VARIABLE CROSSING OVER ARISING IN DIFFERENT STRAINS OF DROSOPHILA PSEUDOOBSCURA[1]

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IT HAS been shown (LEVINE and LEVINE 1954) that the amount of crossing over in the X chromosome of *Drosophila pseudoobscura* can be modified by different third chromosome inversion homozygotes and heterozygotes. Thirty-five different combinations of inversion homo- and heterozygotes were found to be capable of a complex genetic interaction giving rise to variable crossover frequencies in the X chromosome. On the basis of this observation an hypothesis of genotypic control of crossing over was proposed to explain, at least in part, the significant variations in crossing over. The hypothesis proceeds from the assumption adumbrated in the work of GOWEN (1919) that crossing over is a character subject to genetic modification. Since the hypothesis was derived from a study of the effect which presumably genetically different third chromosomes had on crossing over in the X chromosome, it was of importance to analyze crossing over in the different third chromosomes themselves. If there is genetic control of crossing over one would predict that analysis of a number of different strains of wild type flies would yield significantly different results for crossing over in any region of a chromosome under consideration.

The present communication utilizing *D. pseudoobscura* third chromosome gene arrangements deals with experiments designed to test the hypothesis of a genetic control of crossing over.

MATERIALS AND METHODS

D. pseudoobscura provides excellent material for an analysis of variation in crossing over since there are a number of strains of third chromosome inversion types available. Since each strain of any given inversion had its origin from a single female caught in nature, it has a great probability of being genetically distinct and might therefore provide a characteristic pattern of crossing over.

The third chromosome strains used in this study were the gene arrangements known as Standard (ST) and Arrowhead (AR). They were obtained from a 1952 collection made by PROFESSOR CARL EPLING and DR. DONALD F. MITCHELL in the San Jacinto Mountains of southern California.

The ST gene arrangement carries the only markers suitable for crossover analysis. These markers are orange eye (*or*), Blade wings (*Bl*), Scute bristles (*Sc*) and purple eye (*pr*). Crossing over between this marked chromosome and five strains of ST and three of AR was analyzed. Three of the ST and two of the AR strains correspond to those used in the crossing over study recently reported (LEVINE and LEVINE 1954).

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The experimental procedure was to cross males of a given third chromosome strain to virgin *or Bl Sc pr* (ST)/lethal females. From each cross at least ten *or Bl Sc pr*/+ + + + females were recovered and mated singly to *or pr* males for the test generation. The parents were then permitted to remain and lay eggs in 25 × 95 mm shell vials containing a corn meal, molasses, agar, and yeast medium for three days whereupon they were transferred to a new vial. This transfer was carried out so that there were five three-day laying periods. Records were kept on each female for each laying period thus permitting an analysis of crossing over as it varied from female to female and variation in crossing over between laying periods. All cultures were maintained at 25°C.

RESULTS

The results illustrating the amount of crossing over for each of the ST third chromosome strains studied are given in table 1. Recorded in the table are the total number of flies counted in each strain for the second through the fifth laying periods. The first laying period has not been included since few and occasionally no eggs were laid. The total number of flies fall into the classes of non-crossovers; single crossovers between *or* and *Bl* (region 1), *Bl* and *Sc* (region 2), and *Sc* and *pr* (region 3); double crossovers in regions 1 and 2, regions 1 and 3, and regions 2 and 3; and triple crossovers in regions 1, 2, and 3. These same data but expressed in terms of the total percent of crossing over for each strain are shown in table 3.

Table 1 shows that the crossing over for the different ST strains is variable. Such variations in crossing over might be due to differential viability of the complementary classes of flies (e.g. + and *or Bl Sc pr*, *or* and *Bl Sc pr*, *or Bl* and *Sc pr*, etc.). In order to test for this possibility a series of homogeneity chi-square tests was carried out. First, a homogeneity chi-square showed no significant variation between females of any strain for a given laying period. Thus, for each strain it was possible to consider the data at a given laying period as the total from all females involved. To further ascertain whether differential viability might account for the observed variation in crossing over between strains a test was made of the homogeneity of the complementary genotypes of each class of flies given in table 1, through all laying periods of the five strains. The chi-square values for 19 degrees of freedom along with their respective P values are given under each class at the bottom of table 1. It is clear from these values that the proportion of the complementary genotypes for a given class is constant between strains. Thus, it is improbable that the differences in crossing over result solely from differential viability. The results obtained with the AR chromosomes are summarized in tables 2 and 3. Data for individual egg laying periods are not presented since so few crossovers occur between the AR and the *or Bl Sc pr* (ST) chromosome.

