STERILITY, CHROMOSOME BREAKAGE, X-RAY-INDUCED MUTATION RATES AND DETECTED MUTATION FREQUENCIES IN *DROSOPHILA MELANOGASTER*[1]

G. LEFEVRE, JR.

Department of Biology, San Fernando Valley State College, Northridge, California 91324

Received September 8, 1966

AMONG the enduring problems of radiation genetics is the quantitative evaluation of mutational damage elicited in germ cells that have been exposed to a given dose of radiation. The significant question at issue is what proportion of all irradiated germ cells actually suffer an induced mutation, i.e., what is the *mutation rate*? This is the question that must be accurately answered before the consequences of exposure under different experimental conditions can be properly interpreted. The pertinent data emerging from mutation experiments reflect, rather, the proportion of the progeny of irradiated parents that are demonstrably mutant, i.e., the *detected mutation frequency*. There is, unfortunately, no assurance that the mutation rate and the detected mutation frequency are equivalent, or that a change in the mutation frequency reflects a change in the mutation rate.

In Drosophila, mutations can be identified among only those functional derivatives of mutated male germ cells that complete their maturation, are transferred to the female, are stored in the ventral receptacle or spermathecae, are successful in fertilization, and give rise to viable zygotes that, in turn, are capable of developing to the stage where a mutant phenotype can be expressed and identified. Then, if a mutant individual either dies before reproducing or is sterile, the investigator interested in obtaining quantitative data finds himself in the difficult position of being unable to validate his phenotypic classification. Now, he is faced with two alternatives: (1) count the mutant even though unverifiable, or (2) restrict himself to information that can be derived entirely from progeny which are both viable and fertile.

When sex-linked recessive lethal mutations are detected, all embarrassment is avoided; these mutations, by their very nature, can be detected *only* among those progeny that are both viable and fertile. Despite the fact that lethal changes result from both chromosome aberration and gene mutation, most studies on the mutagenic effects of radiation involve the detection of recessive lethal mutations. Not only are they numerous, but the question of the randomness of their distribution between the fertile and sterile classes of progeny does not arise. The question of their genetic nature is usually ignored.

[1] This investigation was aided by research grants from the Public Health Service (GM 07667 and GM 13631) and from the National Science Foundation (GB 2097).

The present study was undertaken to compare the frequency of sex-linked visible mutations detected in fertile progeny as compared with that detected in sterile progeny derived from male germ cells irradiated at different stages of maturity, thereby permitting an assessment of the quantitative validity of mutation data that are based entirely on fertile progeny. The results demonstrate that many types of visible mutants commonly detected in radiation experiments are, in fact, far more frequent among sterile than among fertile F_1 individuals. Further, the frequencies of both viable and lethal mutants that result from chromosome aberration are much more affected by the irradiation of different germ cell stages than is that of mutants with cytologically normal chromosomes.

MATERIALS AND METHODS

Two stocks provided all of the flies used in the present experiments. A mass-bred wild-type stock, M56i, originally obtained from Amherst College, was the source of males. The second stock, *y*^{2} *sc*^{8} *f dl-49 v w*^{a} (yellow-2, inversion scute-8, forked, inversion delta-49, vermilion, white-apricot) provided the females. All markers are sex linked and are fully described by BRIDGES and BREHME (1944).

Both virgin males and inseminated females of known age were irradiated with a Keleket constant-potential 250 kv X-ray machine, operated at 15 ma and provided with a 1 mm aluminum filter. Flies to be irradiated were placed in size 000 perforated gelatin capsules. At 45 cm target distance, the dose rate was approximately 300r/min. All exposures were timed to deliver 4000r and were carried out at room temperature.

After irradiation of males, successive broods were obtained by mating the males with a series of individual females. Inseminated females, after irradiation, were subcultured at three day intervals without males. Techniques and procedures followed for both male and female irradiations have been fully described in earlier publications (LEFEVRE and JONSSON 1964a, b; LEFEVRE 1965a, b; 1966); thus, they will not be repeated here.

The data presented in the publications just cited consist of sex-linked recessive lethal mutation frequencies detected at successive intervals after irradiation of virgin males of different ages which were subjected to different mating schedules, and after irradiation of inseminated females. Although not reported in these earlier studies, all F_1 females were examined for the presence of visible mutants at the *y*, *w*, *v* and *f* loci and for Notch (*N*) and white-mottled (w^m) mutations.

Each visibly mutant female was bred to appropriate males and was recorded as sterile or fertile. If fertile, the mutant was further classified as male viable or male lethal. Because every mutant was heterozygous for an inversion chromosome when found, mutants could not be safely classified as male lethal when no mutant F_2 males appeared until outcrosses had been completed. Then, any possible independent recessive lethal mutation could be separated from the mutant locus and its true nature assessed. Further, salivary chromosome analyses were carried out on all lethal mutants, including Notches.

