By determining just what feature of the chemistry of peroxides is responsible for their mutagenic action one might hope to shed light on the nature of the mutation process. It seems unlikely that this action is simply related to oxidizing power, since oxidizing agents are common and organic peroxides are not especially effective ones. Of more interest is the characteristic decomposition of peroxides by which free radicals are produced. If this is the essence of peroxide action non-peroxidic free radical sources (e.g., diazomethane) should show similar effects. It should be noted that irradiation of a cell could produce free radicals directly as well as by peroxide formation.

Besides affording a basis for speculation on the nature of the mutation process, the discovery of the mutation-inducing power of organic peroxides substantially increases the number of known mutagenic agents. Organic peroxides of widely varied structure can be prepared. It will be of interest to compare the action of these various agents on different genes and to search for agents having pronounced effects on particular genes.

* This investigation was supported in part by a research grant from Merck and Co.

† DuPont Predoctoral Fellow in Chemistry 1948–1949.

‡ The authors wish to thank Professor Norman H. Horowitz for valuable assistance and advice.

[1] Wyss, O., Stone, W. S., and Clark, J. B., *J. Bact.*, **54**, 767–772 (1947).

[2] Wyss, O., Clark, J. B., Haas, F., and Stone, W. S., *J. Bact.*, **56**, 51 (1948).

[3] Milas, N. H., *J. Am. Chem. Soc.*, **53**, 221–233 (1931).

[4] Giles, N. H., Jr., and Lederberg, E. Z., *Am. J. Bot.*, **35**, 150–157 (1948).

[5] In an unpublished investigation this method for detecting chemical mutagens was developed by M. Westergaard and H. K. Mitchell to whom the authors are also indebted for the stock.

[6] Delbrück, M., *Ann. Mo. Bot. Gard.*, **32**, 223–233 (1945).

[7] Hadorn, E., Rosin, S., and Bertani, D., *Proc. 8th Int. Congr. Gen.*, 256–266 (1949).

[8] Rapoport, I. A., *Acad. Sci. U.R.S.S.*, **54**, 65–67 (1946).

[9] Dickey, F. H., Rust, F. F., and Vaughan, W. S., *J. Am. Chem. Soc.*, **71**, 1432–1434 (1949).

CROSSING-OVER BETWEEN ALLELES AT THE LOZENGE LOCUS IN DROSOPHILA MELANOGASTER

BY M. M. GREEN AND K. C. GREEN

DEPARTMENT OF ZOOLOGY, UNIVERSITY OF MISSOURI, COLUMBIA, MISSOURI

Communicated by L. J. Stadler, July 22, 1949

It has been reported that females of *Drosophila melanogaster* having one *X*-chromosome containing the lozenge allele glossy (lz^g) and the other *X*-chromosome the lozenge allele spectacle (lz^s), crossed to either lz^g or lz^s males, occasionally produce individuals wild type (lz^+) in appearance.[1,2]

The occurrence of wild-type progeny from this cross is unexpected since all the progeny of both sexes should have the phenotype of either lz^s or lz^g. Furthermore, it has been noted that the production of the non-mutant X-chromosome has resulted when crossing over has occurred in the vicinity of the lozenge locus. These results suggest that either the lz^+ chromosome occurred as a result of unequal crossing over between lz^g and lz^s in the same manner as Bar in *D. melanogaster*,[3] or that lz^g and lz^s are in fact not alleles in the usual sense but represent two closely linked loci, such that an ordinary crossover between them would yield an lz^+ chromosome.

In view of the information already obtained, experiments were set up to determine (1) whether the phenomenon of crossing over between lz^g and $lz^s \rightarrow lz^+$ was peculiar to these lozenge alleles, or whether it occurred between other lozenge mutants as well; (2) whether the crossing over involved is equal or unequal; and (3) what is the nature of the complementary crossover X-chromosome, presumably bearing two lozenge mutants.

TABLE 1

RECOMBINATION RESULTS FROM ♀ ♀ HETEROZYGOUS FOR TWO DIFFERENT LOZENGE ALLELES CROSSED TO ♂ ♂ *In(1)dl 49, v lz*^{g}

GENOTYPE OF ♀ ♀	TOTAL F_1 ♂ ♂ AND ♀ ♀	lz^+ ♂ ♂ AND ♀ ♀	lz^s-LIKE ♂ ♂	PER CENT CROSSING-OVER*
$lz^{BS}/sn^3\ lz^{46}\ ras^4\ v$	20,554	9†	5‡	0.09
$lz^{BS}/ec\ ct^6\ lz^g\ v\ f$	16,255	13§	5‖	0.14
$lz^{46}/ec\ ct^6\ lz^g\ v\ f$	16,098	4§	3‖	0.06

* See text for method of calculation.
† All carried sn^3. ‡ All carried $ras^4\ v$.
§ All carried ct^6. ‖ All carried v.

