THE PROBLEM OF SYNTHETIC LETHALS IN DROSOPHILA MELANOGASTER

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THE "classical" concept of the lethal is one of a genetic condition present at a specific locus which in homozygous or hemizygous state causes death before completion of development. In contrast to this, DOBZHANSKY in 1946 described "synthetic" lethal chromosomes which he discovered in *Drosophila pseudoobscura* while studying variation presumably due to recombination between different second chromosomes. If lethal chromosomes can originate not only in consequence of mutational events at specific loci but also by synthesis through crossing over of new chromosomes, the question arises: how frequent are classical and synthetic lethals relative to each other? Data bearing on this question have been provided by several authors.

DOBZHANSKY's synthetic lethals arose from crosses of flies one of which carried a second chromosome that was normal in viability when homozygous and the other carried a second chromosome which in the homozygous condition was lethal at 25.5°C but viable at 16.5°C. Three of the resultant chromosomes were lethal to homozygotes at 16.5° as well as at 25.5°. These new chromosomes were interpreted by DOBZHANSKY as being recombinants of the two original chromosomes. Crossing over, it was assumed, had brought together complementary loci or regions which separately, permitted survival at 16.5°, but which together, in homozygous state, produced lethality.

In 1949 MISRO published results of experiments to test for lethality due to recombination between pairs of normal chromosomes in *D. melanogaster*. Flies from wild populations were used, females heterozygous for two different wild type second chromosomes serving as the source of recombinants. The controls with recombination either absent or irrelevant were males heterozygous for the same two chromosomes and females homozygous for each wild type chromosome. MISRO found that lethals appeared about four times as frequently in heterozygous females as in homozygous females. This statistically significant difference was attributed to recombinational lethals.

WALLACE, KING, MADDEN, KAUFMANN and MCGUNNIGLE, (1953) testing variability arising through recombination of second chromosomes in *D. melanogaster*, found that lethals arose with higher frequency in the females heterozygous for two different wild type second chromosomes than in the controls which were homozygous for each of the chromosomes. This difference in the lethal frequency was again explained as being due to recombinational lethals. Twenty-eight combinations of the 45 heterozygotes produced lethals. The total frequency of lethals in all 45 was 2.96%. The control frequency was 1.20% in the nine homozygous classes tested.

According to WALLACE *et al.*, "selection favors particular heterozygotes and thereby

builds up selected series of alleles at all (or at least many) loci—the particular alleles at any locus being determined in each population by those present at all other loci." It is this heterozygosity that accounts for variability when crosses are made between different chromosomes and may in extreme cases form lethal chromosomes. This is contrasted with the theory that selection always favors a particular allele at a particular locus, thus selecting for homozygosity in a population.

The following report deals with tests for synthetic lethals in the X and third chromosomes of *Drosophila melanogaster.*

X CHROMOSOME LETHALS

In 1955 it was reported (HILDRETH) that tests for synthetic lethals in the X chromosome of *D. melanogaster* produced results in contrast to those of the above-mentioned authors. Ten different wild type laboratory stocks were used for the experiment with the ten homozygous classes serving as controls for the 45 possible heterozygous classes. One lethal was found in the 994 control chromosomes tested and 13 were found in the 5186 chromosomes from the recombination line. Two of the 13 lethals were allelic and arose in one culture, indicating an earlier nonrecombinational origin of these two lethals. The difference between the lethal frequencies of the control and experimental groups was not significant, indicating that recombination had a small role, if any, in the increase of the lethal rate in the experimental group. When the 1955 report was published, complete localization tests on the lethals had not been carried out; therefore the results of the localizations are presented here.

Even though the results did not require the assumption that a substantial fraction of the lethals arose by recombination, there remains the possibility that some of the lethals may be recombinational ones. This can be tested by genetic localization of the lethals. If a lethal is at a specific locus on a chromosome, localization tests with different markers will give consistent results. If, however, the lethal is due not to one particular locus or gene but to the interaction of two or more specific alleles of different genes, then nonlethality will be restored to the chromosome by any crossing over that removes any of these alleles from the lethal combination. This will confuse the genetic localization analysis since separate tests with different markers may place the lethal locus at a different region in each test. In a test of a female containing a single locus lethal, theoretically 50% of the chromosomes carry the lethal. With a recombinational lethal the nonlethal chromosomes will be more frequent owing to crossing over between the different loci that interact to form the lethals. Of course, the closer the loci are, the more difficult it will be to tell such a lethal from one that arose at only one locus. However, if the loci are very close together then the frequency of the origin of such a lethal will be low.