Mutant F_1 females that failed to produce mutant progeny were recorded as "sterile." Reasons other then genetic sterility obviously contributed to the failure to obtain progeny from a mutant female. Occasionally, a female was lost in handling, was overetherized, or became stuck on the food surface and died. It is impossible to judge what proportion of all mutant females failed to produce progeny solely because of genetic sterility; however, the levels of sterility varied in a characteristic fashion from brood to brood. Thus, handling errors, which would be expected to be made at random throughout the experiment, could not have been responsible for all of the "sterility" encountered. Further, sterility, as defined above, was much higher in mutant than in nonmutant F_1 females, despite the fact that mutant females received careful and individual attention. Altogether, then, the relative frequency of failure to produce progeny can be taken to indicate the level of genetic damage associated with different kinds of mutants and with F_1

females derived from male germ cells irradiated at different stages of maturity. Hereafter, the term sterility is used in a broad sense: failure to produce progeny, for whatever reason.

As shown in the earlier studies, the frequency of sex-linked recessive lethal mutations detected at successive intervals after irradiation of adult males exhibits a variable but characteristic pattern, being initially high, then falling to about half that value before rising again 4 or 5 days after irradiation. This pattern suggested that the mutability of fully mature sperm is approximately twice that of immotile, immature sperm in the "bundle" stage, but that early spermatids again have a high mutability (LEFEVRE and JONSSON 1964a). The mutation frequency observed in any particular brood after irradiation was considered to reflect the relative proportions of the different germ cell stages sampled in that brood. For example, progeny which are obtained from the first mating after irradiation of 3-day-old males, and which show a high frequency of sex-linked lethal mutations, are derived from sperm that were fully mature at the time of irradiation; so, too, are progeny from the first three matings after irradiation of 7-day-old males. By contrast, no mature sperm are present when males are irradiated at emergence; thus, their first mating after irradiation gives rise to progeny which are derived from irradiated immature sperm, and which show a low frequency of lethal mutations.

Based on the frequency of lethal mutations present and its sequence in the mating schedule, every brood produced in the various earlier mutation experiments was assigned to one of the following categories: (A) mature sperm irradiated in females, (B) mature sperm irradiated in males, (C) mixture of mature and immature sperm, (D) immature sperm, (E) mixture of immature sperm and spermatids, and (F) spermatids. Mutants identified in each brood were thoroughly analyzed and their frequencies computed. The data are presented below.

RESULTS

The magnitude of the experiment: In Table 1 are recorded the total number of F_1 males and females counted in the successive brood categories following irradiation with 4000r. Column A relates to progeny derived from the irradiation of inseminated females; all mutants recorded for this class of progeny were produced in the stored mature sperm, not in eggs. Columns B through F relate to progeny produced in successive, but not necessarily daily, broods following irradiation of wild-type males, such that column B represents the most mature sperm

TABLE 1

F_1 offspring examined and mutants identified following exposure to 4000r of postmeiotic male germ cells at different stages of spermiogenesis

Brood categories	Mature sperm irradiated in ♀♀ A	Mature sperm irradiated in ♂♂ B	Mixture of mature and immature sperm C	Immature sperm D	Mixture of immature sperm and spermatids E	Spermatids F	All postmeiotic stages in ♂♂ (B-F)
Total F_1 males	20,516	19,035	35,690	81,334	25,284	5,522	166,865
Total F_1 females	22,351	19,000	36,736	84,748	26,222	5,718	172,424
Hyperploid males	25	32	47	56	50	14	199
Mutant females*							
1. *y, w, v, f*	43	38	43	93	36	7	217
2. w^m	4	9	7	11	5	1	33
3. *N*	33(1)†	33(10)	31(6)	48(14)	16(6)	3(1)	131(37)
4. Total mutants	80	80	81	152	57	11	381

* Including both fertile and sterile mutants.
† Figures in parentheses show number of *N* mutants that were simultaneously either *w* or w^m.

stages and column F the least mature surviving postmeiotic stages. In addition to the regular expected F_1 males and females (y^2 w^a v f males and wild-type females), hyperploid males and phenotypically mutant females were detected and their numbers are also recorded in Table 1.

Hyperploid males result from the fertilization of a normal egg by a sperm carrying a highly deleted X chromosome that retains only a short distal euchromatic section and proximal heterochromatin containing the centromere. In these experiments, y^+ w^a v f and y^+ w^+ v f males were classified as hyperploid. Such males, lacking a Y chromosome, are sterile so that their identification cannot be verified by genetic test.

All F_1 females that exhibited a phenotype differing from wild type at the marked loci, y, w, v or f, or that showed Notch wings or mottled eye pigmentation were identified and saved for breeding tests. Hereafter, only these individuals will be referred to, collectively, as "mutants." Although many other kinds of mutants of one sort or another, mostly dominant, were also found, they have not been recorded in Table 1. All recorded mutants were observed initially in F_1 females as they were examined; mutants identified only in the F_2 generation have been excluded from Table 1.

