THE GENOTYPIC CONTROL OF CROSSING OVER IN DROSOPHILA PSEUDOOBSCURA[1]

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THE adaptability of a population will be a function of its capacity to produce genotypes which will be adapted to conditions with which it need not ordinarily cope. Crossing over is one means whereby such new gene combinations arise; it is therefore a process of great evolutionary significance. Our knowledge of the mechanism of crossing over and of the factors which modify it is still far from satisfactory. The purpose of the present paper is to summarize the results of an extended analysis of interchromosomal effects of inversions on crossing over and how they come about.

R. P. LEVINE and DICKINSON (1952) have recently shown that crossing over in the X-chromosome of *Drosophila pseudoobscura* can be modified by the different inversions found in the III chromosome. Their results are in agreement with those of MORGAN, BRIDGES and SCHULTZ (1933) who found that crossing over in the II chromosome of *D. melanogaster* could be significantly increased in the presence of inversions in the X or III chromosomes of that species. Similar results were also obtained by STEINBERG (1936). More recently, CARSON (1953) has observed the same phenomenon in *D. robusta*. The present study compares the effect of three different III chromosome gene arrangements, the result of inversion, on crossing over in the X-chromosome of *D. pseudoobscura*.

MATERIALS AND METHODS

Three III chromosome inversions of the Piñon Flat population in the San Jacinto Mountains, southern California, were chosen for this study. Three structurally homozygous inversions known as Standard (ST), three of Chiricahua (CH) and two of Arrowhead (AR) obtained by PROFESSOR CARL EPLING and DR. DONALD F. MITCHELL in 1950 were tested. They are among those recently used by DOBZHANSKY and PAVLOSKY (1953) and EPLING, MITCHELL and MATTONI (1953). Each strain had been derived by pair matings of the offspring of a single female caught in nature. Each is thus presumed to be genetically different. Chromosomes derived from natural populations of *D. pseudoobscura* are known to vary genetically as shown by the investigations of DOBZHANSKY, HOLZ and SPASSKY (1942), DOBZHANSKY and SPASSKY (1944), and DOBZHANSKY (1946) in that they carry either recessive genes or gene complexes which, " when homozygous, modify the

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viability, fertility, or development rate of the flies" (WRIGHT and DOBZHANSKY 1946).

The eight III chromosome strains referred to were combined in all possible ways on a background of an X-chromosome marked with yellow body (*y*), singed bristles (*sn*) and vermillion eye (*v*). The inversion combinations which are given in the first column of table 1 have been subdivided into three major classes. The first class is that of the structurally homozygous III chromosomes, in which both chromosomes of the pair have the same origin in that they were derived by the method described above (e.g., ST-1/ST-1 or CH-a/CH-a). These will be referred to as homozygotes. The second class also consists of structural homozygotes but the two chromosomes of the pair have had a different origin, having been obtained from two different wild females (e.g., ST-1/ST-2 or CH-a/CH-b). These are called hetero-homozygotes. The third class, called heterozygotes, comprises the ST/AR, ST/CH and AR/CH structural heterozygotes in all possible combinations of the homozygous strains. A total of eight homozygotes, seven hetero-homozygotes and twenty-one heterozygotes were accordingly analyzed for crossing over in the X-chromosome.

A standard procedure was followed in order to determine crossing over in the X-chromosome. Virgin females of a desired III chromosome combination, and heterozygous for a *y sn v* X-chromosome, were mated to *y sn v* males. The females were allowed to oviposit in 25 × 95 mm shell vials on the usual cornmeal, molasses, agar and yeast medium. The parents were transferred to new food vials at three-day intervals to prevent overcrowding of the vials and to permit, if necessary, an analysis of the effect of female age on crossing over. Each of these 36 matings was replicated at least five times, and an attempt was made to obtain a total of at least 1000 flies in the test generation of each combination. Replication of each combination also permitted an analysis of the variation in crossing over from female to female within any given combination. All cultures in this experiment were maintained at 23°C.

RESULTS

The data are listed in table 1. Included in the table are the total number of flies counted, the number of non-crossover types, number of single crossovers in the *y-sn* region (region 1), the number of single crossovers in the *sn-v* region (region 2), and the number of double crossovers (regions 1 and 2). In addition, the percents of crossing over in regions 1 and 2 are given considering each double crossover twice along with the average total percent for the replications of a given combination. The values for each replication of the 36 combinations have not been given in order to conserve space. Three possible combinations, all involving CH-c, have been omitted because they did not produce 100 flies or more, for unknown reasons. These combinations are not, therefore, comparable with the others.