The nature and significance of the variation in crossing over between the strains can be best demonstrated by an analysis of variance. In this analysis the components of variance are the variations between strains, between the three chromosome regions, and between egg laying periods; and the interactions of strains and chromosomal regions, of strains and laying periods and of chromosomal regions and laying periods.

The analysis of variance of the five ST strains is given in table 4 while that of the

TABLE 1

Crossing over in the or Bl Sc pr *regions of the third chromosome for ST gene arrangements*

Period	0		1		2		3		1-2		1-3		2-3		1-2-3		Total
	+	*or Bl Sc pr*	*or*	*Bl Sc pr*	*or Bl*	*Sc pr*	*or Bl Sc*	*pr*	*or Sc pr*	*Bl*	*or pr*	*Bl Sc*	*or Bl pr*	*Sc*	*or Sc*	*Bl pr*	
ST-1																	
II	276	160	34	35	88	61	109	96	1	1	3	17	12	14	0	0	907
III	230	191	23	28	77	66	100	79	1	1	14	17	7	21	0	0	855
IV	208	116	25	22	70	48	98	80	2	2	10	14	20	24	0	0	739
V	283	155	35	33	103	79	137	107	1	2	15	21	12	29	0	0	1012
Total	997	622	117	118	338	254	444	362	5	6	42	69	51	88	0	0	3513
ST-2																	
II	267	184	17	33	61	46	79	86	1	1	5	10	11	8	0	1	810
III	261	211	27	40	83	76	98	103	0	2	5	8	9	3	0	1	927
IV	274	189	28	28	81	77	103	115	2	0	7	7	13	9	1	0	934
V	358	248	42	35	97	77	137	139	0	0	6	9	15	18	0	1	1182
Total	1160	832	114	136	322	276	417	443	3	3	23	34	48	38	1	3	3853
ST-3																	
II	201	144	27	21	80	41	96	101	1	0	5	10	12	8	0	0	747
III	197	116	20	21	73	59	90	100	1	0	4	3	8	12	0	0	704
IV	252	165	33	24	87	66	117	100	0	1	12	6	14	12	1	0	890
V	238	158	31	33	112	67	120	126	0	0	7	12	19	21	1	0	945
Total	888	583	111	99	352	233	423	427	2	1	28	31	53	53	2	0	3286
ST-4																	
II	208	163	26	26	64	36	60	63	0	1	5	4	8	10	0	0	674
III	313	232	43	35	78	66	110	99	0	1	7	9	5	10	0	0	1008
IV	275	166	27	25	57	62	91	82	0	2	6	7	8	10	1	0	819
V	235	144	32	31	61	68	94	73	1	0	9	6	7	13	0	0	774
Total	1031	705	128	117	260	232	355	317	1	4	27	26	28	43	1	0	3275
ST-5																	
II	169	122	21	15	54	49	77	79	0	0	3	6	6	15	0	0	616
III	249	148	38	22	86	71	101	113	0	2	6	13	11	17	0	0	877
IV	202	138	27	15	71	62	102	96	1	0	7	9	5	4	1	0	740
V	164	102	20	15	38	36	58	65	0	0	7	9	8	10	0	0	532
Total	784	510	106	67	249	218	338	353	1	2	23	37	30	46	1	0	2765
χ^2	24.49		20.60		22.63		16.24				12.29		26.00				
P	0.17		0.35		0.25		0.65				0.85		0.16				

TABLE 2

Crossing over in the or Bl Sc pr *regions of the third chromosome for AR gene arrangements*

Period	0		1		2		3		1-2		1-3		2-3		1-2-3		Total
	+	*or Bl Sc pr*	*or*	*Bl Sc pr*	*or Bl*	*Sc pr*	*or Bl Sc*	*pr*	*or Sc pr*	*Bl*	*or pr*	*Bl Sc*	*or Bl pr*	*Sc*	*or Sc*	*Bl pr*	
AR-1 II–V	1426	980	30	24	7	6	0	13	0	0	0	0	0	0	0	0	2486
AR-2 II–V	1839	1596	17	12	4	3	0	8	0	0	0	0	1	0	0	0	3480
AR-3 II–V	2136	1870	54	70	20	16	0	10	1	0	0	0	2	0	0	0	4179