TABLE 2

Sterility of phenotypically normal and mutant F_1 females

Brood categories	Mature sperm irradiated in ♀♀ A	Mature sperm irradiated in ♂♂ B	Mixture of mature and immature sperm C	Immature sperm D	Mixture of immature sperm and spermatids E	Spermatids F	All postmeiotic stages in ♂♂ (B-F)
Phenotypically normal F_1 females							
No. examined	22,271	18,920	36,655	84,596	26,165	5,707	172,043
No. sterile	3,228	1,568	2,834	3,495	1,297	399	9,593
No. tested	21,457	13,452	34,200	52,262	18,890	4,164	122,968
(% sterile)	(15.04)	(11.66)	(8.29)	(6.69)	(6.87)	(9.58)	(7.80)
Calculated no. sterile	3,350	2,205	3,037	5,657	1,797	547	13,243
Calculated no. fertile	18,921	16,715	33,618	78,939	24,368	5,160	158,800
Phenotypically mutant F_1 females							
1. Non-Notch mutants							
No. sterile	23	22	18	46	14	2	102
No. tested*	47	47	50	104	41	8	250
(% sterile)	(48.9)	(46.8)	(36.0)	(44.2)	(34.1)	(25.0)	(40.8)
2. Notch mutants							
No. sterile	15	15	17	18	4	3	57
No. tested*	33	33	31	48	16	3	131
(% sterile)	(45.5)	(45.5)	(54.8)	(37.5)	(25.0)	(100.0)	(43.5)
3. All mutants							
No. sterile	38	37	35	64	18	5	159
No. tested*	80	80	81	152	57	11	381
(% sterile)	(47.5)	(46.3)	(43.2)	(42.1)	(31.6)	(45.5)	(41.7)

* All mutants found were tested.

The sterility of F_1 females: Insofar as possible, every emerging F_1 female, whether mutant or nonmutant, was tested for fertility. Table 2 presents the results of these breeding tests. Since it proved to be impossible to test literally all of the phenotypically normal F_1 females examined, a calculation has been made, based on the percent of tested wild-type females that proved to be sterile, of the number of originally examined wild-type females that were sterile. As more than 70% of all F_1 females were tested, this calculation provides a reasonably accurate measure of the incidence of sterility among the nonmutant daughters of males exposed to 4000r. The variation in sterility of the nonmutant F_1 females is plotted in Figure 1.

Every mutant female was tested. As Table 2 shows, both Notch and non-Notch mutant F_1 females were much more highly sterile than were nonmutant F_1 females. Further, in both phenotypically normal and mutant females, the amount

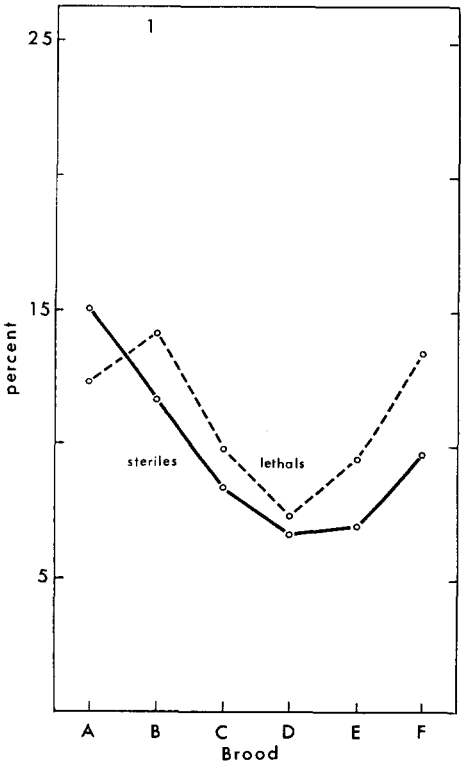

FIGURE 1.—The frequency of recessive sex-linked lethal mutations and the incidence of sterility exhibited by phenotypically normal F_1 females following exposure to 4000r of male germ cells at different stages of maturity. (Brood categories are the same as in Table 1; data from Tables 2 and 3.)

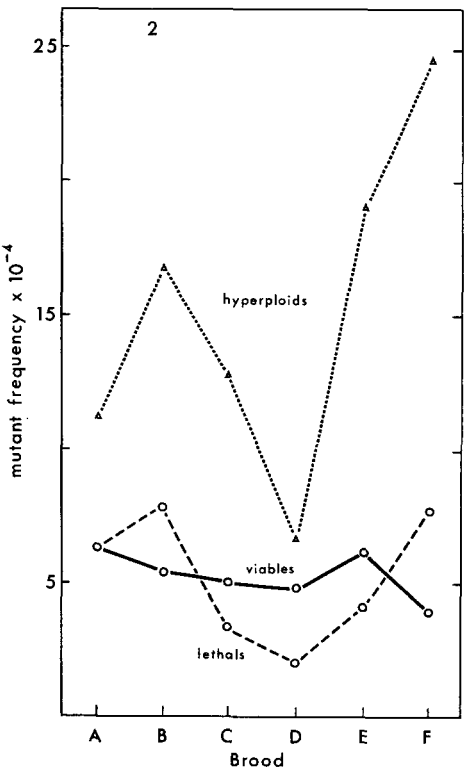

FIGURE 2.—The frequency of hyperploid males, and of both male-lethal and male-viable (white-mottleds excluded) mutants detected in breeding tests of fertile non-Notch F_1 mutant females following exposure to 4000r of male germ cells at different stages of maturity. (Brood categories are the same as in Table 1; data from Table 4.)

