THE NATURE OF LETHALS IN *DROSOPHILA WILLISTONI*¹

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IT is well established that Mendelian populations carry a large amount of concealed variability. Chromosomes which are lethal when homozygous constitute an important part of the concealed variability in cross-breeding populations as shown, among others, by PAVAN *et al.* (1951) in *Drosophila willistoni*. Recessive lethal second chromosomes had a mean frequency of .286 in seven Brazilian populations of *D. willistoni* studied by them. The ultimate origin of the lethal chromosomes is mutation. However, as shown for the first time by DOBZHANSKY (1946), lethal chromosomes can arise also by crossing over between nonlethal chromosomes. Such lethal chromosomes are called "synthetic lethals", as they are formed by recombination from preexisting genetic components and are not produced suddenly by mutation. Synthetic lethals may also be broken by recombination, giving nonlethal chromosomes (DOBZHANSKY and SPASSKY 1960). Production of synthetic lethals has been shown in *D. pseudoobscura* by DOBZHANSKY (1946) and by SPASSKY *et al.* (1958), in *D. prosaltans* by DOBZHANSKY *et al.* (1959) and in *D. willistoni* by KRIMBAS (1961). The situation of synthetic lethals in *D. melanogaster* is not clear. While MISRO (1949), WALLACE *et al.* (1953) and GIBSON and THODAY (1962) obtained synthetic lethals in *D. melanogaster*, HILDRETH (1956), GANTNER (1958) and SPIESS and ALLEN (1951) obtained no evidence of their occurrence. The situation in *D. persimilis* is also doubtful (SPIESS 1959).

The work we are doing on mapping second chromosome lethals in *D. willistoni* produced some data, which we will report below, bearing on the nature of lethals and the relations between "synthetic" and "point mutation" lethals.

MATERIALS AND METHODS

The work was planned in order to obtain the relative distances between the *D. willistoni* second chromosome lethals n_1 , n_2 , n_3 , n_4 , r_1 , r_2 , r_3 and r_4 . The lethals n were obtained from a natural population living on the island of São João, Angra dos Reis. The lethals r were induced by irradiation in the laboratory, in chromosomes of normal viability also isolated from flies caught in the island of São João. The irradiation was 800R from a caesium-137 source of 50 curies. The lethals, with the exception of r_1 and r_3 , are nonallelic. However, even though r_1 and r_3 are alleles, they were used separately in the mapping in order to see whether they behaved differently in relation to the other lethals.

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The eight lethals were maintained in the laboratory in stocks balanced with *St Hk 207 abb bw* second chromosomes. *St* (Star) is lethal when homozygous. *Hk* is the symbol for Hook, *abb* for abbreviated, and *bw* for brown (SPASSKY and DOBZHANSKY 1950). The number 207 designates a long pericentric inversion which prevents most recombination. For simplicity, the whole *St Hk 207 abb bw* chromosome will be called simply 207.

The first step in determining the relative distances among the lethals was to obtain flies hybrid for two lethal chromosomes. Flies having different lethals balanced with 207 were crossed $l_1l_2+/207 \times l_1+l_2/207$, in order to obtain the hybrids for two lethals l_1l_2+/l_1+l_2 . Afterwards l_1l_2+/l_1+l_2 females were crossed with 207/broad males, broad being lethal. The F_1 males from these crosses had one 207 chromosome from the father and one chromosome from the mother l_1l_2+/l_1+l_2 , which could be l_1l_2+ , l_1+l_2 or the recombinants l_1l_2 and l_1+l_2+ . To identify the four types of chromosomes from the female l_1l_2+/l_1+l_2 and to estimate their frequencies, F_1 males were crossed individually with females from the stocks $l_1l_2+/207$ and $l_1+l_2/207$ in succession. The absence of wild-type flies in the offspring of these crosses indicates the presence, in the tested chromosome, of a lethal that is allelic with the lethal already present in the stock. The presence of wild-type flies is evidence of the presence of the normal allele l_1+ or l_2+ . At least 100 flies were examined from the offspring of each cross. The number of males tested gives the number of chromosomes analysed.

According to what is known about linkage and crossing over, if the two lethals l_1 and l_2 are at two different loci, and if, in the crosses above, the frequencies of the chromosomes l_1l_2+ , l_1+l_2 , l_1l_2 and l_1+l_2+ are respectively x_1 , x_2 , x_3 and x_4 , the following relations should be observed:

- (1) $x_1 + x_3 = 0.5$;
- (2) $x_2 + x_3 = 0.5$;
- (3) $x_1 = x_2$;
- (4) $x_3 = x_4$;
- (5) $x_1 + x_2 \geq x_3 + x_4$.

