BACK MUTATION IN DROSOPHILA MELANOGASTER. II. DATA ON ADDITIONAL YELLOW AND WHITE MUTANTS

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THE previously reported X-ray-induced back mutations of the recessive sex-linked mutants yellow-2 (y^2), scute (sc) and white-apricot (w^a) (GREEN 1961a) supplemented and extended existing data for *Drosophila melanogaster* on induced back mutations of the forked mutants f^1 and f^{3N} and white-ivory (w^i) (MULLER and OSTER 1957; GREEN 1959a; LEFEVRE and GREEN 1959; LEWIS 1959) and leave little doubt that X rays can induce back mutations. An appraisal of all of these data suggests the following general conclusions. First, those mutants which spontaneously back mutate are the more prone to induced back mutation. Second, the frequency of X-ray-induced back mutation is a characteristic of the particular mutant rather than of the locus. Third, the qualitative nature of the induced back mutant, i.e., whether full or partial, is also a property of the individual mutant. Fourth, the back mutation pattern of partial back mutants appears not to differ from that of the mutants from which they arose. Fifth, premeiotic cells are more back mutation sensitive than are meiotic cells.

The data of TIMOFÉEFF-RESSOVSKY (1933, 1939) conflict with the fourth generalization. He reported that whereas back mutation to wild type could be induced after irradiating white-eosin (w^e) males, comparable back mutants could not be obtained from w from which w^e arose. Furthermore, his recovery of a substantial number of back mutations after irradiating males is contrary to the fifth generalization. Therefore, back mutation studies of w^e and equivalent partial back mutations of w were undertaken. Additional yellow body color (y) mutants and the mutant acheate (ac) for which GOLDAT (1936) had recovered a single induced back mutant were simultaneously studied.

MATERIALS AND METHODS

Three spontaneous, partial back mutations of the original w mutant were available for study: w^e, w^{e2}, and w^h. Since mutants phenotypically equivalent to w^e and w^{e2}, especially their nondosage compensation property, are extraordinarily rare, it is very likely that either w^e or w^{e2} is identical to the w^e of TIMOFÉEFF-RESSOVSKY. Stocks obtained from the California Institute of Technology were those originally maintained by C. B. BRIDGES. Genetical evidence has already been presented which establishes the spatial (recombinational) identity of w^e,

w^{e2}, and w^h (GREEN 1959b). In addition, the mutant wch recombinationally and phenotypically inseparable from w^e or w^{e2} but which arose by direct mutation and the mutant w^{bf} for which one instance of spontaneous back mutation has been described (REDFIELD 1952) were included for study. Among the y mutants, the following were included: y^{519}, a y^2-like mutant of spontaneous origin (RED-FIELD 1952) which recombinationally appears to be identical to y^2 (GREEN 1961b); y^{59b}, a y^1-like mutant derived from y^2 by X rays (GREEN 1961b); y^{50k}, an X-ray-induced y^1-like mutant; and y^{60b}, a y^1-like mutant of X-ray origin occurring as a mosaic male among the progeny of X-rayed wild-type males crossed to attached-X females. Procedures were identical to those employed previously (GREEN 1961a) and can be summarized as follows. Attached-X females of the following homozygous genotypes were synthesized: $y^{59b} w^e$, $y^{60b} w^{e2}$, $y\ ac\ w^{ch}$, $y^{519} w^h$ and $y^{50k} w^{bf}$. In addition, a quintuplication of the w locus carrying five of the same w mutants on one X chromosome was kindly made available by PROFESSOR E. B. LEWIS. This quintuplication, designated $Qn(w)_5$, was introduced and made homozygous in attached-X females such that ten w mutants can be simultaneously tested for back mutation. The w mutant is either a recurrence of the w mutant studied earlier or identical to it (GREEN 1961a) and is allelic to w^e. Females were irradiated with 5000r X rays and crossed to males of identical genotype. Their female progeny were scored for back mutations as were the males which served as controls. Where exceptions were found, they were progeny tested and subjected to genetic tests as will be outlined below.