The variation in crossing over in the replications of each inversion combination has been tested by chi-square, and the P values are given in the last

column of table 1. The P values range from less than 0.01 to 0.95. A chi-square test of the distribution of P values was performed by dividing the values into three groups: 1) P between 1.0 and 0.67; 2) P between 0.66 and 0.34; and 3) P less than 0.34. Thus, with 33 inversion combinations there is an expectation of 11 P values in each group. The actual distribution of P

TABLE 1

X-Chromosome crossing over in the presence of different third chromosome inversion combinations.

	Combination	Total	Non-crossovers	Single c.o. region 1	Single c.o. region 2	Double c.o. regions 1 + 2	% c.o. region 1	% c.o. region 2	Avg. Total % c.o.	P
Homozygotes	ST-1/ST-1	2704	2427	228	49	0	8.43	1.81	11.00	0.55
	ST-2/ST-2	2204	1986	181	37	0	8.21	1.68	9.79	0.75
	ST-3/ST-3	2260	2011	217	31	1	9.65	1.42	10.50	0.50
	CH-a/CH-a	1772	1516	212	44	0	11.96	2.48	13.82	0.70
	CH-b/CH-b	2374	2055	265	54	0	11.16	2.27	13.90	0.34
	CH-c/CH-c	....	....	....	....	....	....	....	....	....
	AR-e/AR-e	2091	1822	226	43	0	10.81	2.06	12.70	0.35
	AR-f/AR-f	3080	2594	410	73	1	13.34	2.47	15.75	0.95
Hetero-Homozygotes	ST-1/ST-2	2513	2213	246	54	0	9.79	2.15	12.04	0.65
	ST-1/ST-3	3533	3132	326	71	4	9.34	2.12	11.32	0.35
	ST-2/ST-3	1731	1511	188	32	0	10.86	1.85	12.78	0.25
	CH-a/CH-b	1984	1713	226	45	0	11.39	2.27	13.75	0.75
	CH-a/CH-c	1347	1090	208	49	0	15.44	3.64	19.00	0.23
	CH-b/CH-c	....	....	....	....	....	....	....	....	....
	AR-e/AR-f	1698	1436	219	42	1	12.96	2.53	14.82	<0.001
Heterozygotes	ST-1/CH-a	2411	2027	305	79	0	12.65	3.28	15.58	0.55
	ST-1/CH-b	1401	1189	179	33	0	12.78	2.36	15.29	0.13
	ST-1/CH-c	2048	1734	252	62	0	12.30	3.03	15.12	0.12
	ST-2/CH-a	997	872	107	18	0	10.73	1.81	10.58	0.008
	ST-2/CH-b	2555	2220	269	60	6	10.76	2.58	13.32	0.43
	ST-2/CH-c	2272	1984	269	49	0	11.84	2.16	14.25	0.06
	ST-3/CH-a	2709	2408	252	48	1	9.34	1.81	11.34	0.43
	ST-3/CH-b	2835	2412	346	75	2	12.28	2.72	15.11	0.50
	ST-3/CH-c	2048	1780	223	45	0	10.89	2.20	12.95	0.65
	ST-1/AR-e	2117	1817	234	66	0	11.05	3.12	13.44	0.08
	ST-1/AR-f	2048	1718	279	51	0	13.62	2.49	16.48	0.07
	ST-2/AR-e	2304	1978	262	62	2	11.46	2.78	15.78	<0.001
	ST-2/AR-f	1262	1048	175	38	1	13.95	3.09	17.19	0.45
	ST-3/AR-e	2387	2050	289	48	0	12.11	2.01	14.65	0.08
	ST-3/AR-f	2144	1793	289	60	2	13.57	2.89	16.20	0.18
	CH-a/AR-e	2442	2075	289	78	0	11.83	3.19	15.02	0.85
	CH-a/AR-f	2286	1925	291	68	2	12.82	3.06	15.93	0.26
	CH-b/AR-e	1424	1239	151	33	1	10.67	2.39	13.11	0.90
	CH-b/AR-f	1505	1298	170	36	1	11.36	2.46	13.96	0.48
	CH-c/AR-e	1757	1510	208	39	0	11.84	2.22	14.40	0.55
	CH-c/AR-f	....	....	....	....	....	....	....	....	....

values is 6 in group one, 14 in group two, and 13 in group three. The P value of such a distribution is 0.17, a reasonable fit.

The results set forth in table 1 will be discussed under three major groups of combinations; homozygotes, hetero-homozygotes and heterozygotes. This will be followed by a general analysis. When P values are mentioned they will have been derived by the Brandt-Snedecor chi-square test for homogeneity

(SNEDECOR 1946). Calculations for the chi-square comparisons between combinations are based on the actual crossover values from the replications of each combination. For the crossing over values of a given combination reference is to be made to table 1.

