THE FREQUENCY OF LETHALS IN CROSSOVER AND NONCROSSOVER SECOND CHROMOSOMES OF *DROSOPHILA MELANOGASTER**

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IT has long been known in different species that some individuals carry lethals even though they were descended from lethal-free ancestors. These lethals usually have originated by mutation at various loci. There exists, however, a second possible source for new lethals. Since individuals in a population are heterozygous for a large array of loci, recombination, it has been argued, might give rise to ill-adapted assortments of pre-existing alleles some of which might give lethal effects.

Evidence for the production of such "synthetic" lethals has been presented by WRIGHT and DOBZHANSKY (1946); MISRO (1949); and WALLACE, KING, MADDEN, KAUFMANN and MCGUNNIGLE (1953). The extensive work of the last named group of investigators concerned recombination among second chromosomes of *Drosophila melanogaster*. They found a considerable number of lethals among the offspring of females which had been made heterozygous for different lethal-free second chromosomes, in contrast to a small number of lethals from corresponding heterozygous males. In the females the presence of crossing over was considered to have been responsible for the origin of synthetic lethals while the absence of crossing over in the males did not lead to their occurrence.

Recent work by HILDRETH (1955, 1956) on the X chromosome and on the third chromosome of *Drosophila melanogaster* shows none of the marked deviations in the frequency of lethals from recombinant as compared to that from nonrecombinant chromosomes. Further, HILDRETH was able to localize at single loci the lethals which did occur in recombinant X and third chromosomes indicating that they were not due to lethal complexes synthesized by recombination.

In none of the former studies had there been a direct check on crossing over. The present experiment was so designed that specific crossover and noncrossover chromosomes could be selected and then tested for the presence of lethals. All data refer to the second chromosome of *D. melanogaster*.

PROBLEM AND METHODS

This test duplicated, in part, the techniques used by WALLACE *et al.* (1953). In each of seven "wild type" populations of *D. melanogaster* a single second chromosome was selected for the test. Flies that were homozygous for these "selected"

* This work paralleled other studies in this laboratory carried out with the support of the U.S. Atomic Energy Commission.

second chromosomes from each population were mated with flies from a single common population. Nonrecombinant and recombinant chromosomes were recovered from the interpopulational hybrids. Zygotes that were homozygous for these chromosomes, were tested for the presence of lethals.

Six of the "wild type" stocks used in this work were populations of diverse origin and histories which had been kept in the laboratory for long periods. Brief descriptions of these stocks are as follows: Stock 1. *Corona*, originally from Riverside, California; 2. *Formosa*, originally from Formosa, February 1950; 3. *Salta*, collected in Argentina, February 1950; 5. *Canton*, from the California Institute of Technology at Pasadena, 1939, originally 1A Canton S; 6. *Florida*, collected in Columbia, Florida. These stocks had all been kept by mass matings in the Genetics and Zoology Departments of the University of California at Berkeley. Stock 4. *Samarkand* was kept by brother-sister matings for 217 generations until June, 1953 and then by mass matings. The seventh stock, 7. *Napa*, was collected September 12, 1954 and kept by mass matings until the beginning of the test. This stock was established so that some tests could be made on a population not adapted to laboratory conditions.

The presence of crossovers in the interpopulational hybrids and the subsequent recovery of recombinant chromosomes were checked through the use of a second chromosome containing mutant genes. This "marked" chromosome was introduced into the test from the "common" population with which the other populations were hybridized. This chromosome contained the recessive mutants *aristaless* (*al* 2-0.0), black (*b* 2-48.5), cinnabar (*cn* 2-57.5), curved (*c* 2-75.5), and brown (*bw* 2-104.5). To a certain degree these mutants permitted the selection of nonrecombinant and recombinant chromosomes during the test.