TABLE 3

Percent crossing over by regions

	ST-1	ST-2	ST-3	ST-4	ST-5	Mean	AR-1	AR-2	AR-3	Mean
or-Bl	10.2	8.2	8.3	9.3	8.6	8.9	2.17	0.83	2.99	2.00
Bl-Sc	21.1	18.0	21.2	17.4	19.8	19.5	0.52	0.23	0.93	0.56
Sc-pr	30.1	26.1	30.9	24.3	30.0	28.3	0.52	0.26	0.29	0.36

TABLE 4

Analysis of variance of crossing over in ST chromosomes

	ss	*df*	*ms*	*F*	*P*
Strains (S)	18,474.10	4	4,618.525	17.52	<0.001
Regions (R)	259,326.43	2	129,663.215	491.76	<0.001
Periods (P)	11,624.33	3	3,874.777	14.70	<0.001
S × R	5,206.40	8	650.800	2.47	0.01–0.05
S × P	25,380.17	12	2,115.014	8.02	<0.001
R × P	2,454.37	6	409.062	1.55	>0.05
Remainder	6,328.13	24	263.672	—	—
Total	328,793.93	59			

TABLE 5

Analysis of variance of crossing over in AR chromosomes

	ss	*df*	*ms*	*F*	*P*
Strains (S)	757.56	2	378.780	40.01	<0.001
Regions (R)	1,468.23	2	734.115	77.54	<0.001
Periods (P)	216.11	3	72.037	7.61	0.007
S × R	623.27	4	155.818	16.46	<0.001
S × P	64.22	6	10.703	1.13	>0.10
R × P	173.55	6	28.925	3.06	0.05
Remainder	113.62	12	9.468	—	—
Total	3,416.56	35			

AR strains is shown in table 5. Considering the ST strains first it is seen that the main effects (those of strains, regions, and periods) are significant. Thus, as shown in table 3, the five strains produce significantly different amounts of crossing over in the *or Bl Sc pr* region of the third chromosome. In addition the total amount of crossing over is significantly different between the four laying periods. Each strain, therefore, has its characteristic pattern from period to period. The significant P value for regions is to be expected since the four loci under consideration are widely separated along the length of the chromosome.

The interactions are of particular interest in that they show that differences in crossing over need not arise merely in the total amount of crossing over between the five strains. The interaction of strains and regions is significant. Thus, the strains may not respond similarly in their total percent crossing over for these specific third chromosome regions.

The second interaction, that of strains and periods, is highly significant and indicates that the strains respond differently during the four laying periods. The interaction of regions and periods is not significant; therefore the regions as a whole regardless of the strain have the same mean percent crossing over at each laying period.

The analysis of variance for the three AR strains as presented in table 5 is substantially the same as the ST analysis. The variations between strains, regions, and periods all contribute significantly to the variance as does the interaction of strains and regions. However, the interaction of strains and periods is not significant and the regions by periods interaction is just significant at the 5 percent level. As was the case with ST chromosomes, different AR chromosomes behave differently with respect to the amount of crossing over per region. However, the strains do not behave differently with respect to the several laying periods. There is variation in the regions with the laying period which was not present in the ST strains. Unfortunately, the very small number of crossovers produced in the AR/*or Bl Sc pr* (ST) heterozygotes makes interpretation of the interactions difficult. In general it may be said that the three strains differ from each other markedly in their amount of crossing over and that there is a significant effect of the laying period on crossing over.

DISCUSSION

Crossing over between an *or Bl Sc pr* (ST) third chromosome and five strains of ST chromosomes of natural origin results in significant variations. The analysis of variance shows that the strains differ in their total percent of crossing over. Secondly, differences in crossing over are exhibited by the strains for the *or-Bl*, *Bl-Sc*, and *Sc-pr* regions of the chromosome. Thus it would appear that each third chromosome strain determines to some extent its percent of crossing over. In addition, a significant difference arises between the five egg laying periods and in the interaction between strains and egg laying periods. Accordingly, it may be assumed that the age of the female has an effect on crossing over. Though BRIDGES (1915) reports an effect of age on crossing over in *D. melanogaster* it is of a distinctly different character from that observed here for *D. pseudoobscura*. BRIDGES' data showed that third chromosome crossing over underwent a marked drop in the first nine days of the female's egg laying followed by a gradual rise to a level never exceeding the initial peak.