Crossing over in homozygous inversion combinations

AR homozygotes were found to have a significantly higher percentage of crossing over in the X-chromosome than the ST homozygotes. Neither the three ST homozygotes nor the CH homozygotes differ significantly in their X-chromosome crossover values ($P = 0.45$ and 0.31, respectively). Since no differences of statistical significance were found between the different ST homozygotes they may be considered as a group. The same is true for the CH homozygotes. The two AR homozygotes gave a P value of 0.004, indicating a strong statistical difference in their effect upon X-chromosome crossing over, with AR-f/AR-f having the greater percent of crossing over.

Comparisons can also be made between the three homozygous classes. The differences in effect of the grouped ST homozygotes, compared with the grouped CH homozygotes had a P value less than 0.0001, indicating that the X-chromosome crossing over is significantly greater in CH homozygotes than it is in the ST homozygotes. The differences of the grouped ST homozygotes compared with AR-e and AR-f separately were also highly significant, P values in both cases being less than 0.0001. Thus, the AR homozygotes have a significantly greater amount of crossing over in the X-chromosome than do the ST homozygotes. The CH homozygotes had a significantly lower percent of crossing over than did the homozygote AR-f ($P = 0.025$), but they did not differ significantly from the AR-e homozygote ($P = 0.28$).

These results indicate that different homozygous inversions in the III chromosome have different effects on crossing over in the X-chromosome and that in this case CH and AR homozygotes increased it more than ST. They also suggest that crossing over in the X-chromosome may be modified by particular gene orders found in the ST, CH and AR homozygotes. This could be ascribed to a position effect, but if it were the case one would not expect the significant differences between the two AR homozygotes, which have the same gene order. The evidence suggests, therefore, that different genetic constitutions in the III chromosome have differential effects on crossing over in the X-chromosome. Further indication of this was gained from the study of the hetero-homozygous and heterozygous combinations.

Crossing over in hetero-homozygous inversion combinations

No significant differences were found between the three ST hetero-homozygous combinations ($P = 0.35$). As indicated in table 1 only two of the three CH hetero-homozygous crosses were successful and these differ significantly ($P < 0.0001$). The crossover value obtained for the CH-a/CH-c hetero-homozygote was in fact the highest obtained for all combinations tested. Comparisons have been made between the ST, CH and AR hetero-homozygotes, and the hetero-homozygotes compared with the homozygous combinations.

The grouped ST hetero-homozygotes differ significantly from both the CH and the AR hetero-homozygous combinations. This is consistent with the results of the preceding section in which it will be recalled that the ST homozygotes differed significantly from both the CH and the AR homozygotes. The two CH hetero-homozygote combinations, however, compare differently with the AR hetero-homozygote, CH-a/CH-c being significantly higher than AR-e/AR-f in X-chromosome crossing over. Unfortunately there are no data for the CH-c homozygote and therefore little can be learned from this last comparison.

The comparison between the various homozygotes and the hetero-homozygotes shows that the ST hetero-homozygotes did not differ significantly from the ST homozygotes ($P = 0.75$). The grouped CH homozygotes compared with the CH-a/CH-b hetero-homozygote gave no significant difference ($P > 0.95$). The AR homozygotes, however, compare differently with the AR hetero-homozygous combination. AR-e/AR-e has a lower X-chromosome crossing over percentage than does the AR-e/AR-f hetero-homozygote. AR-f/AR-f, on the other hand, does not differ significantly from AR-e/AR-f.

From these results it is apparent that both the ST homozygotes and hetero-homozygotes used here have similar effects on crossing over in the X-chromosome regardless of their genetic make-up. The same is true for the CH chromosomes. However, each of the AR homozygotes differ in their effects but not significantly from those of their hetero-homozygous combinations. Thus, these results again suggest that particular gene combinations in the III chromosome may modify crossing over in the X-chromosome. For example, the ST hetero-homozygotes differed markedly from both the CH and AR hetero-homozygotes just as the ST homozygotes differed from the CH and AR homozygotes.