All lethals of the X chromosome, with one exception, were tested, each with two different groups of markers and the localization tests showed no discrepancy that would necessitate an explanation of lethality due to interaction of a combination of loci. The exception was one lethal which was lethal in the tests but became semilethal in stock; i.e., males carrying this mutant occasionally survived. This lethal was not localized; since the survivors had no distinguishing phenotype. One other lethal (L # 3) at first gave strange results because of occasional survivors. However, further

TABLE 1

X chromosome lethals from heterozygous females as localized in individual tests with markers shown

Lethal no.	Class	Markers used in localizations				
		y cv v f	*y rb*	*y sn v*	*g sd B*	*f car*
1	Sal/Fres*					
2	Sal/Fres	65.6				65.7
3	Sal/Cor	48.0			49.6	52.2
5	Sal/Ore	67.4				63.4
9	Sam/Tem	50.2			50.7	
11A	Sam/Ore**	56.7			56.8	
B	Sam/Ore**	56.3				
12	Sam/Cant	49.6			50.2	
13A	Fla/Cor	0.0	0.0			
B	Fla/Cor	23.5		21.0		
14	Fla/Sac	56.7			56.5	
15	Sal/Fla	0.3	0.0			
16	Sal/Ore	61.2				62.5

* Semilethal, not tested.

** Allelic.

localization tests were made and the results were compatible with the theory that the lethal was at a single locus on the chromosome. The lethals and their loci as determined by separate tests are given in table 1. The lethals were kept in stock balanced with the "Binscy" (*y* sc^{s1} *B In49* sc^{s}) chromosome so there would be little chance of losing a lethal by crossing over especially if the lethals were recombinational lethals.

THIRD CHROMOSOME LETHALS

Because the tests with X chromosomes indicated that recombination played no role in the production of lethals, it was decided to study the same problem for the third chromosome.

Material and method

Eight different stocks were used for the third chromosome experiments. Three of these were stocks that had been kept for several years in the laboratory. These three were cultures from which X chromosomes had been isolated and tested and they were chosen for this reason. They include a stock from Formosa (For); one from Salta, Argentina (Sal); and an inbred stock, Samarkand (Sam). To minimize the possibility of selection for specific genotypes in the course of laboratory culturing five wild type stocks from nature were also chosen for the experiments. One of the freshly caught wild types (A) was trapped in Berkeley, California in October 1954. The second and third (B and C respectively) were trapped at different localities, separated by more than two miles, in Orinda, in October and November 1954. The fourth (E) was trapped at Riverside in December 1954, and the fifth (F) was trapped in September 1954 in Napa. A crossover suppressor chromosome was needed and for this the *DcxF* chromosome was chosen. This will be indicated by *D* in the crosses. The

experiments to determine the frequency of lethals produced in recombinational and nonrecombinational series were started in July 1954 and terminated in July 1955.

From each of the eight stocks one nonlethal third chromosome was isolated and all the chromosomes in the control and recombinational tests were descended from these. Salivary chromosome analyses did not reveal any detectable chromosomal aberrations. It is important that there be no obvious aberrations, since crossing over should be normal. Each chromosome was isolated by mating a wild type male to virgin *D/Sb* females. One *D/+* male from the F_1 generation was mated with virgin *D/Sb* females.

Among the offspring of this mating would be males and females of the constitution *D/+* whose wild type third chromosomes were all derived from a single sperm in the P_1 male. Matings of these *D/+* males and females would produce wild type (+/+) offspring if the original + chromosome was not lethal. From cultures in which +/+ flies appeared stocks were obtained in which the wild type chromosomes were kept balanced with the *DcxF* chromosome. Twelve generations elapsed between trapping of the fly bearing the first of the third chromosomes tested (A) and the mating of the last heterozygote (A/F) bearing this initial chromosome. Three more generations elapsed between the mating of the fourth and fifth wild types, the last in the series.