TABLE 3

Incidence of sex-linked recessive lethal mutations among fertile phenotypically normal and non-Notch mutant F_1 females

Brood categories	Mature sperm irradiated in ♀♀ A	Mature sperm irradiated in ♂♂ B	Mixture of mature and immature sperm C	Immature sperm D	Mixture of immature sperm and spermatids E	Spermatids F	All postmeiotic stages in ♂♂ (B-F)
Phenotypically normal F_1 females							
No. lethal	2,240	1,668	3,073	3,576	1,679	504	10,500
No. fertile	18,229	11,884	31,366	48,767	17,593	3,765	113,375
(% lethal)	(12.28)	(14.04)	(9.80)	(7.33)	(9.54)	(13.39)	(9.26)
Phenotypically mutant (non-Notch) F_1 females							
No. lethal	12	13	11	16	10	4	54
No. fertile	24	25	32	58	27	6	148
(% lethal)	(50.0)	(52.0)	(34.4)	(27.6)	(37.0)	(66.7)	(36.5)

of sterility observed in the different broods was not constant, tending to be higher among progeny derived from the most mature and least mature male germ cells than among progeny derived from germ cells irradiated at intermediate stages of maturity.

Sex-linked recessive lethal mutation frequencies: Those phenotypically normal females that proved to be fertile in the breeding tests permitted the detection of sex-linked recessive lethal mutations. Table 3 records the number and percentage of such lethals induced in male germ cells at different stages of maturity, and the values are shown graphically in Figure 1. Recessive lethal mutations were also identified in the tests of some of the fertile mutant F_1 females. Since the mutants detected included Notch, which always breeds as a recessive sex-linked lethal, lethal mutations produced only by non-Notch mutant females have been entered in Table 3. Quite similar patterns (but not levels) of lethal mutation frequencies were exhibited by both phenotypically normal and non-Notch mutant females.

Mutant frequency calculations: In Table 4, the frequencies of hyperploid males and of the various kinds of visible mutants have been calculated. The frequency of hyperploid males was calculated by dividing their number by the total number of X-chromosome-bearing male germ cells tested (i.e., total F_1 females plus hyperploid males). The frequencies of visible mutants, which can be identified in both fertile and sterile females, have been calculated in three different ways: (1) as the proportion of all mutants (both fertile and sterile) among all F_1 females examined (both normal and mutant); (2) as the proportion of fertile mutants among all fertile F_1 females; and (3) as the proportion of sterile mutants among all sterile F_1 females. These frequencies are shown for non-Notch mutants, for Notch mutants, and for all mutants.

In the particular case of fertile *y*, *w*, w^m, *v*, and *f* mutants, the mutation frequency has been partitioned to show, separately, the incidence of such mutants that bred as male viable and as male lethal. Since the distinction between these

TABLE 4

Frequencies of hyperploid males and visible mutants

Brood categories	Mature sperm irradiated in ♀♀ A	Mature sperm irradiated in ♂♂ B	Mixture of mature and immature sperm C	Immature sperm D	Mixture of immature sperm and spermatids E	Spermatids F	All postmeiotic stages in ♂♂ (B-F)
Hyperploid males							
No.	25	32	47	56	50	14	199
Total X chromosome	22,376	19,032	36,783	84,804	26,272	5,732	172,623
(frequency)	(11.2)	(16.8)	(12.8)	(6.6)	(19.0)	(24.4)	(11.5)
Non-Notch mutants							
(1) All *y,w,w^m, v, f*	47	47	50	104	41	8	250
All F_1 ♀♀	22,351	19,000	36,736	84,748	26,222	5,718	172,424
(frequency)	(21.0)	(24.7)	(13.6)	(12.3)	(15.6)	(14.0)	(14.5)
(2) Fert. *y, w,w^m, v, f*	24	25	32	58	27	6	148
Fert. F_1 ♀♀	18,963	16,758	33,664	79,027	24,407	5,166	159,022
(frequency)	(12.7)	(14.9)	(9.5)	(7.3)	(11.1)	(11.6)	(9.3)
a. ♂-viable (frequency)	(6.3)	(7.2)	(6.2)	(5.3)	(7.0)	(3.9)	(5.9)
b. ♂-viable, *w^m* excluded (frequency)	(6.3)	(5.4)	(5.0)	(4.8)	(6.1)	(3.9)	(5.1)
c. ♂-lethal (frequency)	(6.3)	(7.8)	(3.3)	(2.0)	(4.1)	(7.7)	(3.4)
(3) Ster. *y,w,w^m,v,f*	23	22	18	46	14	2	102
Ster. F_1 ♀♀	3,388	2,242	3,072	5,721	1,815	552	13,402
(frequency)	(67.9)	(98.1)	(58.6)	(80.4)	(77.1)	(36.2)	(76.1)
Notch mutants							
(1) All *N*	33	33	31	48	16	3	131
All F_1 ♀♀	22,351	19,000	36,736	84,748	26,222	5,718	172,424
(frequency)	(14.8)	(17.4)	(8.4)	(5.7)	(6.1)	(5.2)	(7.6)
(2) Fert. *N*	18	18	14	30	12	0	74
Fert. F_1 ♀♀	18,963	16,758	33,664	79,027	24,407	5,166	159,022
(frequency)	(9.5)	(10.7)	(4.2)	(3.8)	(4.9)	(0)	(4.7)
(3) Ster. *N*	15	15	17	18	4	3	57
Ster. F_1 ♀♀	3,388	2,242	3,072	5,721	1,815	552	13,402
(frequency)	(44.3)	(66.9)	(55.3)	(31.5)	(22.0)	(54.3)	(42.5)
All mutants							
(1) All mutants	80	80	81	152	57	11	381
All F_1 ♀♀	22,351	19,000	36,736	84,748	26,222	5,718	172,424
(frequency)	(35.8)	(42.1)	(22.0)	(17.9)	(21.7)	(19.2)	(22.1)
(2) Fert. mutants	42	43	46	88	39	6	222
Fert. F_1 ♀♀	18,963	16,758	33,664	79,027	24,407	5,166	159,022
(frequency)	(22.1)	(25.7)	(13.7)	(11.1)	(16.0)	(11.6)	(14.0)
(3) Ster. mutants	38	37	35	64	18	5	159
Ster. F_1 ♀♀	3,388	2,242	3,072	5,721	1,815	552	13,402
(frequency)	(112.2)	(165.0)	(113.9)	(111.9)	(99.2)	(90.6)	(118.6)
Freq. ster. muts. / Freq. fert. muts.	5.1	6.4	8.3	10.1	6.2	7.8	8.5