Limitations to the recognition of recombination are based on the fact that the five mutant markers are not sufficient to exclude the occurrence of undetectable types of multiple crossovers. Groups of chromosomes in which all markers had remained together while primarily consisting of nonrecombinants would also contain some double crossover strands in which the two crossovers had occurred between the adjacent markers. Even occasional quadruple crossovers consisting of two doubles, each between adjacent markers, might have been present among the "nonrecombinants." Similarly, recombinant chromosomes, such as $+ b \text{ } cn \text{ } c \text{ } bw$, while usually the products of a single exchange between the loci of *al* and *b* might occasionally be the products of triple or even higher multiple exchanges which were so located as to leave the sequence *b cn c bw* unchanged. These reservations should be kept in mind when in the following pages the terms noncrossover, single crossover and, multiple crossover classes are used. These terms apply to the recognizable events only.

As a first step six separate lines were established from each of the seven wild type stocks. One second chromosome in each line contained the mutants, Curly (*Cy*) and Lobe (*L*⁴), and an inversion in each arm as well as the recessive genes *al*², *lt*³, and *sp*². The genotype of this chromosome, designated in this paper as *Cy al L*, is actually *Cy al*² *lt*³ *L*⁴ *sp*² with inversions *In*(2L)*Cy al*², *In*(2R) *Cy lt*³

$L^4 sp^2$. The other second chromosome in each line was the descendant of a single second chromosome from the original wild type population. This chromosome was isolated by mating wild type flies with *Cy al L* flies and remating one $+ / Cy al L$ male offspring with *Cy al L* females.

The *Cy al L* chromosome used in the crosses to isolate the single wild type second chromosome also served to keep that chromosome intact—inhibiting crossing over until flies were used in the final stages of the test for recombinational lethals.

Among the six lines from each stock one was selected for the main experiment. This selection was made: (1) upon the basis of the absence of inversions as indicated by salivary smears and (2) upon the absence of lethals in the isolated chromosome at this point in the crosses. The tests for prerecombinational lethals were made by mating *Cy al L* females with $+ / Cy al L$ males from each line in each stock. The $+ / Cy al L$ offspring were then mated. The development of wild type adults among the offspring of this last cross indicated the absence of a lethal while the presence of a lethal would lead to the appearance of Curly Lobe individuals only.

These tests for prerecombinational lethals at this stage served also as tests for the viability of the various nonlethal second chromosomes when in homozygous constitution. Taking the viability of the Curly Lobe class as unity the viability of the wild type homozygotes could be estimated by the deviation of the $+ / Cy al L$ to $+/+$ ratio from the theoretical 2:1 ratio (Table 1).

While these selected wild type chromosomes assuredly were initially free of lethals the possibility existed that mutational lethals might arise in some of them. In order to distinguish such later prerecombinational lethals from lethals originating during the essential part of the experiment a special test was made. From each line, ten females heterozygous for a wild type chromosome and *al b cn c bw* were obtained and used separately to produce the next generation from which recombinant and nonrecombinant chromosomes were to be taken. If, as turned out to be the case in the crosses with chromosomes from the Canton population,

TABLE 1
Records of viability tests on second chromosome lines— F_1 of $Cy al L/+ \times Cy al L/+$

Populations	Totals of flies produced in test of each line and percent of wild type flies in each test											
	A		B		C		D		E		F	
	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%
1. Corona	263	23	283	19	40	17	219	27*	154	26	244	25
2. Formosa	402	24	256	0†	229	18	156	0†	115	20	285	39*
3. Salta	140	34*	81	19	135	40	157	42	138	23	82	0†
4. Samarkand	119	31	94	24	157	33*	122	27	116	26	48	6
5. Canton	111	29	198	29	68	25	115	21	77	32*	81	23
6. Florida	138	31*	25	20	173	27	109	25	34	8	99	23
7. Napa	93	22	134	28	173	27	303	34*	150	28	240	32

* Used in subsequent tests for recombinational lethals.

† Absence of wild type indicates presence of a lethal.

several lethals appeared at the end of the succeeding crosses and they could all be traced back to one heterozygous female, it was assumed that the lethal had been present in this female and could not be attributed to recombination. Such prerecombinational lethals were not included in the totals used in the analysis of the results of the tests.