The experimental procedure for determination of lethals is outlined below. Since the experiments were carried out over a period of weeks it was necessary to check for spontaneous lethals that might arise in the stocks and needlessly complicate the results. The outline below assumes that each chromosome of cross G1 was not carrying a lethal. The experiment was also set up so that heterozygous males (G2), serving as noncrossover controls, would be brothers of the heterozygous females thus making the genotypes of the two as nearly identical as possible. Throughout the crosses, except in the heterozygous wild type females, recombination between the third chromosomes of females was minimized by the crossover suppressor *DcxF* chromosome. The outline of the experimental procedure uses the Formosa (For) and Salta (Sal) chromosomes as an example.

G1:	1 *D*/Sal ♀	×	1 *D*/For ♂
	Experimental Series of Crosses		Control Series of Crosses
G2:	4(1 Sal/For ♀ × 2 *D/Sb* ♂♂)		4(2 *D/Sb* ♀ ♀ × 1 Sal/For ♂)
G3:	100(2 *D/Sb* ♀ ♀ × 1 *D*/+ ♂)		100(2 *D/Sb* ♀ ♀ × 1 D/+ ♂)
G4:	100(2 *D*/+ ♀ ♀ × 2 *D*/+ ♂♂)		100(2 *D*/+ ♀ ♀ × 2 *D*/+ ♂♂)
G5:	*D*/+ and +/+		*D*/+ and +/+

In the experimental series some of the +/+ flies will be homozygous for recombinant chromosomes while in the control series the individuals will carry only noncrossover chromosomes. It is the recombinants which may account for the production of possible synthetic lethals.

All experiments were carried out at 26°. The G5 cultures were determined to be lethal or nonlethal containing 15 days after the cultures were started. Under ideal conditions one would expect approximately two thirds of the offspring to be Dichaete and one third normal wild type since Dichaete is lethal in homozygous condition. In the tests for lethality a minimum number of *D*/+ flies per culture with no +/+ flies present had to be set as a standard. If there were at least the minimum number and

no +/+ flies, the culture was to be scored as a lethal. If there was not the minimum number and there were no +/+ flies the culture was scored as doubtful. The presence of one or more +/+ flies, no matter what the total number of flies in the culture, would mean the culture was to be scored as nonlethal bearing. The minimum number per culture was set at 20 Dichaete flies and none wild type. Of the 20 flies, all could be offspring or one to four could be parents since the parents were not removed from the cultures. A χ^2 test, using the maximum (20) and the minimum (16) when one third of the flies are expected to be wild type (13.7 Dichaete to 6.3 wild type and 10.7 Dichaete to 5.3 wild type, respectively) indicates that such results would be found by chance less than once in 100 times (χ^2 = 9.19 and 7.99 respectively). If there were at least 20 flies present and none was wild type, the culture was assumed to be carrying a recessive lethal in the + chromosome. Three new creamers were then started from this culture, each with three pairs of *D*/+ flies. Fifteen days later these cultures were examined for the presence of wild type flies. If any were present (one or more) in any creamer the original culture was declared not lethal. If there were no wild type flies in any creamer and there were more than 50 flies in at least one creamer, then the culture was scored as lethal. If there were less than 50 flies the culture would be scored as doubtful and three creamers would be started again. However, in these experiments no cultures had to be tested a third time. The minimum of 50 *D*/+ flies with no +/+ flies present was chosen to eliminate as much as possible the scoring of semilethals as lethals. The probability of finding a culture with 50 *D*/+ and no +/+ flies when the expected ratio is 33.7 to 16.3 would be much less than one in 100 with a χ^2 value of 24.18. (Six of the 50 flies may be parent flies. In this case one would expect 29.3 Dichaete to 14.7 wild type flies. The probability of obtaining such a culture by chance alone would still be much less than once in 100 times.) When lethals were found in the second check, cultures were started keeping the lethals balanced with the *DcxF* chromosome.

If in the first check a culture had less than 20 flies and none was wild type the culture was labelled "doubtful" and either new matings of the *D*/+ sibs were made or the original parents were transferred to a fresh creamer. These were again examined 15 days later. Only six "doubtful" cultures occurred, and all were found to belong to the nonlethal class on the second check.

Only five cultures were sterile. In each case their sterility was due to early death of the G4 flies.

Lethal frequency

The results of the tests for lethal frequency are presented in table 2. In the table three groups (For/A, A/F, E/F) marked with daggers produced several lethals (8, 8, and 9, respectively). The lethals within each group were found to be allelic by crossing together and their occurrence can best be explained as due to descent from a single nonrecombinational lethal arising in an earlier generation.