Mutant frequencies, given separately for non-Notch, Notch, and all mutants, show the proportion of: (1) all mutants among all F_1 females, (2) fertile mutants among fertile F_1 females, and (3) sterile mutants among sterile F_1 females. (All frequencies × 10^{-4}.)

two kinds of mutants can be made only when mutant F_1 females are fertile, no attempt has been made to calculate their proportions among all F_1 females or among sterile F_1 females. Any such calculation would require unverifiable assumptions about the genetic nature of sterile mutants.

The frequencies of hyperploid males and of both male-lethal and male-viable non-Notch mutations induced in the different male germ cell stages are presented in Figure 2. Of interest is the much greater variability in the frequency of genetic effects that are produced exclusively by chromosome breakage (hyperploid males) as compared with the frequency of recessive sex-linked lethals, some, but not all, of which are associated with chromosome breaks. SLIZYNSKI (1938) reported that 30 to 40% of induced and spontaneous sex-linked recessive lethals exhibit chromosome abnormalities detectable in salivary gland chromosome preparations. Of 238 randomly selected recessive sex-linked lethals not associated with any mutant phenotype that were analyzed in the present experiment, 63 (26.5%) had a gross chromosome aberration in the euchromatic region of the X chromosome. The frequency patterns of recessive lethals and male-lethal *y*, *w*, *v*, *f*, and w^m mutants are remarkably similar, as can be seen by comparing Figure 1 with Figure 2. Most of the latter, however, exhibit detectable chromosome aberrations. The frequency of male-viable mutations, few of which are associated with detectable chromosome aberration, is appreciably more uniform from stage to stage than is that of male-lethal mutations. With the white-mottled mutants, nearly all of which result from translocation, excluded from the male-viable mutant class, the mutation frequency, as seen in Figure 2, varies relatively little, regardless of stage irradiated.

DISCUSSION

Are mutation rates different when mature sperm are irradiated in the female and in the male? In Tables 3 and 4, detected mutation frequencies recorded in Column A (mature sperm irradiated in the female) are, with one exception, lower than the corresponding values in Column B (mature sperm irradiated in the male). The reduction is appreciable for hyperploid males and sterile mutants (Table 4); less pronounced for sex-linked recessive lethals (Table 2); and of dubious significance for fertile mutants, especially male-viable fertile mutants. In fact, with white-mottled mutants removed from the male-viable class, the frequency following female irradiation is actually somewhat higher than that observed in male irradiations. At the same time, the sterility of phenotypically normal F_1 females, as shown in Table 2, is appreciably higher in column A than in column B.

Since different categories of detected mutations do not respond similarly to irradiation of mature sperm in the female as compared with irradiation in the male, the results cannot be explained by an intrinsic difference in the mutability of mature sperm irradiated in the two environments, nor by a uniformly operating repair mechanism of greater efficiency in one environment than in the other. The effect is most pronounced on mutants that result exclusively from chromosome breakage and rearrangement (hyperploid males and white-mottleds); less

on mutants that result only in part from chromosome breakage (sex-linked lethals); and is not evident for mutants that are rarely associated with chromosome breakage (male-viable *y*, *w*, *f*, and *v* mutants). Therefore, chromosomal mutants produced in irradiated mature sperm must be subject to increased elimination when irradiated eggs are fertilized, presumably owing to the added burden on the developing zygotes of being heterozygous for a second irradiated genome. (See discussion, Lefevre 1966). When irradiated mature sperm fertilize normal eggs, chromosomal mutants have a greater chance of completing development to be detected. In short, even in heterozygous condition, chromosomal mutants of themselves contribute to zygotic inviability. The relative fitness of such mutants is surely a function of the overall genetic constitution of the zygote. When both paternal and maternal genomes are irradiated, the detected frequency of chromosomal mutants induced in mature sperm will be reduced in comparison with that resulting when only the paternal genome is irradiated.

Further, if chromosome damage, as opposed to dominant mutation of specific fertility genes, contributes significantly to sterility as well as to inviability of F_1 females, then the frequency of detected recessive sex-linked lethal mutations should also be reduced following female irradiation. Because many recessive lethal mutations result from chromosome rearrangement, these, if not made inviable, should be disproportionately frequent in sterile F_1 females (which cannot be tested) when both genomes are irradiated; but the frequency of chromosomally normal lethals, like male-viable mutations, should not be affected. Therefore, the reduction in the frequency of lethals should be less pronounced than that of hyperploid males or white-mottled mutants.

