CROSSING OVER IN THE SEX CHROMOSOME OF RACIAL HYBRIDS OF DROSOPHILA PSEUDOOBSCURA

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INTRODUCTION

LANCEFIELD (1929) studied crossing over in the sex chromosome of racial hybrids of *Drosophila pseudoobscura*. He found that in F_1 females crossing over in the central region, between yellow and bubble, was approximately the same as in race A, while distally there was a strong reduction in both limbs. In females resulting from repeated backcrosses to race B but hybrid for the sex chromosomes, that is, having one X of race A and the other of race B, he found no crossing over between yellow and vermilion, an interval which in the F_1 female gave about ten percent crossing over. The low values of crossing over in the more distal portions of both limbs in the F_1 hybrid female were attributed to the presence of an inversion in each limb. For the subsequent reduction of crossing over in the central region after repeated backcrossing there was no apparent explanation. Professor TH. DOBZHANSKY has recently observed a similar phenomenon in the autosomes, and has kindly permitted me to publish certain data which he has obtained (tables 3 and 4). At Professor LANCEFIELD'S suggestion I undertook in November, 1934, a study of this anomaly as observed in the sex chromosome.

TAN (1935) studied the salivary chromosomes of *Drosophila pseudoobscura* and described their pairing in the racial hybrid. My observations are in complete accord with his: 1) there is an inversion in each of two autosomes, and an inversion in each limb of the X; 2) there is a strong tendency towards asynapsis in certain regions of the left limb of the X. These regions, however, have been shown by DOBZHANSKY and TAN (1936) to contain no chromosomal rearrangements. The four inversions by which the Columbia *P v* (A) stock and *sg bu* (B) stock differ are similar in size and position to those described by TAN, and considering the origin of the stocks are presumably identical with them.

STURTEVANT (1919) found that females heterozygous for the Nova Scotia second chromosome, which has an inversion in each arm, gave only 1 or 2 percent of recombination between purple and curved unless a factor suppressing crossing over in the third chromosome (C III-II) was present in heterozygous condition, in which case the purple-curved interval gave about 20 percent of recombination, which is about the standard amount. MORGAN, BRIDGES, and SCHULTZ (1930, 1932, 1933) and STEIN-

BERG (1936) have since published data showing an increase in crossing over in one chromosome produced by an inversion in another.

It seemed possible that crossing over in the central region of the X in the F_1 hybrid females might be about as great as normal only by virtue of the presence of heterozygous autosomal inversions. This suggestion has also been made independently by STURTEVANT and DOBZHANSKY (1936). Thus the observed decline in crossing over with repeated backcrossing might be due to the elimination of the heterozygous autosomal inversions, a hypothesis agreeing with the observation of KOLLER (1931) that, "As was found by LANCEFIELD, the crossing over values were in such repeated backcrosses, distinctly variable." If this were the case one would expect the F_2 females hybrid for the sex chromosomes to fall into four equally numerous classes with regard to the amount of recombination in the X which they gave and to the autosomal inversions for which they were heterozygous. Those with both inversions should give as much recombination in the X as did the F_1 females. Those with neither should give as little as females resulting from repeated backcrosses. Those with one or the other should give an intermediate amount. Experiments were made to determine whether or not this is the case. The results show that it is.

REDUCTION OF CROSSING OVER WITH REPEATED BACKCROSSING

Pointed vermilion females of race A were crossed to singed bubble males of race B. The genes are arranged in the following order: *P sg v bu*. Pointed is at the left end of the genetic map of the X; singed and vermilion are somewhat to the left of the middle, and bubble is somewhat to the right. In the F_1 female bubble gives about five percent of recombination with short, which is at the right end of the genetic chromosome (LANCEFIELD 1929).

The F_1 females were crossed (F_1 Backcross) to *sg bu* (B) males (see table 1, F_1 ♀ ♀, for offspring; see also fig. 1, F_1BC), and Pointed virgins were selected in the progeny. These virgins in turn were crossed (F_2 Backcross) to *sg bu* (B) males. Their constitution with respect to autosomal inversions was determined as described below, and their offspring were classified. The classification of *sg* in females was uncertain, as the character overlapped wild type. For this reason *sg* has been neglected in computations based on females. All cultures were kept at 17°–18°C.