The total number of lethals found was 15 out of 2771 chromosomes tested in the recombinational line, and 18 out of 2693 chromosomes in the nonrecombinational control line. From these raw data there is no reason to believe that recombination plays any role in producing lethals since the frequency is higher in the control line.

TABLE 2

Frequency of lethals

Classes indicate combinations of wild type third chromosomes. Numbers in columns indicate chromosomes tested. Superscripts indicate numbers of lethals found among chromosomes tested. Where superscript is absent no lethals appeared. Fractions show total lethals over total chromosomes tested. Roman numerals indicate G2 females or males. Within any class each row represents a separate experiment which lists the G2 flies derived from a single pair of G1 individuals. (Those marked * are probably premeiotic clusters arising in the G2 germ lines; † probably prerecombinational lethals and not included in totals.)

Class	Females							Males					
	I	II	III	IV	V	VI	Total	I	II	III	IV	V	Total
For/Sal	21	9	16	14	14^{1}	21		21	22	32	22		
	15	9	12	33				26	18	15			
	13^{1}	17	11	17			2/222	16^{4*}	18	22			4/212
Sam/For	10	13	25	17	29			39	21	25			
	31	30	25					19	31^{1}	28	19		
	11	17	22	13	14		0/257	13	10	15	13	16	1/249
Sam/Sal	14	19	11	31	6	12		24	15	27	20	12	
	29	22^{1}	27	23			1/194	15	32	22	16	24	0/207
Sal/A	29	20	10	15	10			18	14	19	10	23	
	21	21	24	33			0/183	28	21	14	37		0/184
For/A	19^{1}	28	13	22	23			30	23	28	14		
	31	34	18^{8}†	19			1/189	20	29	20	27		0/191
A/B	30	23	21	32				29	16	34	18	9	
	17	32	30	27			0/212	26	18	43^{1}	22		1/215
A/C	36^{1}	39	30^{4*}					25^{1}	16	18	28	11	
	23	35^{1}	20^{1}	32^{1}			8/215	31	15	24	30^{1}		2/198
B/C	13	20	21	14	14			16	22^{1}	19	20		
	20	18	16	15	22		0/173	15	21	21	24	23	1/181
A/E	13	12	20	29	22			28	25	9	20		
	25	26	34				0/181	19	30	15	24^{1}		1/170
B/E	23	21	28	21				20	24	20	16^{5*}		
	12	22	14	31^{1}	10		1/182	22	21^{2*}	27	14		7/164
C/E	20	24	36	20				22	28	31			
	35	22	31				0/188	37	25	35			0/178
A/F	19	13	21^{1}	16	23			21	25	18	19^{8}†		
	25	27	21	21			1/186	21	31	18			0/134

TABLE 2.—*Cont.*

Class	Females							Males					
	I	II	III	IV	V	VI	Total	I	II	III	IV	V	Total
B/F	10	15	18					24	8	18			
	19	21	8	14			0/105	19	17	16	16		0/118
C/F	13	8	23	26				29	24	15			
	14	37	19	9			0/149	20	20[1]	23	28	*	1/159
E/F	17	11	15	27	20			16	15[9]†	19	23	14	
	10	12	5	5[1]	5	8	1/135	12	9	17	11	12	0/133
Total Lethals							15/2771						18/2693

However, it is necessary to consider a source of error other than the presence of prerecombinational lethals in the heterozygous wild type experimental females or control males of G2. This is the possible occurrence of premeiotic lethals arising in the germ line of these G2 females and males. Such lethals would give rise to clusters of lethals, the number within each cluster being dependent upon the stage in development of the germ tract when the lethal arose and upon the sampling technique (SPENCER and STERN 1948; CASPARI and STERN 1948).

In table 2 the clusters of lethals are marked with an asterisk. All lethals within each cluster were tested by intercrosses and found to be allelic in each case, indicating that common origin of the lethals was possible. Since it will be shown below that all lethals formed must be regarded as having arisen by mutation, not by recombination, the allelism within clusters means either a single common origin or that several mutations occurred at the same loci. The latter occurrence is extremely unlikely.