The results show that irradiation of inseminated females rather than males reduced the frequency of hyperploid males from 16.8×10^{-4} to 11.2×10^{-4} (Table 4), a reduction of 33.3%; of sex-linked recessive lethals from 14.04% to 12.28% (Table 3), a reduction of 12.5%; whereas male-viable mutants, excluding white-mottleds, increased in frequency from 5.4×10^{-4} to 6.3×10^{-4} (Table 4). Of further interest is the fact that among the 80 visible mutants derived from mature sperm irradiated in the male were 9 white-mottled, 4 white-mottled-Notch, and 6 white-Notch mutants (which result from relatively long deficiencies), of which five were sterile. Among the 80 visible mutants derived from mature sperm irradiated in the female, there were only 4 white-mottleds and 1 white-Notch, of which two were sterile. Yet, in each series, exactly 12 of the 80 mutants proved to be male viable. Thus, irradiation of mature sperm in the female, rather than in the male, reduces the frequency with which chromosomal mutants are detected and increases sterility, without appreciably affecting the frequency of chromosomally normal mutants.

Since the germ cell is the same—a mature sperm—in both modes of exposure, even though in different locations, it seems unreasonable to conclude that primary chromosome breakage is significantly less frequent or that chromosome breaks are preferentially repaired when sperm are irradiated in the female, rather than in the male. More plausible is the conclusion that a zygote containing a gross chromosome rearrangement or deletion is less likely to complete development

(or, if it does, to be sterile) when derived from two irradiated, genetically damaged gametes than when derived from one irradiated and one normal gamete. That is to say, induced mutants, including recessive lethals, that have abnormal chromosome do not invariably survive to be detected as fertile heterozygotes. Consequently, the detected mutant frequency fails to be a reliable measure of the mutation rate. This must be true even when only one genome is irradiated, but the selective loss of chromosomal mutants through inviability or sterility is exaggerated when both genomes are irradiated.

In sum, the reduced frequency with which sex-linked lethals and other kinds of mutants are detected when mature sperm are irradiated in the female does not force the conclusion that mutation rates are lower. Mutation rates in mature sperm irradiated in the male and in the female are, in all probability, exactly the same; only the detected mutation frequencies are affected.

Are mutation rates higher in mature sperm than in immature sperm? In Tables 3 and 4, mutation frequencies recorded in Column B (mature sperm) are, without exception, higher than the corresponding values in Column D (immature sperm); as seen in Table 2, the sterility of both phenotypically normal and mutant F_1 females is also higher in Column B than D. The differences are, in general, much more extreme than are the corresponding differences between the values in Columns A and B, discussed above. Once again, the greatest change in mutant frequencies is exhibited by hyperploid males (16.8×10^{-4} *vs.* 6.6×10^{-4}), a 60% reduction. Sex-linked lethals (14.04% *vs.* 7.33%) are 48% less frequent; visible mutants (42.1×10^{-4} *vs.* 17.9×10^{-4}), 57% less; sterility of phenotypically normal F_1 females (11.66% *vs.* 6.69%), 43% less; and male-viable mutants (7.2×10^{-4} *vs.* 5.3×10^{-4}), 26% less. With white-mottleds removed from the male-viable class, the reduction in frequency (5.4×10^{-4} *vs.* 4.8×10^{-4}) is only 11%.

The mutant frequency differences associated with mature *vs.* immature sperm have the same character as the differences associated with irradiation of mature sperm in the male and in the female. That is to say, the frequency of chromosomal mutants is more strongly affected than is that of chromosomally normal mutants. In this case, however, all categories of mutants, even male-viables, show some degree of reduction when immature, rather than mature, sperm are irradiated. This implies that the intrinsic mutability of immature sperm is somewhat lower than that of mature sperm, or that a more effective repair mechanism exists in immature sperm. In any event, the much greater effect on the frequency of chromosome mutants unquestionably demonstrates either that primary chromosome breakage is significantly reduced in immature sperm, or that breakage restitution without rearrangement is favored in immature sperm.

Whatever the answer may be, these results call attention to the need for care in interpreting experiments dedicated to the question of repair *vis-à-vis* differential mutability in germ cells at dfferent stages of maturity. Since the detection of sex-linked lethals, a category of "impure" mutants, is commonly employed (for examples, see SOBELS 1963) in such experiments, conclusions regarding the efficacy of repair mechanisms must be viewed with some reservation, unless

clearly restricted to chromosome breakage. Although mutation rates may be somewhat lower in immature than in mature sperm, the large reduction in the frequency of sex-linked lethal mutations reflects, primarily, a difference in chromosome breakage or restitution; it is not evidence for the repair of induced gene mutation.

Are mutation rates higher in spermatids than in immature sperm? As shown in Tables 2 and 3, both sterility of F_1 females and the frequency of lethal mutations is higher in Column F (spermatids) than in Column D (immature sperm). Moreover, as seen in Table 4, the frequency of hyperploid males in Column F is nearly four times higher than that in Column D; but mutant frequencies, for the most part, show little difference. Male-viable mutants, however, appeared somewhat less frequently among progeny derived from irradiated spermatids than among those derived from immature sperm, and male-lethal *y*, *w*, *v*, and *f* mutants occurred with greater frequency in Column F than in Column D.