Crossing over in the heterozygous inversion combinations

Crossing over in the X-chromosome was found to vary markedly in the presence of different III chromosome inversion heterozygotes. Reference to table 1 will show that in certain heterozygotes, for example ST-1/CH-a and ST-3/AR-f, crossing over in the X-chromosome was increased over that common to the homozygotes from which the heterozygotes had been constructed. This is in agreement with the findings for *D. melanogaster* (MORGAN, BRIDGES and SCHULTZ 1933) in which the presence of an inversion heterozygote in one chromosome pair is related to increased crossing over in another chromosome pair. Other heterozygous combinations, however, showed an increased crossing over over one but not over *both* of the homozygotes. For example, ST-2/CH-a gave 10.58% crossing over while ST-2/ST-2 gave 9.79% and CH-a/CH-a gave 13.90% crossing over. Comparisons between heterozygotes and their respective homozygotes are presented in table 2 for each case in which a significant difference was found between a heterozygote and its respective homozygotes when tested together for homogeneity. Only thirteen of the twenty-one possible heterozygous combinations are listed. Three gave non-significant results and are excluded, namely those involving CH-a

TABLE 2

Comparison of X-chromosome crossing over in homozygous and heterozygous III chromosome combinations.

	Percent crossing over		Percent crossing over		Percent crossing over	
1	ST-1/ST-1	11.00	ST-1/ST-1	11.00	ST-2/ST-2	9.79
2	CH-a/CH-a	13.82	CH-b/CH-b	13.90	CH-a/CH-a	13.82
3	ST-1/CH-a	12.04	ST-1/CH-b	15.29	ST-2/CH-a	10.58
1-2	*<0.0001*		*0.0005*		*<0.0001*	
1-3	*<0.0001*		*<0.0001*		*0.026*	
2-3	0.20		0.15		0.17	
1	ST-2/ST-2	9.79	ST-3/ST-3	10.50	ST-3/ST-3	10.50
2	CH-b/CH-b	13.90	CH-a/CH-a	13.82	CH-b/CH-b	13.90
3	ST-2/CH-b	13.32	ST-3/CH-a	11.34	ST-3/CH-a	15.11
1-2	*0.0002*		*0.001*		*0.016*	
1-3	*0.0006*		0.95		*<0.0001*	
2-3	0.75		*0.0008*		0.13	
1	ST-1/ST-1	11.00	ST-1/ST-1	11.00	ST-2/ST-2	9.79
2	AR-e/AR-e	12.70	AR-f/AR-f	15.75	AR-e/AR-e	12.70
3	ST-1/AR-e	13.44	ST-1/AR-f	16.48	ST-2/AR-e	15.78
1-2	*0.0045*		*<0.0001*		*0.0002*	
1-3	*<0.0001*		*<0.0001*		*<0.0001*	
2-3	0.20		0.75		0.20	
1	ST-2/ST-2	9.79	ST-3/ST-3	10.50	ST-3/ST-3	10.50
2	AR-f/AR-f	15.75	AR-e/AR-e	12.70	AR-f/AR-f	15.75
3	ST-2/AR-f	17.19	ST-3/AR-e	14.65	ST-3/AR-f	16.20
1-2	*<0.0001*		0.06		*<0.0001*	
1-3	*<0.0001*		*0.0002*		*<0.0001*	
2-3	0.35		0.24		0.60	
1	CH-b/CH-b	13.90				
2	AR-f/AR-f	15.75				
3	CH-b/AR-f	13.96				
1-2	*0.017*					
1-3	0.80					
2-3	0.062					

and AR-e; CH-a and AR-f; and CH-b and AR-e. Combinations involving CH-c were also excluded because no data are available for the CH-c homozygote with which to make comparisons. The remainder gave P values of less than 0.01 except for the combination of CH-b and AR-f which had a P value of 0.036.

Table 2 shows the observed total average percent crossing over for each combination (lines 1, 2, 3) and the P values for the comparisons between each pair (lines, 1-2, 1-3, 2-3). It will be seen that the ST homozygotes differ significantly from the CH homozygotes (comparison 1-2). They also differ significantly from the ST/CH heterozygotes except in the case of ST-3/CH-a (comparison 1-3). The CH homozygotes, however, do not differ significantly from the ST/CH heterozygotes except in the case of ST-3/CH-a (comparison 2-3). Thus, it would appear that the CH chromosomes, which as CH homozygotes differ significantly from the ST homozygotes but not from the ST/CH heterozygotes, are modifying the X-chromosome crossing over in

the ST/CH heterozygotes. A similar situation obtains in the case of the ST-1 and ST-2 comparisons with AR-e and AR-f, and ST-3 with AR-f. Again the ST homozygotes differ significantly from both the AR homozygotes and the ST/AR heterozygotes while the AR homozygotes do not differ from the heterozygotes. Thus, the CH and AR chromosomes may be contributing in a major way to X-chromosome crossing over in ST/CH and ST/AR heterozygotes. There are three exceptions to this possibility. The ST-3 homozygote is significantly different from the CH-a homozygote, but it does not differ significantly from the ST-3/CH-a heterozygote while the CH-a homozygote does. In this case the CH-a homozygote has a greater percent crossing over than does the ST-3/CH-a heterozygote. The second exception is that of ST-3 and AR-e. In this case only the ST-3 homozygote differs significantly from the ST-3/AR-e heterozygote. However, the difference between the ST-3 and AR-e homozygotes is just above the 5% level of significance. The last exception is that of CH-b and AR-f. The only difference of significance is between the CH-b and AR-f homozygotes. The difference between the AR-f homozygote and the CH-b/AR-f heterozygote is just above the 5% level of significance.