If each cluster is considered as one lethal the number of lethals in the recombination line will drop from 15 (.541%) to 12 (.433%) in 2771 while the control will decrease from 18 (.668%) to 10 (.371%) in 2693 chromosomes tested. It is not entirely accurate to correct in this way since some of the nonlethals have arisen from single gonial cells which then form nonlethal clusters, but the error has the same effect on experimental and control groups.

In the study of recombinational lethals we are ideally concerned only with those lethals which arise as a result of crossing over in meiosis. In addition, we must expect lethals which arise independently of crossing over and occur either as singles or in clusters. The experimental lethal frequency should then include the normal spontaneous lethals plus those which became lethal during meiosis as a result of recombination. Probably the best estimate of lethal frequency in our case would be to eliminate all clusters from the calculations despite the inaccuracies involved. If this is done the experimental values listed in table 2 will be lowered to 11 lethals in 2741 (.401%) and the controls to seven lethals in 2640 (.265%) chromosomes tested. These values are not significantly different (P value = .5 − .3) using a χ^2 test. Another method of treating these results would be to test for significance of difference between the number of mothers producing lethals in the experimentals and the number of

TABLE 3

Lethal frequency of experimentals (female) and controls (males) from tests on third chromosome. A = total lethals; B = each cluster of lethals counted as one lethal; C = all groups containing clusters of lethals discarded from totals. In the fractions the numerator is the number of lethals and the denominator the number of chromosomes tested. LW = laboratory wild type stocks and FW = fresh caught wild type. Figures in parentheses are percent lethal

	A		B		C	
	Females	Males	Females	Males	Females	Males
LW × LW	3/673 (.445)	5/668 (.748)	3/673 (.445)	2/668 (.299)	3/673 (.445)	1/652 (.153)
LW × FW	1/372 (.269)	0/375 (0.0)	1/372 (.269)	0/375 (0.0)	1/372 (.269)	0/375 (0.0)
FW × FW	11/1726 (.637)	13/1650 (.788)	8/1726 (.463)	8/1650 (.485)	7/1696 (.413)	6/1613 (.372)
Total	15/2771 (.541)	18/2693 (.668)	12/2771 (.433)	10/2693 (.371)	11/2741 (.401)	7/2640 (.265)

fathers producing lethals in the controls. In the experimental series 11 mothers out of 137 produced single lethals while in the controls 7 fathers out of 124 produced lethals. A χ^2 test of these data gives a P value of .5 — .3. If we used either of the other methods of including lethals, (table 3) 15 in the female line to 18 in the male line (A) or 12 in the female line to 10 in the male line (B), the results would be still less compatible with the hypothesis that lethals often are formed by recombination. Table 3 compares the number of lethals if (A) all lethals are included, or (B) a cluster is counted as one lethal, or (C) clusters are eliminated from the analysis. This table also compares the results with flies from laboratory stocks and flies freshly caught in nature. The samples are small but no evidence appears in favor of a higher frequency of lethal formation in the freshly caught wild types due to recombination.

Localization of lethals

The lack of statistical significance of the difference in frequency of lethals between the experimental and control tests for recombinational lethals in the third chromosome of *D. melanogaster* does not necessarily mean that none of the lethals in the experimental group could have been formed by crossing over. The possibility remains that some of these lethals derived from the heterozygous females could have been produced by recombination between the third chromosomes.

Genetic localization tests were conducted on the third chromosome lethals derived from the heterozygous females. Two separate tests were carried out for each lethal but one, using different markers in each test. One of the lethals became semilethal in the stock bottles and was not tested for its location on the chromosome. The lethals were kept balanced with the *DcxF* chromosome so that there was little opportunity for crossing over to restore normality to a lethal chromosome. The method of localizing the lethals is more complicated and more time- and space-consuming than the method used for X chromosome lethals. The method used in the third chromosome

tests is outlined below:

$$\text{G1: } 1\ \frac{D}{\text{lethal}}\ ♀ \qquad \times 2\ \frac{h\ st\ sr\ ca}{h\ st\ sr\ ca}\ ♂♂$$

$$\text{G2: } 4\left(1\ \frac{\text{lethal}}{h\ st\ sr\ ca}\ ♀ \qquad \times 5\ \frac{D}{\text{lethal}}\ ♂♂\right)$$

$$\text{G3: } \pm 100\left(2\ \frac{h\ st\ sr\ ca}{h\ st\ sr\ ca}\ ♀♀ \times 1\ \frac{\times\ -\text{over?}}{\text{lethal}}\ ♂\right)$$