The details of the mating scheme may be followed in Figure 1. Males homozygous for originally lethal-free, selected second chromosomes were mated with *al b cn c bw* females. F_1 females, heterozygous wild type ($+/al b cn c bw$), were mated with males from a specially prepared stock made up from the *al b cn c bw* laboratory line. In this stock the factors Star and asteroid (*S 2-1.3 ast 2-1.34*) were inserted into the marked second chromosome. This stock (*al S ast b cn c bw/Cy al L*) permitted the selection of offspring with nonrecombinant and recombinant chromosomes in the next generation and the factors Star and asteroid were used at a later point in the crosses as a necessary tool for classification (see F_3 offspring below). The F_2 generation contained flies with noncrossover and crossover second chromosomes over the marked chromosome *al S ast b cn c bw*. Fifty F_2 males of the noncrossover class (+), 50 males of each of the single crossover classes (*al, b cn c bw, al b, cn c bw, al b cn, c bw, al b cn c, bw*) and, a total of 50 males from various multiple crossover classes (usually seven each from double crossovers *al bw, b cn c, al b bw, cn c, al c bw, b cn* and two to three from triple crossovers *al c, al cn c, b cn bw*) were selected and individually mated with *al b c sp/Cy al² lt³ L⁴ sp²* females. Thus a total of 500 males from each of the seven test series were selected from the F_2 offspring and mated in individual "creamers" (one ounce glass containers).

It was not possible to maintain a constant number of tests for each population—or constant number of tests for each wild type or crossover class—tested for recombinant lethals. During the course of the tests with each population a few of the cultures did not produce any flies, or not enough flies in some cases. In the experiments with the Samarkand population the bottles became heavily infected with mold and considerable numbers of the cultures did not develop. The tests using the Napa population had to be repeated because excessive heat which occurred during the time of the F_2 cross rendered a great number of the test cultures sterile.

The Curly Lobe F_3 offspring in each test consisted of two types, namely flies with (1) a wild type, noncrossover, chromosome or with one of the various recombinant chromosomes respectively, and (2) flies with the *al S ast b cn c bw* chromosome. Flies of group (1) were selected for the next mating; those of group (2) which showed the dominant mutant Star were discarded. The selected F_3 flies were mated among themselves, several males to several virgin females. In the next and last generation, zygotes homozygous for the wild type or the various recombinant chromosomes were produced. Provided no lethal was present and assuming full viability, the expected ratio of Curly Lobe heterozygotes to wild type, or recombinant, homozygotes was two to one. The absence in any of these F_3 cultures of wild type or recombinant offspring indicated a lethal. Curly Lobe

FIGURE 1.—Mating scheme for test for recombinational lethals in the second chromosome of *D. melanogaster*. The mating scheme presented here shows the test with the nonrecombinant chromosome and one example, only, with a recombinant chromosome.

heterozygotes from tests indicating the presence of lethals were remated and a final check for lethals was made in the succeeding generation.

At the conclusion of these tests it was felt that the significance of the results was limited by the small number of "control", or noncrossover, chromosomes that had been included. A series of crosses generally comparable to those used in the foregoing tests were designed and carried out to test the frequency of lethals in additional noncrossover chromosomes. In these crosses the noncrossover chromosomes came from males and thus were not only phenotypically noncrossovers but also did not include undetectable multiple crossovers. Males from six of the seven wild type lines that had been selected earlier were mated with *al b cn c bw* females. The $+ / al\ b\ cn\ c\ bw$ male offspring were mated with *Cy al L* virgin females. At the next step a viability test was introduced into the procedure and at the same time the test for lethals in the nonrecombinant chromosomes was begun for each of the wild type chromosomes by mating approximately 100 $+ / Cy\ al\ L$ males from the preceding cross with virgin females containing the "marked" second chromosome carrying the mutants Star and asteroid. The viability test was similar to the one performed in the first series of tests where *Cy al L* females were mated with $+ / Cy\ al\ L$ males, the $+ / Cy\ al\ L$ offspring were mated and the subsequent development of wild type offspring indicated the absence of a lethal. If this viability test indicated the absence of lethals in the chromosome being tested then the final cross was made using males and virgin females of the genotype $+ / Cy\ al\ L$ from the $+ / Cy\ al\ L \times al\ S\ ast\ b\ cn\ c\ bw / Cy\ al\ L$ mating. The absence of flies homozygous for the nonrecombinant wild type chromosome in the offspring indicated presence of a lethal. As in the preceding tests all suspected lethals were retested for another generation.