Unfortunately, the number of progeny derived from irradiated spermatids is small because early spermatids (and spermatocytes) are much more radiosensitive than are more mature germ cell stages. After exposure to 4000r, the earliest postmeiotic stages fail to complete their transformation into functional sperm, and those stages that do survive transmit a high incidence of dominant lethality. As a consequence, relatively few mutants (only 11 altogether) were detected, and after dividing them into sterile, male-lethal, and male-viable classes, the calculated frequencies are based on two few individuals to be reliable. Only the sex-linked lethal frequency and the incidence of sterility in phenotypically normal F_1 females are based on sufficient numbers to be meaningful. In both instances the frequencies in spermatids (Column F) are rather similar to those in mature sperm (Column B). Only the frequency of hyperploid males is higher in progeny derived from irradiated spermatids than in those derived from mature sperm.

These facts support the contention of LÜNING (1952) that early spermatids sampled 7 to 10 days after irradiation are especially sensitive to chromosome breakage. The present data can be interpreted to mean that chromosomes in early spermatids, like those in mature sperm, are more readily breakable than are chromosomes in immature sperm; alternatively, spermatids have an ineffectual mechanism for restituting chromosome breaks, as compared with that in immature sperm. In any case, only chromosome mutants show a marked change in frequency when spermatids rather than immature sperm are irradiated. Thus, insofar as chromosomally normal mutants ("true" gene mutations?) are concerned, X-ray-induced mutation rates in all postmeiotic male germ cell stages, mature sperm, immature sperm and spermatids, in all likelihood are little, if any, different.

Are all kinds of mutants more frequent among sterile than among fertile F_1 females? All categories of visible mutants are appreciably more frequent among sterile F_1 females than among fertile ones (Table 4). Since sterile mutants cannot be tested, suspicion may arise that the phenotypic classification of sterile mutants is unreliable. When an F_1 female is sterile, perhaps a variety of ab-

normalities appear, which are occasionally mistaken for mutant phenotypes. In some small measure, this may be true of Notch and white-mottled mutants. Yet, never has it been necessary to change the original classification of any presumptive *y*, *w*, *v* or *f* mutant when the mutant later was able to produce progeny. Either there are no dominant mimics of *y*, *w*, *v*, or *f*, or they are so infrequent that they may be safely disregarded. To be sure, there are Notch mimics, but even these fail to exhibit the typical microchaete and wing-vein abnormalities that are always found in true Notches. Only white-mottleds, of all the kinds of mutants identified in F_1 females in this experiment, can be successfully mimicked; dominant brown-mottleds (Plum mutants) may be mistaken for white-mottleds. A breeding test, then, is required for their accurate classification. Thus, the frequency of the sterile class of white-mottleds may have been somewhat overestimated. Fortunately, Plum mutants, like white-mottleds, arise solely as the result of chromosome rearrangement, so that both belong to the same category of mutation. Among the sterile Notch mutants, too, some few may have been erroneously classified, but this is not true of *y*, *w*, *v*, or *f* mutants detected in F_1 females. For these mutants, phenotype reliably indicates genotype. A breeding test is not essential in order to validate their original classification.

The overall frequency of non-Notch mutants among sterile F_1 females is approximately eight times higher than that of similar mutants among fertile F_1 females. Eliminating white-mottleds, there is little likelihood of misclassification of the sterile mutants. Only one fertile F_1 female in 1252 was a *y*, *w*, *v*, or *f* mutant; one sterile F_1 female in 149 was such a mutant. Fertile non-Notch mutants breed either as male viable or male lethal. Cytological analysis of the male-lethal *y*, *w*, *v*, and *f* mutants showed virtually all to be associated with detectable chromosome aberrations, mostly deletions. Male-viable mutants (excepting white-mottleds) rarely have abnormal chromosomes. Thus, since chromosomal mutants are more likely to be sterile than are mutants with normal chromosomes, sterile *y*, *w*, *v*, and *f* mutants should consist predominantly of chromosomal aberrations. These, if they had been fertile, would more likely have bred as male lethal than as male viable. Since they cannot, in fact, be tested, there is no safe way to partition sterile mutants into male-lethal and male-viable categories. One can be relatively sure only that their breeding behavior would be different from that of fertile mutants. The sterility of visibly mutant F_1 females is not impressed independently of the effect on the mutant locus; being visibly mutant often announces the fact that the mutant female is sterile since the two effects can result from the same primary cause: chromosome aberration.

It should be noted that different kinds of non-Notch mutants are not uniformly sterile. More than 70% of the initially detected vermilion mutants were sterile (see also GREEN 1960); whereas, only 8% of the yellow mutants were sterile. White and forked mutants showed 30 to 40% sterility. A detailed analysis of the mutants detected in this experiment will be presented elsewhere.

The overall frequency of Notch mutants among sterile F_1 females was about nine times higher than that among fertile F_1 females. Even if some sterile Notch mutants were incorrectly classified, the ratio of the frequency of Notch mutations in sterile to that in fertile females is unlikely to be less than that of non-Notch

mutants. Except for the fact that females heterozygous for Notch can be phenotypically identified in the F_1 generation, Notch mutations are genetically no different from recessive sex-linked lethal mutations. Both are inviable in the male, are associated with abnormal as well as with normal chromosomes, and are equally reduced in frequency when mature sperm are irradiated in the female rather than in the male. The conclusion appears inescapable that sex-linked recessive lethals, like Notches, are disproportionately frequent among sterile F_1 females.