It is in the germ cells of the heterozygous females in generation G2 that crossing over between the lethal-bearing and marked chromosomes will occur. The progeny of the G2 cross will be phenotypically Dichaete and wild type in the ratio of 2 to 1 respectively. All wild type males were taken from each bottle and mated singly in creamers with two virgin *h st sr ca* females. In any one creamer the offspring of the G3 cross will be 50% *h st sr ca*/lethal (wild type) and 50% will be the crossover or noncrossover chromosome over the marked chromosome. If a culture contained *h st sr ca* offspring in addition to wild type offspring, then the test chromosome was a noncrossover chromosome. If a culture contained all wild type offspring, the test chromosome was wild type due to crossing over. If any of the flies in a creamer were any specific combination of three or fewer of the recessives the test chromosome would be a specific cross over type and would be scored as such.

In this way all chromosomes were tested and scored. Each lethal behaved as a single-locus lethal and it was not necessary to assume that any had arisen due to crossing over between chromosomes. Table 4 lists the lethals and their loci as established by two separate tests for each lethal.

TABLE 4

Third chromosome lethals from heterozygous females as localized with markers shown

Lethal no.	Class	Markers used				
		h st sr ca	*ru h*	*jv se by*	*h st p^p ss*	*st p^p ss e^s*
1	Sal/For	46.8			±48*	
2	Sal/For**					
5	Sal/Sam	48.5			48.0	
6	For/A	51.7			±53*	
9	A/C	45.7			46.0	
12	A/C	47.0			48.0	
13	A/C	63.9				69.1
14	A/C	44.0			45.6	
17	B/E	10.8	10.8			
21	A/F	33.1		33.1		
23	E/F	44.0			44.0	

* Test not large enough to localize lethal more exactly. Crossing over occurred on one side of lethal only. Localizations compatible with one locus lethal theory.

** Semilethal, not tested.

DISCUSSION

The results of the work reported here on the X chromosome and the third chromosome in *D. melanogaster* are different from those of other investigators who studied the second chromosome of several other species. Some of the lethals studied in the second chromosome of *D. melanogaster* and in other species are interpreted by the authors as arising by recombination of alleles of different loci which by themselves are not lethal. Lethals evidently are not formed in this way in the X chromosome of *D. melanogaster*. At least neither frequencies nor localization tests gave any indication that lethals arose in this manner in those chromosomes which were tested.

It is possible that the third and X chromosomes behave intrinsically differently from the second chromosome with respect to recombinational lethals. There are, however, no obvious reasons for such differences. Particularly the third and second chromosomes, which are both autosomes and of similar length, would *a priori* not be expected to give opposite results. The X chromosome would be under more rigorous selection pressure than an autosome owing to its appearance in the hemizygous condition in the male, and it might seem that this could be a cause for difference between the second and X chromosomes. However, if it is true that selection favors heterozygosity then one might expect that even in the X chromosome heterozygosity might be favored. The work of KERR and KERR (1952) has shown that there are viability differences among different X chromosomes of *D. melanogaster* and that there are viability differences between the sexes when the same hemizygous X chromosome of the male is made homozygous in the female. This indicates that there is heterozygosity in the X chromosome and that there are different alleles of different loci involved, and perhaps in various combinations. It is, of course, possible that crossing over in the X chromosome does not produce lethals because of the more rigorous selection in hemizygotes permitting heterozygosity to give rise to viable X's only, since frequent occurrence of lethals in the X chromosome would be detrimental to the survival of the species.