<div align="center">RESULTS</div>

In Table 1, the results of a series of irradiation experiments have been summarized. Contrary to the findings of TIMOFÉEFF-RESSOVSKY, no back mutations of w^e, w^{e2}, w^h and wch were found. These experiments, considered together, are more extensive in scope than those reported by TIMOFÉEFF-RESSOVSKY, and had back mutation occurred at a rate comparable to the five reversals per 89,583 chromosomes he reported for w^e, some few should have been found. Of interest is the fact that a number of cream-like mutants, diluters of w^e, w^{e2} and w^{ch}, were found. The failure to recover back mutations of w means that very likely the w mutant studied is not identical to the w mutant studied by TIMOFÉEFF-RESSOVSKY.

<div align="center">TABLE 1</div>

Recovered back mutations following irradiation of attached-X females (dose = 5000r)

Genotype attached-X females	Number back mutations			Total treated genes
	y	ac	w	
$y^{59b} w^e$	.	.	.	101,240
$y^{60b} w^{e2}$	.	.	.	90,638
$y\ ac\ w^{ch}$	.	.	.	97,918
$y^{519} w^h$	.	.	.	168,322
$y^{50k} w^{bf}$	1	.	2	190,230
$Qn(w)_5$	.	.	.	349,520

Of the white mutants studied, only apparent back mutations of w^{bf} were induced. The two found appear to be phenotypically identical, approaching wild type when homozygous. However, they are on phenotypic grounds easily distinguishable from the near wild-type back mutants of w^a and from the spontaneous w^{bf} back mutation found by REDFIELD. The compounds of the different back mutants and w are especially easy to separate from each other. Genetic tests as outlined previously (GREEN 1961a) indicate that the two w^{bf} reversals are free of any apparent minute structural alterations and are delimited to the w^{bf} locus. In addition to the back mutants, a dominant autosomal suppressor of w^{bf} occurred in these experiments.

The comparatively low frequency of induced w^{bf} reversals necessitated the undertaking of complementary experiments to support the reversibility of this mutant. The near white eye color phenotype of w^{bf} as compared to the dark color of the reversals, suggested that a study of induced somatic reversals was both possible and probably profitable. Based on the observations of LEWIS (1959) who has shown that w^i which undergoes both spontaneous and induced back mutation germinally also reverses somatically following X-irradiation, a number of predictions were suggested. Since induced back mutations of w^{bf} are appreciably less frequent than those of w^i (2/190,000 after 5000r vs. 8–9/120,000 after 4000r), parallel somatic back mutation frequencies may be expected. Based upon the phenotypes of females w^{bf}/w^{bf} reversal and w^i/w^i reversal—the former with a clear reddish cast, the latter essentially wild type—the presumed somatic back mutations of w^{bf} should also be phenotypically separable from those of w^i.

Concurrent somatic back mutation experiments of w^{bf} and w^i were conducted in the following way. Fertilized eggs of the appropriate genotypes were collected during ten hour periods at a room temperature of 22–24°C. Twenty-four hours later the hatched larvae were irradiated *in situ* with 1000r and transferred to bottles with fresh media. Upon emergence, the adults were scored for mosaic eye color spots which presumably arose as a consequence of induced somatic back mutation. The results of these experiments are listed in Table 2 and show that one anticipated result was realized. Thus w^{bf} mosaics occur appreciably less frequently than do w^i mosaics in the ratio of ca. 1:5, roughly paralleling the germinal back mutation rates. Phenotypically, the w^{bf} reversal spots were clearly separable from those of the w^i reversals.

Among the y mutants tested, only a single reversal, that of y^{5ok}, was found. This reversal was found to be devoid of any obvious structural change and may therefore be considered a genuine back mutation. It is of interest since it repre-

TABLE 2

Frequency somatic back mutations induced in larvae 0–36 hours old (dose = 1000r)

Genotype treated larvae	Number mosaic flies/total scored	
	Females	Males
$y^{5ok}w^{bf}$	10/7,387	7/6,069
$w^i f^3$	8/1,027	4/912

sents a case of back mutation of an X-ray-induced mutant. Such an event is not particularly unusual since DE SERRES' (1958) detailed demonstration of X-ray reverse mutability of X-ray-induced mutants at the ad-3 loci in Neurospora.

The failure to obtain back mutations of y^{519} deserves brief comment. There is evidence that y^{519} spontaneous in origin and phenotypically resembling y^2 is also probably allelic to y^2 (GREEN 1961b). One dominant sex-linked suppressor, loosely linked to y^{519} was found. The failure to obtain reversals of y^{519} compared to the frequent y^2 reversals means that phenotype and locus are not valid criteria for predicting the potential reversibility of mutants.