The relationship between the III chromosome combination and the X-chromosome crossing over can be conceived as being the result of a number of factors. Certain of the results indicate that a particular chromosome or rather the constellation of genes in that chromosome is affecting crossing over in what seems to be a case of dominance of CH and AR chromosomes over the ST chromosomes. Other results, particularly those which show no obvious correlation between the crossing over of a given homozygote and its heterozygote, suggest that interaction between both members of a III chromosome pair, rather than the action of one chromosome of the pair alone, is responsible, at least in part, for the observed variations in crossing over. There is little doubt that the results presented in this section and in those preceding show a definite relationship between the particular strains of III chromosomes used and X-chromosome crossing over. Such a relationship indicates that X-chromosome crossing over is to an extent controlled by the genetic constitution of different kinds of III chromosomes tested. Two hypotheses amenable to both statistical and experimental test are; first, that one of the III chromosomes in a given combination because of its particular genetic constitution contributes in a major way to X-chromosome crossing over, and second, that it is the interaction between two genetically different III chromosomes of the combination which is most effective. The statistical analysis presented in the next section was attempted in order to determine the applicability of these hypotheses to the observed data.

Statistical analysis of the effects of different III chromosome inversion combinations on crossing over in the X-chromosome

An analysis of variance similar to that of WALLACE, KING, MADDEN, KAUFMANN and MCGUNNIGLE (1953, p. 293) was carried out to test whether the percentage of crossing over in the X-chromosome is due to contributions

made by the particular III chromosomes tested or results from the specific interaction between any two different III chromosomes. The variance of the X-chromosome crossing over has three components; namely, that due to experimental error, that resulting from contributions made by III chromosomes, and that resulting from interactions between III chromosomes. The results of the analysis are given in table 3. The contributions of III chromosomes to the variance (e.g., the contribution of the ST-1 chromosome is $a_1 = -0.62$), and the interaction between chromosomes (e.g., ST-1/CH-a is $g_{1a} = 2.55$) are shown in Part A of the table. The variance analysis, given in Part B of the table, shows that different III chromosomes make statistically significant contributions to the amount of X-chromosome crossing over. However,

TABLE 3

Contributions and interactions of third chromosomes to X-chromosome crossing over

A

Contributions

a_1 −0.62	a_a −0.32	a_e 0.16
a_2 −1.17	a_b 0.73	a_f 1.59
a_3 −1.04		

Interactions

g_{11} −1.73	g_{22} −1.84	g_{3a} −1.27	g_{af} 1.69
g_{12} −0.14	g_{23} 1.02	g_{3b} 1.45	g_{bb} −1.53
g_{13} −0.99	g_{2a} −1.90	g_{3e} 1.56	g_{be} −1.75
g_{1a} 2.55	g_{2b} −0.21	g_{3f} 1.68	g_{bf} −2.33
g_{1b} 1.11	g_{2e} 2.82	g_{aa} 0.49	g_{ee} −1.59
g_{1e} −0.07	g_{2f} 2.80	g_{ab} −0.53	g_{ef} −0.90
g_{1f} 1.54	g_{33} −1.39	g_{ae} 1.11	g_{ff} −1.40

B

	ss	*df*	*Mean square*	*F-ratio*	*P*
Error	1,032.112	141	7.320	....	....
Interaction	418.8311	21	19.9443	2.72	<0.001
Contribution	354.4363	6	59.0727	8.07	<0.001

pairs of III chromosomes also have a statistically significant effect upon crossing over. Thus, crossing over in the X-chromosome is modified both by particular III chromosomes (contributions) and by particular combinations of III chromosomes (interactions).

Accordingly, it is necessary to determine the relative sizes of the two components of variance. The mean square for interaction (19.9443) includes the mean square for error (7.320); therefore, the interaction variance will be the difference between the two mean squares or 12.6243. The mean square for contributions (59.0727) includes the mean square for the interaction. The contributions component of variance is found by dividing the difference between these two mean squares by the number of different kinds of chromosomes tested (in this case seven) and it therefore equals 5.5899. Thus, interactions

have almost twice the effect on the total variance than do contributions and are therefore the major component of variance in the amount of X-chromosome crossing over.