For practical considerations, and for simplicity in handling the lethal studies, it was decided that the measure of lethality would be the absence in the individual tests of any wild type flies or of flies of the various crossover classes. The experimental procedure consisted of placing etherized flies from each F_3 culture on a counting plate. With the microscope, the offspring were examined for the presence or absence of wild type or of recombinational flies. In both test series, the absence of wild type or of recombinational flies in fifty or more flies was considered evidence for the presence of a lethal factor. If individual bottles produced fewer than 50 flies they were recultured and counted for lethals at the next generation.

All tests were made at 25°C. All culture bottles contained cornmeal-agar-molasses medium enriched with brewer's yeast. The F_3 cultures were examined on the 15th day after mating. The ages of the F_1 and F_2 parents were kept within a variable period of four days for each group, i.e. F_1 and F_2 matings began at the 11th day after culturing and ceased at the 15th day in each case.

RESULTS

A total of 23 F_3 creamers from both series of crosses did not contain wild type or recombinational flies among the offspring, indicating the presence of lethals in the selected second chromosomes. Eighteen, or 78.3 percent of the suspected lethals were confirmed in the F_4 . Of this total of 23 lethals, 18 occurred in test 1,

with the heterozygous females. Fourteen of these were confirmed in the F_4 , i.e. they did not produce homozygous offspring. The remaining five lethals occurred in test 2, with heterozygous males, four were confirmed in the F_4 , one was not confirmed.

No attempt was made to measure poor viability. Creamers in which any wild type flies, or flies of the various crossover types, occurred were counted as non-lethal.

The results of the tests are shown in Table 2 where the seven wild type chromosomes used are listed at the left. The frequencies of lethals recovered are represented in fractional form for each of the wild type and recombinant chromosomes used. Recombinant chromosomes from multiple crossover are listed only as "multiple." When a lethal did occur with recombinant chromosomes from multiple crossover, as in the tests with the Samarkand and Salta populations, the particular recombinant second chromosome involved is listed at the bottom of the table.

In one test with the Canton population, several lethals occurred but were traceable to the same F_1 heterozygous female. These are assumed to have been lethals present in that female and not the result of crossing over, i.e. prerecombinational lethals. They are not included in the totals and percentages presented at the right and at the bottom of the table.

After the elimination of the prerecombinational lethals from the Canton test results, the final totals and percentages do not indicate a significant increase over a reasonable expectation for a spontaneous mutation rate. In different sets of tests the frequency of lethals varies from one in 595 chromosomes, or 0.2 percent lethals, to four in 578 chromosomes, or 0.7 percent lethals. DUBININ (1946) gives the figure 0.53 percent lethals expected per generation for the second chromosome.

A comparison between two sets of observations—the frequency of lethals occurring in the controls, noncrossover or wild type flies, and the frequency of lethals occurring in the experimentals, recombinant flies—does not indicate a significant difference in lethals in recombinant flies. The frequency of lethals in the controls is five in 970 chromosomes tested (0.5 percent). The frequency of lethals in the recombinants is 13 in 3063 chromosomes tested (0.4 percent). A homogeneity test of these two sets of data yields a chi square indicating a probability greater than 0.7. The difference between the two lethal frequencies is, therefore, not statistically significant.

The comparison of frequencies of lethals in recombinant chromosomes recovered from apparent single and multiple crossovers in the second chromosome likewise gives a probability greater than 0.7 in favor of a chance deviation.

The comparison of frequency of lethals in recombinant chromosomes recovered from the test performed on the nonlaboratory stock (Napa) with the frequency of lethals in recombinant chromosomes recovered for tests on laboratory stocks shows a probability greater than 0.7 in favor of chance deviation.