Another possible reason for the difference between the X chromosome and second chromosome results could be that the X chromosomes used came from populations that had been in laboratory culture for at least three years when the experiments were started, as compared with the situation in the second chromosome where WALLACE selected 10 chromosomes from large experimental populations. Because each stock was kept in half-pint culture bottles, selection could have worked in such a way that all X chromosomes had become relatively similar to one another, in spite of the fact that they may have been greatly different when the flies were first trapped in various parts of the world. If this were true, then recombination might not produce lethals since the recombinant chromosomes might not be sufficiently different from the noncrossover chromosomes except for possible mutations. But if this explanation for the absence of synthetic lethals in the X chromosome were correct, then it should also hold for the third chromosome. Yet, the third chromosome data show no great difference between the frequencies in the chromosomes from laboratory stocks and in chromosomes from the freshly caught wild types. There is then no ground for assuming that selection within the laboratory stocks has eliminated an otherwise present recombinational effect. Still another reason for the divergent results could

be that in the experiments described here chance alone was responsible for the fact that no chromosomes were selected which in recombination could give lethals. This again seems unlikely since there were 45 heterozygous combinations for the X chromosomes and 11 combinations heterozygous for the third chromosomes. It would not seem probable that chance alone would be responsible for MISRO's obtaining three wild type chromosomes and having the two combinations which he studied produce synthetic lethals if none of the larger samplings of the third and X chromosomes produce them. Nor does it seem likely from chance alone that 28 of WALLACE's 45 combinations should produce lethals by recombination if none appears from the 56 combinations of the X and third chromosomes.

The major difference between DOBZHANSKY's work with *D. pseudoobscura* and that reported here is that one of the *pseudoobscura* chromosomes was initially strikingly subnormal in effect whereas the present studies reported herein began exclusively with "normal" chromosomes (table 5). The *pseudoobscura* chromosome was lethal at 25.5°C in the homozygous state but permitted normal survival at 16.5°. The synthetic lethals obtained were lethal at 16.5°C as well as at the higher temperature. The origin of these lethals was interpreted as resulting from crossing over between the two parent chromosomes.

A similar difference is in part involved between the second chromosomes used by WALLACE *et al.* and the X and third chromosomes. In WALLACE's work four of the ten chromosomes were specifically chosen because of their poor viability in the homozygous condition. One of these gave 6.5% lethals in the homozygous condition. This is a higher frequency than found in any of the heterozygous combinations of the third chromosome. Combinations involving these four chromosomes are responsible for the majority of the lethals observed. One hundred and twenty-two lethals in 3235 tested chromosomes (3.77%) were observed in the groups which were combinations of the four chromosomes having poor viability. It is likely that recombination between the chromosomes involved in these experiments was the source of some of the synthetic lethals observed by WALLACE *et al.* On the other hand, combinations of the chromosomes that had high viability yielded similar frequencies of lethals from homozygous controls and heterozygous experimentals (.49% and 1.31%

TABLE 5

Viability of flies bearing third chromosomes homozygous for each wild type chromosome tested. Results are from crosses of D/+ *females with* D/+ *males. Expected wild type frequency is 33.33%*

Class	No. of cultures	Offspring		Total	% wild type
		D/+	+/+		
A	6	299	198	497	39.84
B	6	241	204	445	45.84
C	6	348	203	551	36.84
E	6	263	155	418	37.08
F	4	148	124	272	45.59
Sal	5	300	162	462	35.06
For	5	324	186	510	36.47
Sam	6	342	217	559	38.82

respectively). A χ^2 test gives a P value of .3 — .2, indicating that chance alone could be responsible for the observed differences in lethal frequency.

MISRO's results from studies of lethality in the second chromosome in *D. melanogaster* were reported in abstract form and few details are given. This makes it impossible to analyze his results adequately and to compare them with those on the X and third chromosomes.

WIGAN (1949) localized the second chromosome lethals that arose in MISRO's experiments as well as some from his own experiments and found that the synthetic lethals differed from the "point" lethals in their distribution along the chromosome. Of the 23 synthetic lethals tested nine were in the left arm between 15.0 and 45.0, seven in the right arm between 70.0 and 95.0, one near the centromere and one near the left end and five seemed to lie beyond the right end of the chromosome. Of the point lethals three were in the left arm, nine in the right, and five near the centromere. Eighteen lethals were collected from wild populations and these had a distribution similar to that of the point lethals mentioned above. He concluded that even though synthetic lethals may occur in wild populations they may not be found often because of their unstable nature. Contrary to this, PAVAN and KNAPP have suggested that the high frequency of lethals found in their sample of *D. willistoni* populations might be due to synthetic lethals.

The results presented here for the X chromosome (table 1) and the third chromosome (table 4) have a distribution similar to that of WIGAN's point lethals. In the X chromosome eight lethals are within approximately 16 units from the centromere (66.0), two at the left end, and one at 21.0. In the third chromosome seven lethals are not more than seven units to the right or left of the centromere (46.0), with two others in the left arm and one in the right arm.