The method of determining what autosomal inversions were carried by a given female was to make salivary preparations of a number (eight or more, or until both heterozygous inversions had been seen) of her offspring. If a female produced eight larvae thus determined as not heterozygous for a given inversion, then (assuming random selection and equal mortality both in the case of the F_2 females and in the case of their off-

TABLE 1

Offspring obtained from hybrid females of various classes. The values for each class are the total progeny of three or more females. The classification of females for sg is not indicated.

$$\frac{P \quad v \quad \text{(A)}}{sg \quad bu \text{ (B)}} \text{♀} \times sg\ bu \text{ (B)} \text{♂}$$

	INVERSION		*P v*	*sg bu*	*P sg bu*	*v*	*P bu*	*sg v*	*P v bu*	*sg*	*P sg v*	*bu*	*P sg*	*v bu*	*P*	*sg v bu*	*P sg v bu*	+	*n*	RECOMB. % *P-bu*	RECOMB. % *P-bu* ♂&♀
F_1♀♀	in II and III	♂	309	202	8	12	3	11	43	83			2	1	1	1			676	23.7	24.4
		♀					145					383			572			171	1271	24.9	
F_3♀♀	none	♂	100	198					4	4									306	2.6	4.4
		♀					18					266			331			15	630	5.2	
F_4♀♀	none	♂	127	117			1			7									252	3.2	3.6
		♀					5					125			135			6	271	4.1	
F_2♀♀	none	♂	48	63					2	1									114	2.6	2.6
		♀					1					93			95			4	193	2.6	
F_2♀♀	in II	♂	85	63	4	1		3	8	15							1		180	17.8	17.4
		♀					19					103			104			24	250	17.2	
F_2♀♀	in III	♂	147	144			1	2	17	22	1	4							338	12.4	11.3
		♀					24					218			251			31	524	10.5	
F_2♀♀	in II and III	♂	57	42	2	1	2		16	19			1	1	1			1	143	28.7	32.1
		♀					33					71			68			40	212	34.4	
F_5♀♀	in II and III	♂	84	66	1	5	1	7	16	19			1	1	2	1			204	24.0	23.6
		♀					34					105			132			38	309	23.3	
F_3♀♀	in III	♂	70	148				1	13	5									237	8.0	8.2
		♀					15					151			182			15	363	8.3	

spring) the probability is 1/257 that she was heterozygous for the inversion. I am indebted to Dr. D. R. CHARLES for pointing out the applicability of Bayes' Theorem to this case. This cytological method is quite laborious, and if genetic markers had been available I would have used them. However, I knew of no data on the frequency of recombination between autosomal genes and the inversions. The small number of offspring which can be obtained from one female and the poor viability are additional experimental difficulties.

From the progeny of F_2 females shown to be heterozygous for all the sex-linked mutants used, and hence probably hybrid for the sex chromosomes, there were selected a number of Pointed virgins. These were found by subsequent test to be heterozygous for neither autosomal inversion but for all the sex-linked mutants, and were crossed (F_3BC) to *sg bu* (B) males (table 1 and fig. 1).

From the progeny of an F_3 female shown to be heterozygous for the sex-linked mutants but for neither autosomal inversion Pointed virgins were selected; these were crossed (F_4BC) to *sg bu* (B) males. Their progenies (table 1 and fig. 1) showed them to have been heterozygous for the sex-linked factors used.

Table 1 and figure 1 show that F_3 and F_4 females give much lower recombination in the X than do F_1 females, even though the sex chromosomes are the same in all of them, the only difference being in the autosomes.

RECOMBINATION VALUES GIVEN BY F_2 FEMALES

In figure 1 recombination percentages between *P* and *bu* are shown for 15 F_2 females, some with neither autosomal inversion, some with one or the other, and some with both. These data are summarized in table 1. It is apparent that in the three F_2 females heterozygous for neither inversion recombination is about the same as in F_3 and F_4 females. In the F_2 females heterozygous for one or the other autosomal inversion recombination is higher than in females heterozygous for neither, but lower than in F_1 females. So far the results are in very close agreement with the hypothesis. But the three F_2 females heterozygous for both inversions gave recombination values not only as high as did the F_1 females, but considerably and consistently (three cases out of three) higher. I know of no basis on which this could have been predicted. Possibly further data might show no such tendency. Taken as a whole the results of this experiment strongly support the hypothesis.

