. ** = Flies from inbred stocks carrying chromosomes with multiple morphological mutants (M); see Table 1 for a listing of these mutant genes.

TABLE 4

Results of chromosome substitution tests: Effect of experimental chromosome from the high recombinant line on crossing over in the X *chromosome*

Cross: Number from Table 2	Treatment (Experimental chromosomes tested)	Number of families	Progeny $\bar{x} \pm SE$	Mean percent recombination					
				Region of *X* chromosome					
				sc–w	*w–bi*	*bi–cv*	*cv–sn³*	*sn³–m*	*m–B*
—	High control	26	261±54	3.1	7.2	8.4	7.9	14.7	18.6
1	High *X*	36	287±42	4.7‡	8.4‡	9.9‡	9.1‡	15.7†	19.9†
2	High *2*	37	292±37	3.8‡	7.4	9.2	8.9	15.4	19.5
3	High *3*	27	270±53	3.9‡	7.8	10.9‡	8.1	15.4	20.1*
4	High *X23*	33	280±52	5.7‡	8.3‡	11.2‡	8.1	15.1	20.9‡

* .025<P<.05 by analysis of variance comparison with control.
† .01<P<.025 by analysis of variance comparison with control.
‡ P<.01 by analysis of variance comparison with control.

TABLE 5

Results of chromosome substitution tests: Effect of experimental chromosomes from the low and high recombinant lines on crossing over in the X *chromosome*

Cross: Number from Table 2	Treatment (Experimental chromosomes tested)	Number of families	Progeny $\bar{x} \pm SE$	Mean percent recombination					
				Region of *X* chromosome					
				sc–w	*w–bi*	*bi–cv*	*cv–sn³*	*sn³–m*	*m–B*
—	Control	19	307±73	3.5	8.4	10.1	8.5	15.3	18.7
5	Low *3,3*	22	299±62	1.0†	4.4†	6.8†	7.9	14.1*	20.7*
5	High *3,3*	23	260±38	4.9†	9.7†	12.9†	8.3	14.5	19.5

* .025<P<.05 by analysis of variance comparison with control.
† P<.01 by analysis of variance comparison with control.

TABLE 6

Results of chromosome substitution tests: Effect of experimental chromosomes from the low and high recombinant lines on crossing over in chromosome 2

Cross: Number from Table 2	Treatment (Experimental chromosomes tested)	Number of families	Progeny $\bar{x} \pm SE$	Mean percent recombination			
				Regions of chromosome *2*			
				al–dp	*dp–b*	*b–cn*	*cn–sca*
6	Low *2*	22	362±57	11.8*	26.3	4.0*	8.2†
7	Low *3*	20	266±66	12.8	26.5	3.6‡	8.6
—	Control	29	355±64	13.1	26.0	5.0	9.4
6	High *2*	21	341±70	13.2	26.2	4.7	8.6
7	High *3*	21	344±62	13.6	27.9	5.4	7.9

* .025<P<.05 by analysis of variance comparison with control.
† .01<P<.025 by analysis of variance comparison with control.
‡ P<.01 by analysis of variance comparison with control.

TABLE 7

Results of chromosome substitution tests: Effect of experimental chromosomes from the low and high recombinant lines on crossing over in chromosome 3

Cross: Number from Table 2	Treatment (Experimental chromosomes tested)	Number of families	Progeny $\bar{x} \pm SE$	Mean percent recombination				
				Regions of chromosome 3				
				ve–h	*h–th*	*th–ss*	*ss–e*^{s}	*e*^{s}*–ro*
8	Low *2*	20	323±61	25.2*	16.7*	11.8*	14.6‡	19.9
9	Low *3*	25	258±75	26.1	13.4‡	7.9‡	13.0	22.0
—	Control	20	364±71	27.2	15.0	10.2	13.1	20.4
8	High *2*	21	310±42	25.2‡	15.2	9.5	13.0	22.4†
9	High *3*	19	284±43	25.1*	14.1	8.5†	11.6‡	23.0‡

* .025<P<.05 by analysis of variance comparison with control.
† .01<P<.025 by analysis of variance comparison with control.
‡ P<.01 by analysis of variance comparison with control.