If a comparison of contribution and interaction values with the observed X-chromosome crossing over percentages for homozygotes and heterozygotes is made, it will be noted that when a heterozygote is greater than either homozygote in its crossing over there is a positive interaction value, but when the heterozygote lies between the homozygotes in its amount of crossing over there is a negative interaction value. This too indicates the importance of the effect of particular III chromosome combinations on crossing over in the X-chromosome.

Thus, the general conclusion drawn from the variance analysis is that crossing over in the X-chromosome can be modified by particular III chromosome gene combinations. Part of this modification is due to a contribution made by one III chromosome in an inversion combination while part is due to the interaction between the two III chromosomes of a combination.

DISCUSSION

Comparisons between D. pseudoobscura and D. melanogaster

Certain of the results obtained here for *D. pseudoobscura* parallel those found for *D. melanogaster*. There are differences, however, which may either be characteristic of these species or they may result from different experimental methods.

Crossing over in uninverted chromosomes in *D. melanogaster* can be increased in the presence of inversion heterozygotes in other chromosomes. Thus, crossing over in the *y-ec* region of the X-chromosome has been found to increase 171.1 percent in the presence of the Curly inversion in the second chromosome (STEINBERG 1936). In the present study increased crossing over was also found. However, the increase in X-chromosome crossing over in *D. pseudoobscura,* when an inversion heterozygote was present in the III chromosome, was not as marked as that found for *D. melanogaster*. In the most extreme case in the present study it amounted to an increase of 8.5% above the average percent crossing over for individuals with the ST chromosome inversion. This difference between the two species may be caused by several factors. The *y-sn-v* region of the *D. pseudoobscura* X-chromosome used in the present study may not be " sensitive " to the enhancing effect which occurs in the presence of an inversion heterozygote. SCHULTZ and REDFIELD (1951) have shown that the significant increases in crossing over in the uninverted chromosomes of *D. melanogaster* arise between loci which are separated by heterochromatin. Thus it was found that in the presence of the *ClB* inversion in the X-chromosome the crossing over in the second chromosome showed the greatest increase between the loci black and cinnabar which are on either side of the centromere and are adjacent to heterochromatin. The *y-sn-v* region in the left arm of the X-chromosome of *D. pseudoobscura*

apparently does not include heterochromatin. Therefore, the relatively small increases in X-chromosome crossing over may be attributed to the fact that heterochromatin is not involved. However, the possibility remains that the inversion effect in *D. pseudoobscura* is not related to heterochromatin. Studies with an X-chromosome having markers on either side of the heterochromatic region, which is associated with the centromere, are necessary before any further statements can be made.

A second explanation may lie in the total amount of chromatin in the inversions. STEINBERG (1937) has shown there is a direct proportionality between the amount of the total chromatin involved in the inversion and the increase of crossing over in uninverted chromosomes in *D. melanogaster.* Thus, crossing over in the X-chromosome of this species is increased more by the Payne inversion than by the Curly inversion. When both are present an even greater X-chromosome crossing over is observed. STEINBERG and FRASER (1944), however, found on further study that there is no absolute relation in *D. melanogaster* between the length of the inversion and the effect upon either X-chromosome or III chromosome crossing over. The increased crossing over in the *y-sn-v* region of the X-chromosome of *D. pseudoobscura* appears to be independent of the size of the III chromosome inversions. The AR inversion of *D. pseudoobscura* is derived from ST by one pair of breaks in regions 70 to 76 and thus involves only a relatively short section of the chromosome. The CH arrangement is an overlapping inversion with respect to both ST and AR and it involves a much greater amount of the III chromosome from region 68 to region 80 (DOBZHANSKY and EPLING 1944). If there is a direct relationship between the length of the inversion and the amount of crossing over in the III chromosome of *D. pseudoobscura,* then it would be expected that crossing over would follow the sequence AR/CH > ST/CH > ST/AR. This, however, was not found to be the case. Reference to table 1 will show that crossing over in the X-chromosome was of similar order in all of the heterozygote combinations. Increased crossing over in other chromosomes of *D. melanogaster* results, in part, from a highly increased frequency of multiple exchanges when an inversion heterozygote is present (STEINBERG 1936). For *D. pseudoobscura* the frequency of double crossovers was not increased above the frequency of single crossovers. Such a result may indicate a basic difference between the two species or it may reflect a different response of the *y-sn-v* region of the X-chromosome of *D. pseudoobscura* to an inversion heterozygote.