The most distinct changes arose between loci just on either side of the centromere. In the case of *D. pseudoobscura* no such pattern appears among the five ST strains. This may result from a fundamental difference between the two species or possibly from the fact that the centromere region only is sensitive to age. If the latter is true it must be pointed out that the third chromosome of *D. pseudoobscura* has an almost terminal centromere, similar in this respect to the X chromosome of *D. melanogaster*. The effect of age on crossing over in general is not clear and it is quite possible that the age of the female may have little to do with crossover variations but that variation is due to differences in culture and other external conditions. Further study of the change in crossover percent in succeeding egg laying periods is clearly indicated. However, for purposes of the present study it is sufficient to state that there are significant variations in crossing over as shown by the interaction of strains and egg laying periods and suggest a dependence of crossing over upon the different strains themselves.

The results obtained with the three AR strains agree in general with the ST strains. The major departures arise among the interactions, but as has been pointed out the relatively low number of crossovers makes interpretations difficult.

The major assumption underlying this and our previous study of the genetic control of crossing over is that the third chromosome strains analyzed are genetically diverse. Though it is impossible to demonstrate conclusively, at least by ordinary genetic tests, that different wild type chromosomes have varied genetic constitutions there is indirect evidence from a number of sources. WALLACE, KING, MADDEN, KAUFMANN, and MCGUNNIGLE (1953) have shown in *D. melanogaster* that five wild type third chromosomes isolated from different flies and then permitted to recombine in 55 different ways produced significantly different phenotypes in terms of viability, fertility, and bristle number. Experiments with numerous heterozygous lethals in *D. melanogaster* (STERN, CARSON, KINST, NOVITSKI, and UPHOFF 1952) and *D. willistoni* (CORDEIRO 1952 and CORDEIRO and DOBZHANSKY 1954) have indicated that the lethal heterozygotes can have wide variations in viability. The *D. willistoni* studies in particular have shown that various lethal chromosomes in combination with a number of wild type chromosomes have different viabilities. Though only casual observations have been made, it has been noted that there are marked differences in the fertility, fecundity, and growth rate in the stocks of the *D. pseudoobscura* third chromosome strains as they are maintained in this laboratory.

Experiments on selection for quantitative traits in *D. melanogaster*, in which it has been demonstrated that wild type chromosomes possess polygenic systems controlling such phenotypes as bristle number (MATHER 1943) and body proportions (ROBERTSON and REEVE 1952), have shown that there is genetic variability between wild type chromosomes. It appears certain, therefore, that different but still supposedly wild type chromosomes produce measurably different phenotypes at least where quantitative traits are concerned. There seems to be no reason, therefore, why crossing over should not likewise be quantitatively different in different wild type chromosomes. Actual demonstration of genetic systems controlling crossing over awaits detailed experimentation on selection for rates of crossing over. The work of DETLEFSEN and ROBERTS (1921) is suggestive in this regard but more precise experiments are desirable.

In lieu of evidence to the contrary, the conclusion to be derived from the present study is that the variation in crossing over for the different ST and AR strains results most probably from differences in their genetic constitutions, and thus is in line with our previously reported hypothesis, that crossing over is at least partially under genetic control.

On the basis of this study we predict that chromosomes isolated from different natural populations of Drosophila are in general endowed with genetic factors which will result in differences in crossing over. An analysis of crossing over in individual chromosomes derived from four natural populations of *D. pseudoobscura* is underway in order to test the extent to which this prediction seems to be true.

SUMMARY

Five strains of the ST and three of the AR third chromosome arrangements of *D. pseudoobscura* obtained from different females caught in nature were analyzed for crossing over with an *or Bl Sc pr* (ST) chromosome. Analysis of the results with the five ST strains has shown the following:

1. The strains differ significantly in their total percent of crossing over.
2. There are significantly different amounts of crossing over in successive egg laying periods and, in addition, each strain has its own pattern of crossing over from period to period.
3. Significant differences in crossing over are exhibited by the strains within the *or-Bl*, *Bl-Sc*, and *Sc-pr* regions of the chromosome.

The results obtained with the three AR strains agree in general with those found for the ST strains.

It is concluded that the variation in crossing over for different ST and AR strains results, in part, from differences in their genetic constitutions. The results are consistent with an hypothesis which suggests that crossing over is at least partially under genetic control.

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