The distance between the two loci l_1 and l_2 will be $x_3 + x_4$ units.

RESULTS

The results obtained in the analysis of the recombination rates between the lethal n_1 and r_1 , r_1 and r_2 , etc. are indicated in Table 1. In each column, are the numbers of the chromosomes found to be of the two parental and the two recombinant classes, and the results of χ^2 tests for conditions (1) to (5) indicated above. The chi-square values significant at the 5% level are indicated by an asterisk.

Seven out of the nine pairs of lethals studied departed significantly from the values expected under the assumption of recombination between two single loci. Even more, the manner in which the results depart from expectation is not uniform. Some pairs depart significantly only from the relation (4), others do not conform to the conditions (3) and (4), while still others do not respect one of the relations (1) or (2) or both. Therefore, the results cannot be explained by simple recombination between two independent loci. Our hypothesis is that one of the lethals, in some of the pairs, or both lethals, in other pairs, are synthetic lethals. It will be shown later that the differences between "synthetic" and "point mutation" lethals are not absolute but relative, and only for the sake of the presentation will they be considered now as different classes.

If a synthetic lethal chromosome is produced in a female heterozygous for two chromosomes, neither of which is lethal when homozygous, it means that at least two chromosome sections exist which must occur together, in the same chromosome, to give a lethal effect. The substitution of one of these sections by its

TABLE 1
Results of the analysis of the recombination between pairs of second-chromosome lethals and the analysis according with the models developed in the text

Frequencies and gametes		Lethals compared							
n_1-r_1	r_1-r_2	n_1-n_2	n_3-r_3	r_3-r_4	n_3-n_4	n_3-r_4	r_4-r_3	n_4-r_4	n_2-n_3
Numbers of chromosomes studied									
$I_1I_2+(x_1)$	$n_1=98$	$r_1=66$	$r_2=82$	$n_1=81$	$n_3=67$	$n_3=63$	$r_3=36$	$n_4=78$	$n_2=38$
$I_1+I_2(x_2)$	$r_1=113$	$r_2=82$	$n_2=83$	$n_2=78$	$n_4=74$	$r_3=83$	$r_4=23$	$r_4=24$	$n_3=95$
$I_1I_2(x_3)$	$n_1r_1=13$	$r_1-r_2=35$	$r_2-n_2=2$	$n_1-n_2=0$	$n_3-n_4=31$	$n_3-r_3=58$	$r_3-r_4=25$	$n_4-r_3=24$	$n_2-n_3=30$
$I_1+I_2+(x_4)$	$++=19$	$++=45$	$++=38$	$++=17$	$++=63$	$++=99$	$++=46$	$++=56$	$++=150$
Total	243	228	205	176	235	303	130	182	313
χ^2 -test for the conditions†									
(1)	1.81	2.96	6.68*	1.12	6.46*	12.28*	0.50	2.66	100.08*
(2)	0.33	0.16	5.96*	2.27	2.66	1.45	8.88*	40.62*	12.68*
(3)	1.07	1.73	0.06	0.06	0.32	2.74	2.86	28.60*	12.21*
(4)	1.13	1.25	32.40*	17.00*	10.88*	10.70*	6.21*	12.80*	80.00*
(5)‡	...	...	...	...	...	0.399	1.10	...	7.04*
Interpretation									
Two single loci	Two single loci	Two synthetic	Two synthetic	n_3 synthetic	n_3 synthetic	r_3 single locus	r_3 single locus	n_4 single locus	Two synthetic
				n_4 single locus	n_4 single locus	r_3 single locus	r_4 synthetic	r_4 synthetic	

* Significant at the 5% level.
† (1) $x_1+x_3=0.5$; (2) $x_2+x_3=0.5$; (3) $x_1=x_2$; (4) $x_3=x_4$; (5) $x_1+x_2 \geq x_3+x_4$.
‡ χ^2 indicated only when $x_1+x_2 < x_3+x_4$. In all other cases $x_1+x_2 \geq x_3+x_4$.

FIGURE 1.—Models of combinations of chromosomes having one unilocal and one synthetic lethal.

homologue, (its allele in the case of being a single locus) will disrupt the lethal combination and reconstitute nonlethal chromosomes. If the components producing lethality are represented by the sign —, and their nonlethal homologous by +, four different types of chromosomes are possible: ++, +—, —+ and ——. Only the chromosome — will be lethal. The heterozygote of the *trans* type +—/—+ may give rise to synthetic lethals at a rate depending upon the distance between the two elements.