If the presence or absence of heterozygous autosomal inversions is the principal factor affecting crossing over in the central region of the X, then females of any backcross generation should have about the same amount

of crossing over as F_2 females of the same constitution with respect to autosomal inversions. Total counts of the progeny of five F_3 females heterozygous for the inversion in chromosome III are given in table 1 (F_3 ♀ ♀, inversion in III), the percentages of recombination between *P* and *bu* in the individual progenies being 5.6, 11.0, 8.1, 12.9, 2.9. While it is evident that these values are, on the whole, lower than those given by F_2 females heterozygous for the same autosomal inversion, they come a little

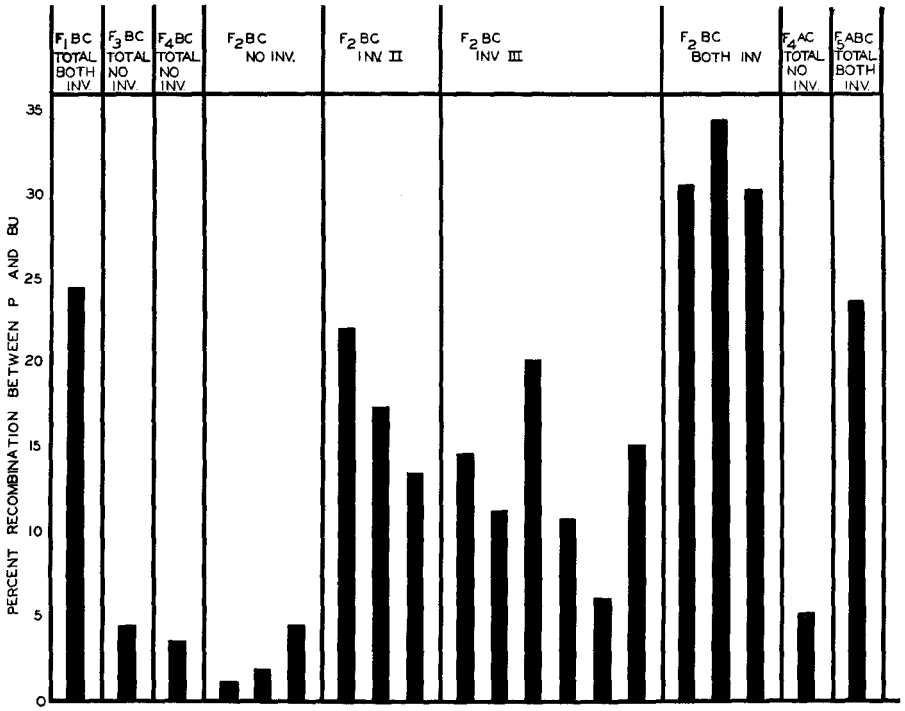

FIGURE 1.—Percentages of recombination between Pointed and bubble obtained by backcrossing various kinds of females. All these females probably had the same sex chromosome constitution, namely *P v* (A)/*sg bu* (B), but differed as to the generation to which they belonged and as to the autosomal inversions for which they were heterozygous. The percentages are computed from male and female offspring combined, except in the one designated as F_4AC, which is based on males only. In the F_2 backcross each column represents the progeny of a single female; in all other cases the value given is based on the progenies of several females.

nearer to them than to the values given by F_3 females heterozygous for no autosomal inversions. I do not think any conclusions can be drawn from this result.

An alternative explanation of the progressive decrease in crossing over in the central region of the X in successive backcrosses might be that zygotes carrying an X which had undergone crossing over were less viable when their autosomal material was predominantly of one race than when

it was derived from the two races in about equal proportions. But this is not in accord with the results obtained by LANCEFIELD (1929), who found that flies with part of an X from one race and the rest from the other had good viability, even when the probability was high that the autosomes were mostly of one race. This explanation is also discordant with the fact that F_4 females crossed to race A gave the same recombination value as F_4 females crossed to race B. These females were the progeny of repeated backcrosses to race B. They were hybrid for the X chromosomes and

TABLE 2

$$F_4 \frac{P \quad v \quad (A)}{sg \; bu \; (B)} \times P\, v\, (A)$$

Male offspring only.