A further source of difference could be in the culture technique used and also in the method of determining lethality. The authors mentioned determined lethality upon the first check of a culture and if at least the minimum number of flies was present and there were no wild type flies the culture was classified as lethal. For this work a more rigorous method was used in that each lethal was tested a second time and the culture was classified as lethal or nonlethal depending on the results of the second check. Only three cultures in the recombinational line and four in the control line became nonlethal on the second check. These would have had little effect on the total results. If localization tests had been done on these "first-check lethals" the analysis would have been confused by the emergence of the unexpected and supposedly absent genotypes of a true lethal.

The divergent results of experiments to determine whether lethals arise as a result of recombination of specific alleles of loci which in their original combination were not lethal may not be as opposed to each other as it might seem. When the results of the experiments are compared one must not lose sight of the intrinsic differences of the chromosomes used by the different authors. It is known that chromosomes in nature range in viability from those that are lethal in the homozygous state to those with normal viability. If any of those are chosen which already carry a recessive lethal or have factors causing poor viability it may not seem so strange that recombinational lethals be produced. On the other hand if chromosomes with high viability are

recombined by crossing over, synthetic lethals may be rare. This has been shown in the work described here on the third and X chromosomes. Thus, synthetic lethals may arise frequently in natural populations, but the phenomenon may not be a universal one. That is, recombinational lethals need not necessarily be expected to arise frequently as a result of crossing over even though the chromosomes involved may be from different populations.

SUMMARY AND CONCLUSIONS

Experiments were carried out on *Drosophila melanogaster* to determine if lethals are formed by recombination between normal X chromosomes of different populations and between normal third chromosomes of different populations.

Statistically the results are in agreement with the hypothesis that recombination played no role in producing lethals in the X chromosome or in the third chromosomes studied. Genetic localization tests, using different sets of markers in at least two separate tests for each lethal, indicate that there is no need to assume that a combination of loci or of regions is involved in any of the lethals.

The results of these experiments on the X chromosome and the third chromosome are different from those of other authors who have interpreted their findings as showing that lethals frequently are produced by recombination in the second chromosome of several Drosophila species. The production of synthetic lethals in the second chromosome of those Drosophila species studied may be due to synthesis of recombinant chromosomes from chromosomes which were originally subnormal in effect.

Crossing over is perhaps a factor in the production of lethals in natural populations, but experiments on the X and third chromosomes indicate that lethality need not be expected to occur frequently as a result of crossing over, especially if the parent chromosomes are free from pre-existing lethal or severe viability defects.

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LITERATURE CITED

CASPARI, E., and C. STERN, 1948 The influence of chronic irradiation with gamma-rays at low dosages on the mutation rate in *Drosophila melanogaster*. Genetics **33:** 75–95.

DOBZHANSKY, TH., 1946 Genetics of natural populations. XIII. Recombination and variability in populations of *Drosophila pseudoobscura*. Genetics **31:** 269–290.

HILDRETH, P., 1955 A test for recombinational lethals in the X chromosome of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U. S. **41:** 20–24.

KERR, W. E., and L. S. KERR, 1952 Concealed variability in the X chromosome of *Drosophila melanogaster*. Am. Naturalist **86:** 405–408.

MISRO, B., 1949 Crossing over as a source of new variation. Proc. Intern. Congr. Genet. 8th Congr.: 629–630.

PAVAN, C., and E. N. KNAPP, 1954 The genetic population structure of Brazilian *Drosophila willistoni*. Evolution **8:** 303–313.

SPASSKY, B., and TH. DOBZHANSKY, 1950 Comparative genetics of *Drosophila willistoni*. Heredity **4:** 201–215.

SPENCER, W. P., and C. STERN, 1948 Experiments to test the validity of the linear r dose/mutation frequency relation in Drosophila at low dosage. Genetics **33:** 43–74.

WALLACE, B., J. C. KING, CAROL V. MADDEN, BOBBIE KAUFMANN, and E. C. MCGUNNIGLE, 1953 An analysis of variability arising through recombination. Genetics **38:** 272–307.

WIGAN, L. G., 1949 Chromosome regions which give new variation by crossing over. Proc. Intern. Congr. Genet. 8th Congr.: 686–687.