No back mutants of ac were found, which was not entirely unexpected since GOLDAT (1936) found but a single reversal.

No instance of spontaneous back mutation of all mutants studied among the male controls equivalent in number to the treated females was found.

<div align="center">DISCUSSION</div>

Since the data presented support the several conclusions considered in detail previously (GREEN 1961a), an extended discussion appears unnecessary here. A few points, however, merit reemphasis. Among the several w mutants studied, a minimum of four distinctive back mutation patterns have been demonstrated. A majority of the w mutants studied make up one class and appear to be refractory to X-irradiation, having thus far yielded neither spontaneous nor X-ray-induced back mutations. These findings need not be interpreted to mean that these mutants are incapable of back mutation. Because spontaneous and/or induced back mutation frequencies may be very low, negative results where between 10^5 and 10^6 genes are scored must be considered as tentative. Furthermore, while a particular mutant may be refractory to X rays, it might yield significant numbers of back mutants following treatment with some other mutagen. Each of three w mutants viz., w^a, w^{bf} and w^i for which back mutants have been obtained is distinctive. These mutants may be readily distinguished from each other either by the frequency with which their back mutants occur or, as judged by the phenotype of the reversals, the qualitatively distinctive states to which they each back mutate. If the ability to back mutate is a function of the specific change in the genetic material (DNA) characteristic of the mutated gene—a conclusion which currently would not be seriously challenged—it follows that differential back mutation patterns reflect qualitatively distinct alterations in the DNA. At a minimum, four types have been found. Clearly this is an underestimate of the possible types of alterations which must occur within a delimited segment of DNA.

The data also reinforce the conclusion that the phenotype produced by a mutant provides no reliable clues either to the capabilities or state to which a specific mutated gene can back mutate. As other studies often have more elegantly shown, a wide variety of genetic alterations may occur within a delimited genetic segment (the locus!), all of which lead to the same end phenotype.

Although the data obtained for the y mutants are certainly less extensive than

those for the w mutants, they support all conclusions based on the w mutants and need no further amplification.

A reconciliation of the failure here to obtain back mutations of the mutants w^h, w^e, w^{e2}, and wch with the early success of TIMOFÉEFF-RESSOVSKY with w^e is indeed difficult. An additional difficulty is the fact that TIMOFÉEFF-RESSOVSKY obtained reversals after irradiating w^e males while it is becoming increasingly apparent that in Drosophila back mutations are more readily obtained from irradiating females than males (LEFEVRE and GREEN 1959; LEWIS 1959; GREEN, unpublished). Admittedly the discrepancy can be dismissed on the grounds that it cannot be unequivocably demonstrated that identical w^e mutants were used and the difference, therefore, rests in employing independent though phenotypically identical mutants. There is good reason to believe that this explanation is not completely satisfactory despite the lengthy time lapse between the two sets of experiments. In this connection, it is not clear precisely how to rationalize some of TIMOFÉEFF-RESSOVSKY's results with current knowledge of the genetical organization of the white locus. He reported (TIMOFÉEFF-RESSOVSKY 1933) recovering back mutations of w to w^e, w^{bl} and w^{bf} and of w^e to w^{bl} and w^+. Recombination analysis of a large number of w mutants (GREEN 1959b; MACK-ENDRICK and PONTECORVO 1952) shows that dosage compensating mutants such as w^{bl} are recombinationally distinct from noncompensating mutants like w^e. If by w^{bl} was meant a mutant equivalent to the standard w^{bl}, then it is difficult to see how w could mutate separately to both w^e and w^{bl} and how w^e could mutate to w^{bl}. Since it is not possible to clarify this nomenclature, a convincing, satisfactory interpretation of the back mutation data is for the present and foreseeable future very unlikely.

SUMMARY

Further attempts to obtain X-ray-induced mutations at the yellow and white loci in *Drosophila melanogaster* are reported. Of five independent white mutants tested, only w^{bf} yielded back mutants. Of the five yellow mutants studied, a single apparent reversal of the X-ray-induced mutant y^{50k} was found. The relationship of these data to previously reported back mutation data in Drosophila are discussed.

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