According to our hypothesis two different situations may be encountered. One model assumes that in a pair of lethal chromosomes, one is a single-locus lethal and the other is a synthetic. The other model assumes that both lethal chromosomes in a pair are synthetic. The first model has two alternatives, the single lethal locus may be placed outside the region comprising the two sections of the synthetic (Figure 1a), or the single locus may be within their limits (Figure 1b). The second model has three alternatives. The two synthetic lethals may be regions apart (Figure 2a), or overlapping (Figure 2b), or one may be included in the region of the other (Figure 2c).

The theoretical consequences of crossing over in the five models are analysed in Table 2. This table gives the frequencies of the four classes of chromosomes $l_1l_2^+$, $l_1^+l_2$, l_1l_2 and $l_1^+l_2^+$ as a function of the distances between the elements which constitute the lethals. The table also gives the theoretical departures from the conditions (1), (2), (3), (4) and (5) expected on the assumption of recombination between single-locus lethals. The way the frequencies were calculated will

FIGURE 2.—Models of combinations of chromosomes having two different synthetic lethals.

TABLE 2
Gamete frequencies and their effects to the conditions (1) to (5) under the several models

Gametes and frequencies	Model				
	Figure 1 a	Figure 1 b	Figure 2 a	Figure 2 b	Figure 2 c
$l_1 l_2^+ (x_1)$	$\frac{1}{2} [1 \cdots d]$	$\frac{1}{2} [1 \cdots (d_1 + d_2) + d_3 d_4]$	$\frac{1}{2} [1 \cdots (D + d_1) + Dd_2]$	$\frac{1}{2} [1 \cdots (2d_3 + d_4 + d_5) + 2d_3 d_5]$	$\frac{1}{2} [1 \cdots (d_5 + d_6 + d_7) + 2d_3 d_5 + 2d_3 d_6 + d_7 d_6]$
$l_1^+ l_2 (x_2)$	$\frac{1}{2} [1 \cdots d]$	$\frac{1}{2} [1 \cdots d_3 d_4]$	$\frac{1}{2} [1 \cdots (D + d_2) + Dd_2]$	$\frac{1}{2} [1 \cdots (d_3 + d_4) + 2d_3 d_4]$	$\frac{1}{2} [1 \cdots (d_3 + d_4 d_6)]$
$l_1 l_2 (x_3)$	$\frac{1}{2} d$	$\frac{1}{2} d_3 d_4$	$\frac{1}{2} (D \cdots Dd_2)$	zero except occurring triple crossingover	$\frac{1}{2} d_5 d_6$
$l_1^+ l_2^+ (x_4)$	$\frac{1}{2} d$	$\frac{1}{2} (d_3 + d_4 \cdots d_3 d_4)$	$\frac{1}{2} (D + d_2 + d_1 \cdots Dd_1 - Dd_2)$	$d_3 \cdots d_3 d_4 - d_3 d_5 + \frac{1}{2} (d_1 + d_2)$	$\frac{1}{2} [d_5 + 2d_2 + d_6 - d_3 d_6 + 2(d_3 d_5 + d_7 d_6)]$
Conditions (1) to (5) for each model*					
(1)	$\sum l_1 = 0.50$	$\sum l_1 = 0.50 - d_3 + d_4 + 2d_3 d_4$	$\sum l_1 < 0.50$	$\sum l_1 < 0.50$	$\sum l_1 < 0.50$
(2)	$\sum l_2 = 0.50$	$\sum l_2 = 0.50$	$\sum l_2 < 0.50$	$\sum l_2 < 0.50$	$\sum l_2 < 0.50$
(3)	$l_1 < l_2$	$l_1 < l_2$	$l_1 \geq l_2, d_1 \geq d_2$	$l_1 \geq l_2, d_3 + 2d_3 d_4 \geq d_4 + 2d_3 d_5$	$l_1 \leq l_2$
(4)	$l_1 l_2 = ++$	$l_1 l_2 < ++$	$l_1 l_2 < ++$	$l_1 l_2 = 0 \leq ++$	$l_1 l_2 \leq ++$
(5)	$P > R$	$P > R$ for $d_3 + d_4 < 0.50$	$P > R$ for $2D + d_2 + d_1 < 0.50$	$P > R$ for $2d_3 + d_4 + d_5 - 2d_3 d_4 - 2d_3 d_5 < 0.50$	$P > R$ for $d_5 + 2d_2 + d_6 < 0.50$

* (1) $x_1 + x_3 = 0.5$; (2) $x_2 + x_3 = 0.5$; (3) $x_1 = x_2$; (4) $x_3 = x_4$; (5) $x_1 + x_2 \geq x_3 + x_4$.