If, in our experiments, recessive lethals had been eight times more frequent in sterile than in fertile F_1 females, then it would be necessary to conclude that, following the irradiation of mature sperm, nearly all of the sterile F_1 females were heterozygous for a sex-linked lethal; even after the irradiation of immature sperm, about 60% of the sterile F_1 females would have been lethal-carriers. Although such extreme frequencies are unlikely, implying that recessive lethals are probably not as much as eight times more frequent in sterile than in fertile F_1 females, the inference remains clear that the same type of genetic damage, presumably chromosomal, that leads to recessive lethality contributes to F_1 female sterility. Where a greater frequency of chromosomal mutations occurs, sterility is higher and detected mutant frequencies are biased so as to be less indicative of the "true" mutation rate.

Experimental procedures that reduce lethal mutation frequencies, such as irradiation of mature sperm in the female rather than in the male, cannot safely be described as affecting mutability or repair mechanisms if, at the same time, sterility is increased. Nor can procedures such as irradiation in nitrogen, unless it is simultaneously demonstrated that there is no increase in sterility. Investigators interested in assessing the effect of modifying the conditions under which irradiation is carried out, including pre- and posttreatments, should measure the frequency of only those kinds of mutations that, like male-viable visibles, are rarely associated with chromosomal abnormality and do not contribute to female sterility. Then, differences in the frequency of detected mutations will be meaningful. The most valid data would result only if, in addition, all detected mutants were fertile.

Sterility presents a serious complication in radiation experiments. Any kind of mutation that contributes to sterility will be detected with reduced frequency in fertile F_1 progeny. The present results strongly indicate that recessive lethals, which can be identified only in fertile progeny, belong to this category of mutation. Consequently, their detected frequency cannot be taken as an explicit measure of mutability, especially if, in different situations, the level of sterility as well as the mutation frequency is affected.

I am much indebted to MISS B. NILSSON and MRS. A. PEABODY for their excellent and cheerful technical assistance.

SUMMARY

In experiments where the frequency of sex-linked recessive lethal mutations was determined following irradiation with 4000r of male germ cells at different stages of maturity, all F_1 females were inspected for the presence of visible

changes at the *y*, *w*, *v*, and *f* loci, as well as for Notch and white-mottled mutations. Further, the occurrence of hyperploid males was noted. The fertility of both phenotypically normal and mutant F_1 females was compared, as was the frequency of mutants in the fertile and sterile classes of F_1 females.—The frequency of both visible mutants and recessive lethals is high following the irradiation of mature sperm in the male, somewhat lower when mature sperm are irradiated in the female, much lower when immature sperm are irradiated (of necessity, in the male), but high again following irradiation of early spermatids. Sterility is highest when mutation frequencies are highest, except in the case of mature sperm irradiated in the female. There, sterility is increased although mutation frequencies are lower. The frequencies of different categories of mutation do not all follow the same pattern. Chromosome mutants (hyperploid males and white-mottleds) are most sensitive, sex-linked lethals (a mixture of chromosomally normal and abnormal mutants) next, and male-viable *y*, *w*, *v*, and *f* mutants least sensitive to the effect of irradiating different germ cell stages and to irradiating mature sperm in the male and in the female. Further, visible mutants, including Notches, are eight times more frequent among sterile than among fertile F_1 females.—These results indicate that differential chromosome breakage or restitution of breaks, not differential gene mutability, is primarily responsible for the differences in lethal mutation frequency detected at successive intervals following irradiation. Further, chromosome mutants contribute to both inviability and sterility of F_1 females. Thus, variations in recessive lethal mutation frequencies do not necessarily reflect true differences in the mutability of different germ cell stages exposed to given doses of X rays. Insofar as mutants with cytologically normal chromosomes are concerned, induced mutation rates are little, if any, different in all postmeiotic male germ cell stages.

LITERATURE CITED

BRIDGES, C. B., and K. BREHME, 1944 The mutants of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. **552**.

GREEN, M. M., 1960 Comparative mutability of wild-type isoalleles at the white loci in *Drosophila melanogaster*. Genet. Res. **1**: 452–461.

LEFEVRE, G., JR., 1965a The mutability of mature sperm of *Drosophila melanogaster* irradiated in the female and in the male. Genetics **51**: 381–390. —— 1965b Factors modifying mutation frequency patterns detected after irradiation of *Drosophila melanogaster* males. Mutation Res. **2**: 22–28. —— 1966 Mutation frequencies detected following irradiation of virgin and inseminated *Drosophila melanogaster* females. Genetics **53**: 137–143.

LEFEVRE, G., JR., and U. B. JONSSON, 1964a X-ray induced mutability in male germ cells of *Drosophila melanogaster*. Mutation Res. **1**: 231–246. —— 1964b The effect of mating intensity on mutation frequency patterns detected after irradiation of *Drosophila melanogaster* males. Genetics **50**: 879–890.

LÜNING, K. G., 1952 The connection between chromosome breaks and apparent gene mutations in *Drosophila melanogaster*. Hereditas **38**: 502–504.

SLIZYNSKI, B. M., 1938 Salivary chromosome studies of lethals in *Drosophila melanogaster*. Genetics **23**: 283–290.

SOBELS, F. H., 1963 *Repair from Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells*. Proc. Int. Symp., Univ. Leiden. Edited by F. H. SOBELS, Pergamon Press. New York.