P v	*sg bu*	*P sg bu*	*v*	*P v bu*	*sg*	*P*	*n*	RECOMB. % *P—bu*
187	206	1	1	8	11	1	415	5.1

homozygous for the race B order of the inverted sections in chromosomes II and III, hence probably for B material throughout these chromosomes; race A material in chromosomes IV and V had probably been eliminated as well. The progeny obtained by crossing these females to B males would be still less likely to have race A autosomal material, while the progeny of the cross to race A would have about equal amounts of autosomal material from each race. If the relative proportions of the autosomal material markedly affected the viability of zygotes with X chromosomes which had un-

TABLE 3

Data of Th. Dobzhansky on recombination in chromosome III. The change in successive generations is what would have been expected on the basis of the theory put forward in the text.

$$\frac{pr \; Sc \quad (A)}{or \; (B)} \text{♀} \times or \; (B) \text{♂}.$$

	or		*Sc*		*or Sc*		+			RECOMB. %
	♀	♂	♀	♂	♀	♂	♀	♂	*n*	*Sc-or*
F_1	62	32	31	9	13	2	26	13	188	28.7
F_2	275	111	220	82	16	7	36	11	758	9.2
F_3	382	345	372	255	6	4	18	23	1405	3.7
F_4	433	384	453	357	7	5	26	29	1694	4.0

dergone crossing over, these two crosses should give markedly different recombination values. But such is not the case (see table 1, F_4 ♀ ♀, no inversions, table 2, and fig. 1, F_4BC and F_4AC).

The female progeny of the cross to race A would all be heterozygous for the two autosomal inversions, and some for the sex chromosomes as well. These latter should, if the hypothesis is correct, give the same value of

recombination as the F_1 females, as in fact they do (see table 1, F_5 ♀ ♀, inversions in II and III, and fig. 1, F_5 ABC).

The data presented in this paper show that the observed variations in the frequency of crossing over between the X chromosomes of the two races are closely correlated with the presence or absence of heterozygous autosomal inversions, which seem, therefore, to be the principal causative factors.

TABLE 4

Data of Th. Dobzhansky on recombination in chromosome IV, which has the same seriation of bands in both races. An increase, rather than a decrease, is observed in successive generations.

$$\frac{tg\ j\ in\ (A)}{(B)}\ ♀ \times tg\ j\ in\ (A)\ ♂$$

	+		*tg j in*		*j in*		*tg*		*tg j*		*in*		*j*		*tg in*		*n*	% RECOMB. *tg-j*	% RECOMB. *j-in*
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂			
F_1	316	95	18	5	24	4	166	39	22	9	18	2	22	7	11	2	760	36.2	12.2
F_2	125	76	149	106	97	73	105	54	30	13	21	23	10	3	8	6	899	39.6	12.7
F_3	219	143	148	112	128	67	179	98	35	25	104	56	29	24	46	36	1449	41.9	24.5

In conclusion I wish to express my indebtedness to Professor D. E. LANCEFIELD, who suggested this problem, and under whose direction it was investigated.

SUMMARY

LANCEFIELD (1929) found that, in the F_1 hybrid female of race A × race B, recombination is strongly reduced in the more distal portion of each limb of the sex chromosome, while in the central region it is about the same as in race A. He correctly inferred the presence of an inverted section in each limb.

He further observed that, in females heterozygous for the X's and resulting from repeated backcrosses to either race, there is far less recombination in the central region of the X than in F_1 females.

It is suggested that the high value in the F_1 female is due to the presence, in heterozygous condition, of the two autosomal inversions by which the stocks of the two races differ. Such an effect would be expected, since it is known (MORGAN, BRIDGES and SCHULTZ, 1930) that crossing over in one chromosome is increased by the presence of a heterozygous inversion in another. Backcrossing would make these inversions homozygous, and crossing over in the X would decline correspondingly.

The evidence from breeding experiments and salivary chromosome study shows that F_2 females heterozygous for the sex chromosomes but for neither autosomal inversion give as little crossing over in the X as females resulting from repeated backcrosses; that those with one or the other autosomal inversion give recombination values intermediate between this and the value in the F_1 female; that those with both autosomal inversions give

values which are actually somewhat higher than the value in the F_1 female (fig. 1). Thus in this experiment (and in others which are described in the text) variations in crossover frequency in the X are closely correlated with the presence or absence of heterozygous autosomal inversions. It is inferred that these inversions are the principal factor responsible for the variations.

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