FIGURE 3.—Scheme of the recombinations, according to Model 1a, analysed in the text.

be exemplified taking one of the models as a paradigm, that in which one of the lethals, l_1 , is synthetic and the other, l_2 , is a single locus outside the range of the synthetic. The distance between the two elements of the synthetic will be taken as d_1 and that between the single locus l_2 and the closest element of l_1 as D . Figure 3 represents the two lethal chromosomes and the recombinations which may occur. Crossing over occurring within the region limited by the elements of the synthetic with the frequency d_1 , will give a normal chromosome and lethal l_2 with frequencies $\frac{1}{2}d_1$ each. Crossing over between the locus l_2 and the proximal element of the synthetic will give two classes of chromosomes, normal and l_1l_2 , with the frequencies $\frac{1}{2}D$ each. Double crossing over, if the distances d_1 and D are large enough to permit it, will produce normal chromosome and l_2 with rates of $\frac{1}{2}d_1D$ each. Table 3 gives the total frequencies of the four types of gametes produced by recombination. $(1 - \text{total frequency of all recombinants}) \times 0.5$ gives the frequencies of the two noncrossover classes, l_1 and l_2 . The addition of the frequencies of recombinant and nonrecombinant chromosomes for each class, gives the frequencies of each of them as a function of the distances d_1 and D . These values permit comparisons between what is expected under the hypothesis of recombination between two single-lethal loci and under the hypothesis presented.

The five alternatives presented in Table 2 and developed from the two models, and their five possibilities shown in Figures 1 and 2, permit the distances to be estimated from the relevant data. It is relatively easy to determine whether one lethal is synthetic and the other is a unilocal one or whether two lethals are both synthetic. However, to recognize in the first case whether the single-locus lethal is inside or outside the range of the synthetic, or in the second case, whether the two synthetic lethals are apart, overlapping or included, is more difficult and

TABLE 3

Types of gametes with their frequencies according with model indicated in Figure 3

Gametes	Frequencies		
	With crossing over	Without crossing over	Total
l_1l_2+	0	$\frac{1}{2} [1 - d_1 - D + d_1D]$	$\frac{1}{2} [1 - d_1 - D + d_1D]$
l_1+l_2	$\frac{1}{2} d_1$	$\frac{1}{2} [1 - d_1 - D + d_1D]$	$\frac{1}{2} [1 - D + d_1D]$
l_1l_2	$\frac{1}{2} [D - d_1D]$	0	$\frac{1}{2} [D - d_1D]$
l_1+l_2+	$\frac{1}{2} [d_1 + D - d_1D]$	0	$\frac{1}{2} [d_1 + D - d_1D]$
Total	$d_1 + D - d_1D$	$1 - d_1 - D + d_1D$	1

laborious. It is necessary to make a comparative analysis of several lethals. This comparative analysis is under way in the laboratory and will be reported in a future paper.

DISCUSSION

A synthetic lethal has at least two chromosomal components, which must be together in a single chromosome to manifest their lethal effect. The synthetic lethal being $--$, the normal chromosomes will be $++$, $+-$ and $-+$. The heterozygotes $+-/-+$ will give rise to synthetic lethals by recombination. This has been demonstrated by GIBSON and THODAY (1962). If a heterozygote $---/+--$ or $---/++$ is studied and the difference between the two chromosomes is in a single element, the result of the analysis will show the lethal effect to be due to a single locus. However, if chromosomes derived from a double heterozygote are analysed, the lethal is found to be due to two elements and therefore to be a synthetic lethal. This was clearly pointed out by THODAY (1963) who says: "Genuine synthetic lethals therefore may often, though produced by recombination, behave in subsequent tests as point mutations". A situation of this type was found by GIBSON and THODAY (1962).

The data reported in the present paper show that the lethals in the heterozygotes n_1/r_1 and r_1/r_2 behave in the analysis as unilocal lethals. However the same chromosomes n_1 and r_2 in the heterozygotes n_1/n_2 and n_2/r_2 behave as synthetic. The lethal chromosome n_1 is $--$ while the homologous region of r_1 is $+-$ or $-+$ and that of n_2 is $++$. The same happens with the lethal chromosome r_2 which is $--$, while the homologous region is $+-$ or $-+$ in r_1 and $++$ in n_2 . These results show that a given lethal chromosome will either behave as having a synthetic lethal, or as having a single-locus lethal, according to the contents of the other chromosomes used in the tests. Synthetic or single-locus lethals are not absolute but relative categories. Any lethal is probably both a single-locus lethal and also a synthetic lethal depending on the rest of the genic constellation.