One unexpected result in the present work was the variation found in X-chromosome crossing over when different III chromosome inversion homozygotes were present. Crossing over was greatest in AR homozygotes, less in CH homozygotes, and least in ST homozygotes. The modification of crossing over in different homozygous inversions of *D. melanogaster* has not been studied, though crossing over has been shown to be one of the most variable genetic phenomena in Drosophila. In an extensive analysis of crossing over in *D. melanogaster,* GOWEN (1919) showed that there were significant variations

in III chromosome crosing over that were not due entirely to experimental error. He attributed the variations to gene differences in the III chromosomes tested; this suggests that crossing over is under genetic control. The results of the present study tend to support this conclusion and as will be pointed out below the genetic constitutions of ST, CH and AR III chromosomes are the basis, in part, of the variation in X-chromosome crossing over.

Our data differ from those obtained for *D. melanogaster* in one other respect. Variation in crossing over was significantly different when heterozygotes were made up of the different strains of ST, CH and AR chromosomes (e.g., ST-1/CH-a, ST-2/CH-a). This result further indicates that the genetic constitutions of the III chromosomes play a role in X-chromosome crossing over. Comparable data are not available for *D. melanogaster* but variations of the sort we have observed have been shown to occur in *D. robusta* (CARSON 1953).

Interpretation of D. pseudoobscura results

Inversion heterozygosis in Drosophila is a means whereby blocks of genes are retained intact as a consequence of the suppression of crossing over in an inversion heterozygote (STURTEVANT and BEADLE 1936). The suppression effect has been noted in *D. melanogaster* (STURTEVANT 1919), *D. pseudoobscura* (DOBZHANSKY and EPLING 1948), *D. robusta* (CARSON 1953), and *D. virilis* (KOMAI and TAKAKU 1940, 1942). In the case of *D. pseudoobscura* and *D. robusta* it has been suggested that inversion heterozygosis is a means whereby combinations of genes imparting a favorable adaptive character to the individuals of a population can be retained without loss through recombination resulting from crossing over (DOBZHANSKY and EPLING 1948; CARSON 1953). R. P. LEVINE and DICKINSON (1952) and CARSON (1953) have also pointed out that inversion heterozygosis is a two-fold system which allows the individuals of a population to exploit ecological niches to a fuller extent because it permits an increased production of recombinants in other chromosomes. The results of the present study indicate, however, that inversion heterozygosis alone is not always a significant factor in the increased production of new genetic types, but that the particular genetic constitutions of the III chromosome combinations are also important. As brought out in the preceding sections, the ST, CH and AR homozygous arrangements have different effects on X-chromosome crossing over. In addition, the two strains of the AR homozygotes differed from each other significantly. The hetero-homozygotes gave results similar to that obtained for homozygotes. Thus, if we can consider crossing over to be dependent upon particular gene combinations, the variations in X-chromosome crossing over reflect what are probably genetic differences between the ST, CH and AR chromosomes. Such differences can arise in nature and in the laboratory since there is a minimum of crossing over between them. Carriers of ST and CH chromosomes have been shown to differ in regard to the responses of their carriers to conditions of temperature and humidity (R. P. LEVINE 1952). Since free genetic exchange can occur between inversion homozygotes of a given sort genetic differences between

chromosomes would be at a minimum. This has been found to be true for certain responses between strains of ST homozygotes to temperature and humidity (R. P. LEVINE unpublished). The effect of both ST and CH homozygotes on X-chromosome crossing over is similar within the strains of ST or CH tested, but different in the two AR homozygotes. This indicates genetic differences between the AR homozygotes.