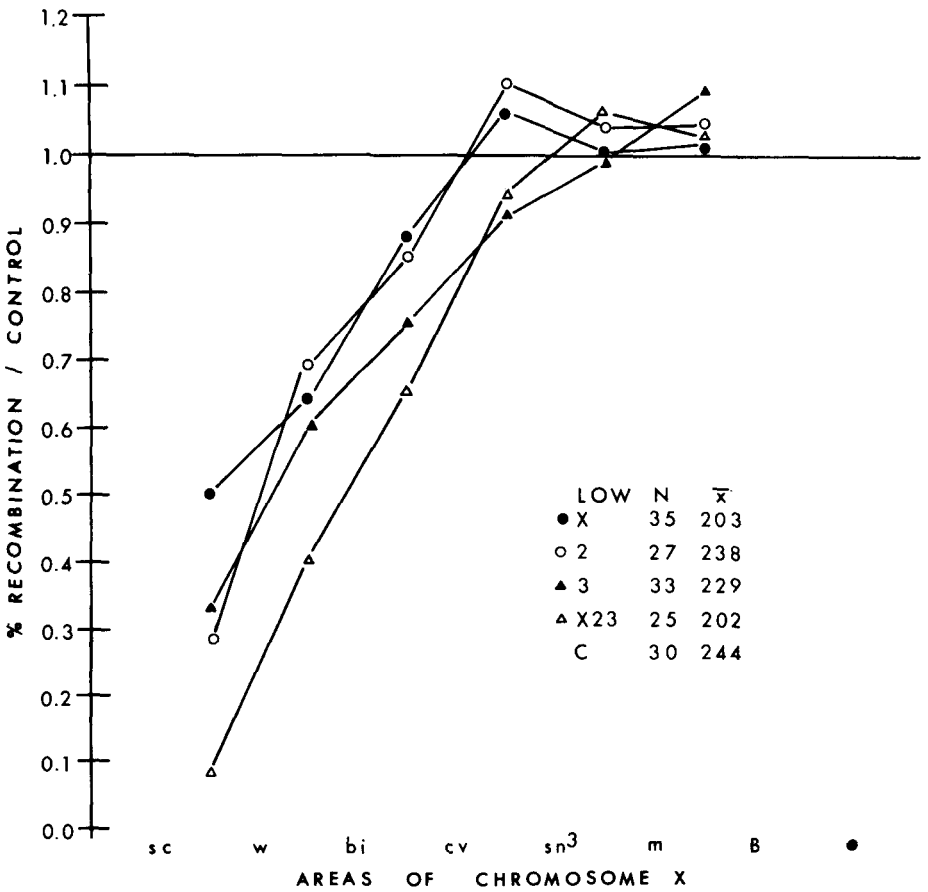


FIGURE 2.—Chromosome substitution results: Effect of chromosomes from the low recombinant line on recombination in the *X* chromosome, expressed in terms of the control values. N = the number of families (single pair matings) set up in each case; $\bar{x}$ = mean number of progeny per family. The black dot on the abscissa indicates the position of the centromere.

values for each region were compared to the appropriate control values by one-way analysis of variance, and the results of these analyses are indicated in the Tables. The magnitudes of mean crossover percentages relative to the control value in each region were determined by dividing the mean crossover value for each treatment by the appropriate control. These values are illustrated in Figures 2, 3, 4, and 5.

Effects of substituted chromosomes on recombination in the X *(crosses 1 through 5 in Table 2):*

(a) Low line chromosomes: Each chromosome combination tested (*X*, *2*, *3*, *33* and *X23*) produced a significant decrease in recombination in the *sc–w*, *w–bi*, and *bi–cv* regions. The *X*, *2*, and *3* chromosomes interact additively to reduce crossing over in these regions, for the result of the *X23* cross approximated the sum of the effects of the individual *X*, *2*, and *3* crosses. None of the chromosomes tested affected recombination in the *cv–sn*^{3} or *sn*^{3}*–m* regions except the *33* cross which caused a significant decrease in recombination in the *sn*^{3}*–m* region. In the *m–B* region, the *X*, *2*, and *X23* crosses showed no effect on recombination frequency, but the *3* and *33* treatments significantly decreased crossing over in this region.