Three lozenge alleles were used in attempting to answer these questions: lz^{BS}, which occurred as a result of X-irradiation in $T(1:4)B^S$ and which has been separated from the translocation; lz^{46}, which is of spontaneous origin, and lz^g, which is the same mutant referred to above except that it has been extracted from *In(1)dl 49* where it originally occurred as a result of X-irradiation. All these mutants are typical lozenge alleles, recessive to wild type, and phenotypically characterized by derangement of the eye facets, reduction in the eye pigment and female infertility related to the absence of spermathecae.[2, 4, 5] All possible heterozygotes of the three alleles (e.g., lz^{BS}/lz^{46}) are lozenge in phenotype and difficult to separate phenotypically from either homozygote. Compounds among the three mutants were made and back-crossed to males of the genotype *In(1)dl 49, v* lz^g. The results are listed in table 1.

From the results tabulated in table 1, it may be noted that in the cross of females $lz^{BS}/sn^3\ lz^{46}\ ras^4\ v\ X$ males *In(1)dl 49, v* lz^g (where sn^3 lies 6.7 units to the left and ras^4 and v lie 5.1 and 5.3 units, respectively, to the right of

lozenge) two unexpected classes of offspring occur. One class is wild type in appearance and carries the marker gene sn^3. These individuals are phenotypically identical to the lz^+ flies recovered from females lz^g/lz^s. The second class comprises individuals which carry the marker genes ras^4 and v and are phenotypically completely separable from both lz^{BS} and lz^{46}, and are in fact identical to lz^s in phenotype. While lz^+ being dominant to both lz^{BS} and lz^{46} was recovered in both males and females, the lz^s-like individuals were males only. The failure to recover the lz^s-like chromosome in females stems from its phenotypic inseparability when compounded to lz^{g3} consequently only one-half the expected number were found. This accounts for the discrepancy between the lz^+ and lz^s-like individuals in table 1 which theoretically should be equal in frequency.

It may be reasoned that the lz^+ flies carry an X-chromosome which as the result of crossing over carries wild-type alleles for both lz^{BS} and lz^{46}, while the lz^s-like flies represent the complementary crossover with lz^{BS} and lz^{46} on the same X-chromosome. If this is the case, then it should be possible to recover both lz^{BS} and lz^{46} separately in the progeny of females of the genotype lz^+/lz^s-like. That this is the case may be determined from the following results. Females were constituted having one X-chromosome carrying lz^s-like (presumably lz^{BS} and lz^{46} together) plus the marker genes ras^4 and v, and the other an lz^+ X-chromosome (derived from crossing over between lz^{BS} and lz^{46}) bearing the marker gene sn^3. Among 12,900 male offspring from these females, three males of the genotype $sn^3\ lz^{46}\ ras^4\ v$ and four males lz^{Bs} were recovered. These results support the hypothesis that the lz^+ chromosome carries wild-type lozenge alleles and the lz^s-like chromosome carries both lz^{BS} and lz^{46} and therefore might be designated as $lz^{BS,\ 46}$. That the lz^+ chromosome does contain only wild-type lozenge alleles is supported by one additional experimental finding. Females were constituted having one X-chromosome carrying $lz^{BS,\ 46}\ ras^4\ v$ and one chromosome derived from the Canton-S wild-type stock and carrying the marker genes sn^3 and v. Among 9100 male offspring of these females, one male $sn^3\ lz^{46}\ ras^4\ v$ and three males $lz^{BS}\ v$ were recovered, thereby confirming the identity of the lz^+ chromosome with a wild-type X-chromosome and therefore as bearing only wild-type lozenge alleles.