It is interesting to note that the lethal chromosomes n_1, n_2, n_3, n_4 were isolated from natural populations, while r_1, r_2, r_3, r_4 are radiation induced, and in both classes, there are chromosomes which behave both as single locus and synthetic lethals.

These results put the problem of the synthetic lethals in a new perspective. Data on the frequency of synthetic lethals are already available with good approximation: 4.9% and 3.3% in *D. pseudoobscura* (SPASSKY *et al.* 1958), 5.0% and 6.4% in *D. prosaltans* (DOBZHANSKY *et al.* 1959), 15% in *D. willistoni* from the island of São João (MAGALHÃES unpublished). However, the relation between the synthetic lethals and the maintenance of the populational variability has not been well understood.

DA CUNHA and collaborators (in preparation) have found, in population cages, that the rate of appearance of new lethals is much higher than expected from mutation alone. Frequencies of new lethal mutations as high 21.3%, 24.6% and 20.7% have been found after seven generations. The rate of accumulation of new lethals is not uniform in the eight populations studied. MAGALHÃES *et al.*

(1965) have also found that suppressors of lethals may be very frequent in populations. The lethals with their suppressors constitute a type of synthetic lethal, as the lethal effect depends on two loci. Synthetic lethals made of a lethal and its suppressor are one of the possible types of synthetic. MAGALHÃES (unpublished) analysing the backcrosses of many synthetic lethals with the parental chromosomes has not found lethality. His results show that the synthetics that are due to the interaction of two elements, neither of which is lethal by itself, are the most frequent. "Synthetic" phenomena are not restricted to lethals, as shown by KRIMBAS (1960), who found synthetic sterility in *D. willistoni*.

All those data indicate that synthetic lethals are very important in the storage of variability in natural populations. Chromosomes which are normal when homozygous produce lethals through recombination, while lethal chromosomes revert to normality through recombination in heterozygotes. The resulting dynamic equilibria permit a selective integration of the variability, being one of the sources of the coadaptation within the gene pools. The relativity implied in the synthetic mechanism gives to the population a much higher plasticity owing to the higher capacity of absorption, storage and release of variability than would be obtained with independent and noninteracting point mutations. The integrations of those genes which affect lethality, due to their relativity, must be very similar to that of polygenes as indicated by MATHER (1943). It is possible that phenomena such as the long range position effect found by GIBSON and THODAY (1962) are due to the integration of genes which affect viability, probably comprising most of the genes, within a chromosome. The far distant genes which seem to interact to produce lethality, are in reality only markers which limit the chromosome pieces involved in lethality. If the genes are integrated in the chromosome, the larger the pieces interchanged between the organized chromosomes the greater the deleterious effects of recombination on viability. The apparent long range position effect may be a deleterious effect due to the disruption produced by recombination within organized chromosomes. The coadaptation of the gene complexes kept intact by paracentric inversions, so common in *Drosophila* populations (see DA CUNHA 1960 for a review), may be due to "synthetic" mechanisms. Inversions have high adaptive values because they prevent destruction, by recombination, of the gene patterns of high viability produced by the recombination mechanism.

Synthetic lethality, synthetic sterility, and all kinds of coadaptations should be much more widespread and important in outbreeding than in inbreeding populations. The data on synthetic lethals obtained in several species of *Drosophila* point exactly in this direction. The rarity or absence of synthetic lethals in *D. melanogaster*, mentioned above, is possibly due to the inbred origin of most populations of this domestic species.

The synthetic lethals are an evidence of integration of the genes within the chromosomes, within the genotypes and within the gene pools. Probably all the genes are integrated and coadapted in systems like those of the synthetic lethals. The lethality of the synthetic systems studied is only a negative aspect of a

coadapted system which has positive adaptive value. For methodological reasons only the negative aspects have been studied so far.

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SUMMARY

The analysis of several natural and radiation induced lethal chromosomes is reported. The same chromosome may behave either as though its lethality were due to a single locus, or as a synthetic lethal, depending upon which chromosome it is tested against. Synthetic lethals are lethal chromosomes produced by recombination between nonlethal chromosomes. Unilocal lethality or synthetic lethality are relative to the rest of the gene constellation, and are not absolute characters. A method is presented for analyzing chromosomes with regard to the locations of lethality factors and their interrelations.

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