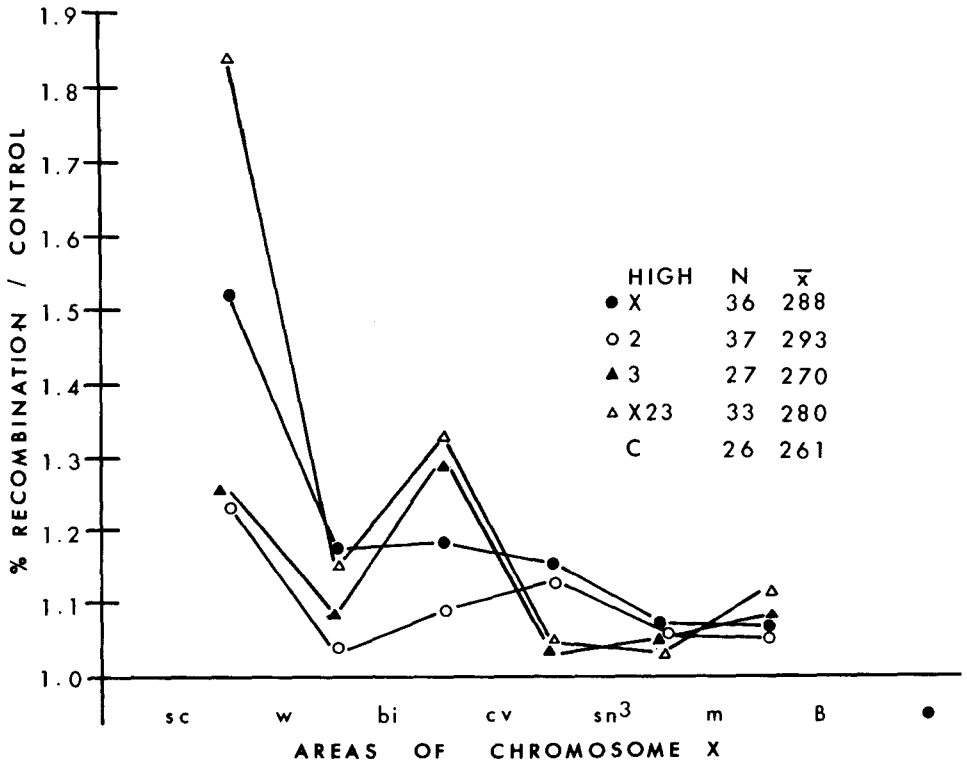
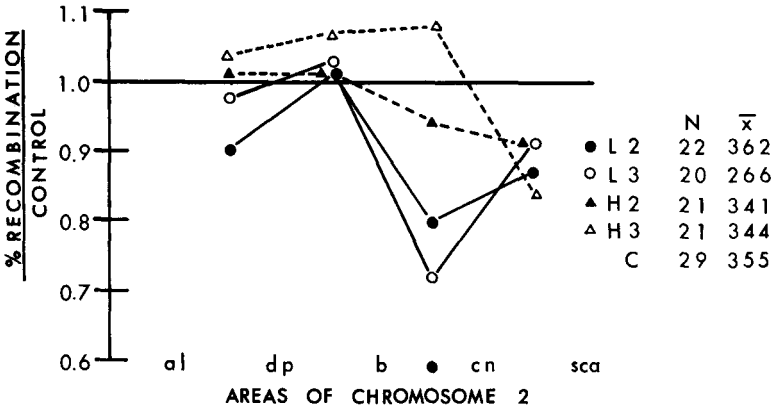


FIGURE 3.—Chromosome substitution results: Effect of chromosomes from the high recombinant line on recombination in the *X* chromosome, expressed in terms of the control values. See Figure 2 for other details.