The results reported thus far indicate that crossing-over is not peculiar to the alleles lz^g and lz^s since it occurs also between other alleles, lz^{BS} and lz^{46}. They indicate also that the crossing-over between alleles is equal and not unequal as in the case of Bar, for the following reasons. First, in no instance among many thousands of flies examined have unexpected individuals (lz^+ or lz^s-like) been observed from females homozygous for lz^{BS} or lz^{46}. Second, the crossover individuals resulting from females lz^{BS}/lz^{46} are directed in so far as the marker genes are concerned. All lz^+ individuals carry the marker sn^3 while all $lz^{BS,\ 46}$ individuals carry ras^4 and v. If un-

equal crossing-over were involved then one would expect to get lz^+ flies carrying either sn^3 or $ras^4\ v$, and not always and only sn^3 as noted here. Similarly, $lz^{BS,\ 46}$ flies would be expected to carry either sn^3 or ras^4 and v, but not solely ras^4 and v. Consequently, it seems reasonable to conclude that regular crossing-over has occurred between two closely linked genes, with lz^{BS} located just to the left of lz^{46}.

It may be noted in the cross of females $lz^{BS}/ec\ ct^6\ lz^g\ v\ f\ X\ In(1)dl\ 49,\ v\ lz^g$ males (where *ec* and ct^6 lie 22.2 and 7.7 units, respectively, to the left and *v* and *f* lie 5.3 and 29 units, respectively, to the right of lozenge) that here too unexpected progeny of two types were obtained (cf. table 1). The lz^+ group is phenotypically identical to the lz^+ flies recovered from females lz^{BS}/lz^{46}, while the complementary crossover individuals, presumably carrying both lz^{BS} and lz^g on the same chromosome, are phenotypically inseparable from flies $lz^{BS,\ 46}$. As in the case of lz^+ flies from females lz^{BS}/lz^{46}, the lz^+ flies derived from females lz^{BS}/lz^g all carry the left marker gene proximal to lozenge, namely ct^6. Similarly, the flies presumably $lz^{BS,\ g}$ carry the marker proximal to lozenge on the right, namely *v*.

These results indicate that the mutant lz^g lies to the right of lz^{BS}. That lz^g also lies to the right of the lz^{46} and represents a third locus in the lozenge group of mutants may be readily deduced from the results obtained when females $lz^{46}/ec\ ct^6\ lz^g\ v\ f$ are crossed to males $In(1)dl\ 49,\ v\ lz^g$. It can be seen that once again two unexpected classes of progeny are obtained (cf. table 1). The lz^+ individuals are phenotypically identical to the lz^+ flies referred to previously and in this cross all carry the marker gene ct^6. The second class of unexpected individuals may again be described as lz^s-like and presumably carry both lz^{46} and lz^g on the same chromosome. In addition all such flies recovered carry the marker gene *v*. On the basis of the directed distribution of the marker genes to the lz^+ and the lz^s-like individuals, it seems logical to represent lz^g as lying to the right of lz^{46}.

Discussion.—These observations may be fitted into a scheme which specifies that the lozenge allelic series is genetically divisible into at least three closely linked loci in the order of lz^{BS}–lz^{46}–lz^g, proceeding from left to right along the *X*-chromosome. The genetic interval separating lz^{BS} and lz^{46} is of the order of 0.09% crossing-over, and the interval separating lz^{46} and lz^g is of the order of 0.06% crossing-over. (The intervals have been calculated after first doubling the crossovers giving two lozenge genes in the same *X*-chromosome since, as noted previously, only one-half of these could be recovered.) If this interpretation is correct, then a wild-type *X*-chromosome may be represented as carrying three wild-type genes each allelic to one of the three lozenge mutant genes. With this as a basis, the *X*-chromosome bearing each of the mutant genes may be represented genotypically as follows: $lz^{BS}\ +\ +$; $lz^{46}\ +$; and $+\ +\ lz^g$.

Phenotypic differences have been noted with combinations of any two

of the lozenge mutants depending on whether the mutants ard located together on the same X-chromosome or separately on homologous X-chromosomes. Using the genotypic notation proposed, the following combinations of mutant genes have been compared and their phenotypes recorded after each genotype:

lz^{BS} + +/+ lz^{46} + (mutant); lz^{BS} lz^{46} +/+ + + (wild type)
+ lz^{46} +/+ + lz^{9} (mutant); + lz^{46} lz^{9}/+ + + (wild type)
lz^{BS} + +/+ + lz^{9} (mutant); lz^{BS} + lz^{9}/+ + + (wild type)