It is not clear wherein the difference lies between the results of WALLACE *et al*,

TABLE 2
Results from the tests for recombinational lethals in the second chromosome of *D. melanogaster*

Frequency of lethals in wild type and various recombinant chromosomes tested																				
Origin of wild type chromosomes	Noncrossovers (+)			%	Crossovers														Grand totals§	%
	Females	Males	Total		<i>al</i>	<i>b cn c bw</i>	<i>al b</i>	<i>cn c bw</i>	<i>al b cn</i>	<i>c bw</i>	<i>al b cn c</i>	<i>bw</i>	Multiples	Total	%					
Corona	0/50	2/98	2/148	1.0	0/50	0/47	0/50	0/50	0/50	0/50	0/50	0/50	0/52	0/499	0	2/597	0.3			
Samarkand	0/28	0/100	0/128	0	1/38	0/33	0/38	0/27	1/49	0/33	0/42	0/31	1/27†	3/346	0.8	3/446	0.7			
Formosa	0/50	0/100	0/150	0.6	0/50	0/48	0/47	0/50	0/50	0/50	0/50	0/50	0/50	0/495	0	1/595	0.2			
Salta	1/56	0/86	1/142	0.7	0/46	0/49	0/48	1/46	0/48	0/53	1/48	0/49	1/49‡	4/492	0.8	4/578	0.7			
Napa ¹	0/43		0/200	0	0/23	0/10	0/3	0/5	0/13	0/16	0/8	1/11	0/22	(1/154	0.6)	2/739	0.3			
Napa ²	0/45	0/112			0/41	0/47	0/50	0/50	0/42	1/50	0/50	0/50	0/48	(1/473	0.2)					
Canton	0/50		0/50	0	0/47	0/45	1/50	0/47	0/50	1/46	0/46	0/49	0/57	2/487	0.4	2/487	0.4			
					2/49	2/47	1/50	1/48	0/50	3/48	0/45	1/50	0/57	10/495*						
Florida	0/50	1/102	1/152	0.6	0/48	1/47	1/50	0/50	0/49	0/50	0/45	1/48	0/52	3/489	0.6	4/591	0.7			
Totals	1/372	4/598	5/970	0.5	1/343	1/326	2/336	1/325	1/351	2/348	1/339	2/338	2/357	14/3435	0.4	18/4033	0.4			

¹ Results 6-4-55.
² Results 10-5-55.
^{*} Eight lethals in the Canton population test (*al* two; *b cn c bw* two; *cn c bw* one; *c bw* two of the three; *bw* one) occurred in the F_3 cultures from F_2 flies produced from the same F_1 female (no. 5). Presence of a precombinational lethal was assumed and these lethals are not included in the final totals or percentages.
[†] Recombinant chromosome contained the mutants *b cn*.
[‡] Recombinant chromosome contained the mutants *b cn c bw*.
[§] From noncrossovers and crossovers.

who reported the finding of large numbers of recombinational lethals and the present study which shows their absence. To some extent the selection of wild type second chromosomes with high viabilities may have reduced the chance of recombinational synthesis of lethals. It may also be argued that six of the wild type chromosomes as well as the marker chromosome came from long established laboratory stocks each of which may have become internally adapted to the laboratory environment. Even if this supposition is accepted one still would expect different coadapted genotypes in these different stocks and a subsequent creation of less adapted genotypes, including lethal ones, by recombination. It is likely that poorly adapted, but still viable, genotypes did indeed originate since the experiments were designed to discover lethal genotypes only. Possibly some of the lethals discovered by former workers would have been semilethals under our own culture conditions and therefore could not be included in the present tally of lethals.

SUMMARY

A test was made for lethals in recombinant second chromosomes of *D. melanogaster*, using techniques similar to those of other authors. In addition, the recovery of recombinant chromosomes was made certain through the use of a chromosome marked with five recessive mutant genes. This allowed for the specific observation of particular second chromosome loci involved in any recombinant chromosome containing a lethal. The experiments were so designed as to permit elimination of prerecombinational lethals from the test results. The wild type second chromosome from each of seven populations was selected for high viability before the test began.

The results indicate no statistical deviation between recombinant and nonrecombinant chromosomes in producing lethals. Furthermore, the frequencies of lethals among the various populations tested show no striking dissimilarities. It seems likely, then, that recombination has played no great part in the production of the lethals recovered in this test.

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