The variation between the heterozygotes strongly suggests that these combinations have varying degrees of influence on X-chromosome crossing over. This is shown by the comparisons given in table 2. Thus, X-chromosome crossing over of the CH-a homozygote is similar to that of the ST-1/CH-a and ST-2/CH-a heterozygotes but it is significantly greater than that of the ST-3/CH-a heterozygote. Increased crossing over in the X-chromosome of *D. pseudoobscura* in the presence of III chromosome inversion heterozygotes is not, therefore, a general rule. We suggest that it is the different gene combinations in the various inversion heterozygotes which modify crossing over in the X-chromosome, rather than inversion heterozygosis alone. The relationship between inversion heterozygotes and increased crossing over in uninverted chromosomes has been interpreted by MATHER (1936) and others as a compensating mechanism for the suppression of crossing over which occurs within the inversion heterozygote itself. This interpretation assumes that there is a characteristic amount of crossing over for the genome as a whole and when it is suppressed in one part of the genome it must increase in another. It must be remembered, however, that the suppression of crossing over within an inversion heterozygote is to a large extent apparent, not real. During meiosis in Drosophila females heterozygous for an inversion, the crossover chromosomes are usually excluded from the egg nucleus and are found in the polar body nuclei (see STURTEVANT and BEADLE 1936 for a full discussion of this phenomenon). If MATHER'S "compensation" hypothesis is tenable then some correlation between the amount of suppression of crossing over in the inversion heterozygote and the increase of crossing over in the other chromosomes should be found. However, it has been shown that there is no obvious correlation between the amount of suppression in the inversion heterozygote and the increased crossing over in other chromosomes. STURTEVANT and BEADLE (1936) found that various inversions in the X-chromosome of *D. melanogaster* suppress crossing over in the following order: $dl\text{-}49 \geqq ClB > sc^7 > bb^{Df} \geqq y^4 > sc^4 = sc^8$. STEINBERG and FRASER (1944) have shown that sc^4 and sc^8, both of which are long inversions in *D. melanogaster,* have little effect on X-chromosome crossing over, but have different effects on crossing over in the III chromosome, sc^8 greatly increasing it and sc^4 having no appreciable effect. Similarly, the y^4 inversion has practically no effect on III chromosome crossing over while the smaller *ClB* inversion has a marked effect. *D pseudoobscura* presents a similar picture. The suppression of crossing over in the overlapping ST/CH inversion heterozygote is almost eight times that of the simple ST/AR inversion heterozygote (DOBZHANSKY and EPLING 1948). However, the effect of both ST/CH and ST/AR on the X-chromosome was found to be the opposite, ST/AR heterozygotes having a slightly greater

X-chromosome crossing over than ST/CH heterozygotes. Thus, it is difficult to accept the " compensation " hypothesis in order to explain the effect of inversion heterozygotes on crossing over in other chromosomes.

Another interpretation of the relationship between inversion heterozygotes and increased crossing over in uninverted chromosomes is that of SCHULTZ (MORGAN, BRIDGES and SCHULTZ 1935) which is based upon the assumption of somatic pairing during the last premeiotic division. If somatic pairing is prevented or disturbed in one chromosome pair as in the case for certain chromosomal segments in an inversion heterozygote, it is assumed that the degree of disturbed pairing will be correlated with increased crossing over in the uninverted chromosome pair. The objections to this hypothesis are the same as those raised for the " compensation " hypothesis. That is to say, no correlation has been found between the kind of inversion present and the increase in crossing over in other chromosomes.

A third interpretation which explains the effect of inversions on crossing over in physiological terms has been offered by STEINBERG and WHITE (1939). They support this interpretation by results obtained from ovarian transplants in *D. melanogaster*. Ovaries carrying the X-chromosome markers *y* and *ec* as heterozygotes were implanted in the oviducts of females heterozygous for both Curly and Payne inversions. STEINBERG (1936) had shown earlier that X-chromosome crossing over was increased 285 percent in the presence of these inversions, but crossing over in the implanted ovaries was not modified by the Payne and Curly inversions of the host. On the basis of this result STEINBERG and FRAZER (1944) suggest that increased crossing over results from " an unspecified physiological effect caused by the inversion." They also state that the effect of inversions on crossing over is the result of a position effect. Though this interpretation is suggestive it lacks sufficient experimental support. Its very general assumptions of unspecified physiological effects of inversions should be tested.

None of the foregoing interpretations completely fit the results we have obtained for *D. pseudoobscura*. We suggest that there is a genotypic control of crossing over in the X-chromosome by III chromosome genes; the control may be mediated either through contributions made to crossing over by genes in a particular III chromosome in a given inversion combination, or through genetic interactions between the two chromosomes of the combination. The statistical analysis testing the two alternatives indicates that both play a significant role in X-chromosome crossing over. We also found that interactions between III chromosomes were more important than contributions made by III chromosomes. Crossing over in the X-chromosome is, therefore, the consequence of the interplay between two different III chromosome gene constellations. How this comes about is unknown but the hypothesis can be tested, and experiments testing it are now underway.

SUMMARY

Crossing over in the *y-sn-v* region of the X-chromosome of *D. pseudoobscura* is modified by different III chromosome homozygous and heterozy-

ous inversion combinations. The inversions studied were three strains of ST and CH and two strains of AR. Individuals homozygous for CH or AR had a significantly higher percentage of crossing over in the X-chromosome than homozygous ST individuals. All possible heterozygous inversion combinations were tested for X-chromosome crossing over. It was found that certain but not all combinations showed increased crossing over. The amount of crossing over in the X-chromosome was found to depend upon the particular III chromosome inversion combination.

The results suggest that crossing over in the X-chromosome depends, in part, upon the different III chromosome gene constellations brought together in the various inversion combinations.

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