It may be noted that in all cases the number of genes, mutant and wild type, is identical. Furthermore in all cases each mutant gene is balanced by a wild-type allele on the homologous X-chromosome. Yet when a lozenge mutant is present on each homologous X-chromosome, the wild-type alleles behave as recessive genes (or the lozenge mutants act as dominant genes), but when the same lozenge mutants are located together on the X-chromosome (even when separated by a wild-type allele) and the homologous X-chromosome carries only wild-type alleles, the wild-type alleles together act as dominant genes (or the lozenge mutants behave as recessives). These dominance relationships may be interpreted as being the result of a position effect. Similar observations have been made in the case of the interactions of the pseudoalleles Star and asteroid in *D. melanogaster*.[6]

A further conclusion which may be drawn in the light of additional information (unpublished) is that the three lozenge loci represent a reduplication of essentially identical genetic material. A number of other lozenge alleles (10 of the 14 which we possess) have been analyzed to determine to which locus they may be assigned.[7] While this analysis is incomplete, the data obtained thus far permit three generalizations which support this conclusion. In the first place, it is clear that the mutants at each locus possess the same array of phenotypic effects, vis., alteration of eye color and structure, infertility of females, etc. Secondly, if the mutants are classified quantitatively (e.g., with respect to the amount of red eye pigment formed), it can be seen that they are distributed at random to the three loci. Lastly, there appears to be no correlation between the mode of origin of the various mutants (i.e., spontaneous, x-ray induced) and their position. These findings are somewhat at variance with the observations made in cotton where three closely linked loci determining anthocyanin pigmentation have been described.[8, 9] In the latter case, although the three loci are in some instances interdependent, it cannot be said that all three possess the same array of phenotypic effects, suggesting that these loci represent three closely linked though not necessarily identical genes.

In addition to the cases cited, additional occurrences of crossing-over between what are apparently alleles have been observed in maize[10] and Drosophila,[11] and possibly in the case of the Brachy series in mice.[12] Just how these cases relate to the lozenge series is not at the present time com-

pletely clear, but it may be that all represent further examples of duplication or reduplication of the same genic material. It is not possible at present to extrapolate the data of the lozenge series to other allelic series. However, they do not justify the conclusion that all multiple allelic series represent cases of multiple loci, for the simplest explanation for the situation within the lozenge complex itself would appear to be that of three closely linked multiple allelic series.

Summary.—1. Crossing-over has been observed between three sex-linked, recessive lozenge alleles, lz^{BS}, lz^{46}, lz^{9} in *D. melanogaster*.

2. From females heterozygous for any two of the mutants, wild type *X*-chromosomes and *X*-chromosomes bearing two lozenge mutants have been recovered.

3. On the basis of the crossover results, the lozenge allelic series may be subdivided into three closely linked loci.

Acknowledgment.—The authors are indebted to Drs. C. P. Oliver, H. J. Muller and E. Novitski for making available mutants used in these experiments.

[1] Oliver, C. P., PROC. NATL. ACAD. SCI., **26**, 452–454 (1940).
[2] Oliver, C. P., and Green, M. M., *Genetics*, **29**, 331–347 (1944).
[3] Sturtevant, A. H., *Ibid.*, **10**, 117–147 (1925).
[4] Oliver, C. P., *U. Texas Publ.*, **4720**, 167–184 (1947).
[5] Green, M. M., *Am. Natl.*, **82**, 188–192 (1948).
[6] Lewis, E. B., *Genetics*, **30**, 137–166 (1945).
[7] Green, M. M., and Green, K. C., unpublished.
[8] Stephens, S. G., *Genetics*, **33**, 119–214 (1948).
[9] Yu, Chi Pao, and Chang, Te Sung, *J. Genetics*, **49**, 46–56 (1948).
[10] Laughnan, J. R., PROC. NATL. ACAD. SCI., **35**, 167–178 (1949).
[11] Lewis, E. B., *Genetics*, **33**, 113 (1948).
[12] Dunn, L. C., and Caspari, E., *Ibid.*, **30**, 543–568 (1945).

EXPERIMENTS ON LIGHT-REACTIVATION OF ULTRA-VIOLET INACTIVATED BACTERIA

BY A. NOVICK AND LEO SZILARD

INSTITUTE OF RADIOBIOLOGY AND BIOPHYSICS, UNIVERSITY OF CHICAGO

Communicated by H. J. Muller, August 15, 1949

Many types of microorganisms are killed by ultra-violet light and the number of survivors falls off with increasing dose. A. Kelner[1] reported recently on his discovery that, if exposure to ultra-violet light is followed by exposure to visible light, the number of survivors is very much larger. A similar discovery was reported by R. Dulbecco[2] for bacteriophage.