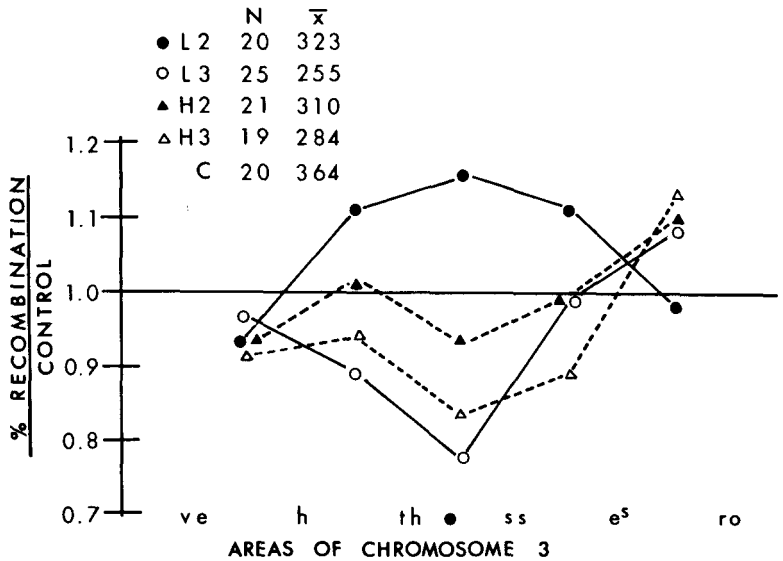


FIGURE 4.—Chromosome substitution results: Effect of chromosomes from the low and high recombinant lines on recombination in the second chromosome, expressed in terms of the control values. See Figure 2 for other details.

FIGURE 5.—Chromosome substitution results: Effect of chromosome from the low and high recombinant lines on recombination in the third chromosome, expressed in terms of the control values. See Figure 2 for other details.

(b) High line chromosomes: All chromosomes tested (*X*, *2*, *3*, *33*, and *X23*) showed a significant increase in recombination in the *sc–w* region. However, only the *X* and *X23* crosses showed a significant increase in *w–bi* recombination, and only the *X*, *3*, and *33* treatments showed significant increases in *bi–cv* recombination. The *X* and *2* crosses caused significant increases in *cv–sn*^{s} recombination, and only the *X* cross significantly increased crossing over in *sn*^{s}*–m*. In the *m–B* region, the *X*, *3*, *33*, and *X23* crosses all caused a significant increase in recombination, while chromosome *2* had no effect. In the *sc–w* region, the chromosomes

interact less than additively since the crossover value for the *X23* cross is less than the sum of the individual *X*, *2*, and *3* values, though significantly higher than the *X*, *2* or *3* treatment values. In the other regions of the *X* chromosome, the *3*, *33*, and *X23* recombination values are as a whole closely correlated, indicating that the third chromosome may be epistatically dominant to chromosomes *X* and *2* in modification of recombination values in the high line.

Effects of substituted chromosomes on recombination in chromosome 2 (crosses 6 and 7 in Table 2):

(a) Low line chromosomes: Both chromosomes *2* and *3* significantly decreased recombination in the *b–cn* region of chromosome *2*, which contains the centromere. Chromosome *2* also significantly lowered crossing over in the *al–dp* region.

(b) High line chromosomes: Chromosome *3* caused a significant increase in recombination in the *dp–b* region and a significant decrease in crossing over in the *cn–sca* region.

Effects of substituted chromosomes on recombination in chromosome 3 (crosses 8 and 9 in Table 2):

(a) Low line chromosomes: Chromosome *2* caused a significant decrease in crossing over in the *ve–h* region, while producing a significant increase in crossing over in the *h–th*, *th–ss* (centromeric region) and *ss–e*^{s} regions. Chromosome *3* significantly decreased crossing over in the *h–th* and *th–ss* regions, the other regions not responding.

(b) High line chromosomes: Chromosome *2* produced a significant decrease in recombination in the *ve–h* regions and a significant increase in crossing over in the *e*^{s}*–ro* region. Chromosome *3* significantly decreased recombination in the *ve–h*, *th–ss*, and *ss–e*^{s} regions, while significantly increasing crossing over in the *e*^{s}*–ro* region.

DISCUSSION

The polygenic nature of the system: LAWRENCE (1963) using 5 different inbred strains of *Drosophila melanogaster* containing the same sex linked mutants, set up a 5 × 5 diallel cross and compared the amount of recombination exhibited by these strains. He found differences among strains in the amount of recombination between the sex-linked genes, and attributed this to genetic differences among the strains. Since the inter-strain differences in recombination were small, it was suggested that the strains differed polygenically for factors modifying crossing over.

The results of the chromosome substitution experiments reported here give the most direct evidence now available that the selected genetic system is truly polygenic. The partitioning of the modification of crossover activity among the *X*, second, and third chromosomes in both lines shows that the genetic system has factors in all three chromosomes. However, this gives no estimate of the actual number of factors present in each chromosome. Further analysis involving methods described by BREESE and MATHER (1957) and THODAY (1961) for analyzing sections of chromosomes would be necessary to determine the approximate number of genetic elements involved.

Regional specificity of crossover modification: The chromosome substitution results from both the high and low lines show that the modification of crossover activity is for the most part regionally specific and not generally distributed over the entire genome. The most striking modifications occur in the *sc–cv* region (*sc–w*, *w–bi* and *bi–cv*) of the *X* chromosome and the centromeric regions of chromosomes *2* and *3*. Since the *sc–cv* region was the region of direct selection (CHINNICI 1971), the fact that each chromosome tested from the high and low lines caused large changes in crossover frequency here is not surprising. However, the responses of the centromeric regions are unexpected, both with regard to the magnitude of the response and to the lack of directional correlation between the *sc–cv* response and the centromeric response.

The centromeric regions (and the entire *Y* chromosome) in *D. melanogaster* are largely heterochromatic as opposed to the arms of the chromosomes which are largely euchromatic. It is well known that recombination in these heterochromatic centromeric regions is considerably more sensitive than euchromatic regions to modification by the presence of heterologous structural changes in other chromosomes (LUCCHESI and SUZUKI 1968), by female aging (BRIDGES 1915; PLOUGH 1921), and by temperature changes (PLOUGH 1921). This may indicate a basic recombinational instability in these heterochromatic regions, allowing them to undergo large changes in recombination frequency in response to genetic as well as non-genetic factors.

The chromosome substitution tests which were performed using chromosomes from the low selected line showed that *sc–w* > *w–bi* > *bi–cv* in the amount of reduction of crossing over. This pattern indicates that the closer the affected region is to the distal tip of the chromosome, the more pronounced is the reduction of crossing over. This corresponds to the pattern of crossover modification seen when the low recombinant line of DETLEFSEN and ROBERTS (1921) was crossed to a stock carrying several sex-linked markers (see DETLEFSEN and CLEMENTE 1923). Their low line was selected for decrease in recombination in the *w–m* region of the *X* chromosome. The F_2 values for the cross between the low line and an unselected mutant stock showed the pattern *sc–cv* > *cv–ct* > *ct–m* > *m–f* in the amount of reduction per standard crossover value. This again shows a decrease in the rate of reduction as the distance from the chromosome end increases. These results suggest that there is a negative correlation between the distance of a chromosome region from the distal end of the *X* chromosome and the degree of response shown to selection for reduction of crossing over. The high line does not show this pattern of response, however, so that the general validity of this correlation is unclear.

Many elaborate theories on the molecular basis of recombination have been postulated in recent years, most notably those of HOLLIDAY (1964, 1968) and WHITEHOUSE (1966, 1970) for higher organisms. These theories do not make provision for fine genetic control of recombination frequency, especially of the type shown to exist in Drosophila, where a polygenic system can modify crossing over specifically in just one small euchromatic segment of the genome. More elaborate models will be necessary to explain such specificity, and it is hoped that the data presented in this paper will stimulate thought in this direction.

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SUMMARY

Using selected lines of *Drosophila melanogaster* showing increased (high line) and decreased (low line) amounts of crossing over between the sex-linked genes *sc* and *cv*, the nature of the genetic system modifying recombination has been analyzed by employing chromosome substitution techniques to study the effects of isolated chromosomes from the high and low lines. In both lines, the activity of crossover modification is present in all chromosomes tested (*X*, *2*, and *3*), and is very specific for the *sc–cv* region. This indicates that the system is polygenic in nature. In the low line, the polygenes from different linkage groups act additively to modify recombination in the *sc–cv* region, for the *X23* treatment roughly equals the sum of the *X*, *2*, and *3* treatments. However, in the high line, chromosome *3* shows epistatis over *X* and *2* in modifying recombination in most regions of the *X* chromosome. These experimental chromosomes cause recombinational instability in the heterochromatic centromeric regions of the autosomes so that significant differences in recombination occur in these areas, the direction of which cannot be correlated with the direction of crossover modification in the *X* chromosome.

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