3000 r X-rays 24 hours after the injection. In the second experiment flies were exposed to 3000 r X-rays and then injected 0.2 micro cc of the saline solution containing 1 mg of aflotoxin dissolved in 1 cc of saline. Twenty-four hours rest was given before they were allowed to mate, in the second experiment (while in the first immediately after exposure to X-rays they were mated) individually with 3 virgin females of Y sc^{S1} In-49 sc^{8};bw;st stock for 3 days only to assess the alteration of genetic damage if any in spermatozoa alone. The F_1 females were mated individually with Y sc^{S1} In-49 sc^{8} males while the males were mated with bw;st females to score for sex-linked recessive lethals and translocations respectively in F_2 generation.

Table 1

Treatment	Sex linked recessive lethals			Translocations		
	T	l	%	T	t	%
3000 r X-rays						
3000 r X-rays	429	28	5.6	429	18	4.2
aflotoxin + 3000 r X-rays	477	17	3.5	411	13	3.1
3000 r X-rays + aflotoxin	468	27	5.7	433	16	4.6

T = total number of X chromosomes or F_1 sons scored
l = lethals recorded
t = translocations recorded

The Chi-square test has been done to compare the following groups: (1) 3000 r X-rays vs aflotoxin + 3000 r X-rays; (2) 3000 r X-rays vs 3000 r X-rays + aflotoxin. The results of the statistical analysis are presented in table 2.

Table 2

Group	Sex linked recessive lethals	Translocations
1. 3000r X-rays vs aflotoxin+3000r	2.139	0.172
2. 3000r X-rays vs 3000r+aflotoxin	0.863	0.137
3. aflotoxin+3000r vs 3000r+aflotoxin	2.358	0.0342

The study indicates that aflotoxin failed to alter the genetic damage induced by X-rays.

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Heterozygous effects in Drosophila melanogaster following treatment with ethyl methane sulfonate (EMS).

In an effort to assess the populational effects of a general rise in mutation rate, experiments are being conducted in our laboratory to measure the viability of heterozygotes carrying second chromosomes recently descended from flies fed with the chemical mutagen EMS. These experiments utilize special stocks isogenized over a period of several years which enable us to study the comparative effects of treated chromosomes in homozygous and heterozygous backgrounds. The isogenic stocks are: 1) cn bw, maintained by brother-sister single pair matings, 2) cn, maintained by backcrossing a cn female to a cn bw male from the above cn bw stock, and 3) bw, by similarly mating a bw female with a cn bw male. A cross between cn and bw flies from these stocks generates cn +/+ bw males, which are fed with EMS according to the procedure given by Lewis and Bacher (1968). Following treatment, these males are divided, without etherization, into three groups for mass matings with either 1) isogenic bw females, 2) isogenic cn, or 3) M-5 females, for a standard test of the induced frequency of sex-linked lethals. The males are removed after two days in order to sample treated mature sperm. From each cross with the isogenic females, wild type sons (cn +/+ bw) were selected and mated individually to either isogenic cn bw females or to cn bw;e females from a non-isogenic stock. In these cn +/+ bw males either the cn or bw chromosome was the one treated, according to whether the mothers were bw or cn, respectively. In the next generation, the progeny, cn +/cn bw and + bw/cn bw, are counted for each of two broods, in both isogenic and non-isogenic backgrounds, at each dose. Comparing the ratio of cn to bw in cultures where cn is treated to the same ratio where bw is treated gives a measure of the effect of treated chromosomes in heterozygotes, each set providing a control for the other, with the viability effects of these markers cancelling out.

Preliminary results pooled for experiments at 3 doses of EMS are tabulated here; each class is expressed as a mean proportion.

Background	cn treated			bw treated			s	σ_s
	# of tests	cn(A)	bw(B)	# of tests	cn(C)	bw(D)		
isogenic	123	.485	.515	119	.509	.491	.049	.010
non-isogenic	135	.493	.507	131	.512	.488	.039	.009

The s value is the mean reduction in viability of heterozygotes, or the heterozygous load and is derived as follows: if we let x be a measure of the relative survival of the treated class, and p and q the relative viabilities of cn and bw flies, respectively, then the expected ratio of cn:bw where cn is treated is px:q and where bw is treated, p:qx. The value x^2 may therefore be estimated from the ratio AD/BC, where these letters represent the observed proportions of flies in the classes as listed in the table. The load s is approximately 1-x, or with a Poisson correction, s = -ln x.

Thus, in the isogenic (cn bw) background the reduction in viability of heterozygotes carrying a treated chromosome was close to 5%, and in the non-isogenic (cn bw;e) background about 4%. Each of these was significantly different from zero, but not different from each other. If the data is subdivided by broods, the effect is consistent: in the isogenic background, s for brood 1 was .053 ± .011, for brood 2, .050 ± .017. In the non-isogenic background, the s values were .040 ± .010 and .025 ±.015 for broods 1 and 2 respectively. Further experiments are planned in which the heterozygous effects will be correlated with homozygous effects. In particular, lethal heterozygotes will be separated from non-lethal heterozygotes and their viabilities compared. In the preliminary studies reported here, a major fraction of the effect may well be due to lethals, based on extrapolation from measurements of lethals induced on the X, as follows. The standard M-5 tests, with an additional generation tested for the presence of mosaic lethals were carried out to establish the lethal rates at three doses. With .017M EMS, there were 28.9% sex-linked lethals (89/219) in the F_2 and an additional 7.3% lethals in the F_3 (15/191). At .021M EMS, the F_2 rate was 37.9% (143/377) and F_3, 3.5% (7/201). At .023M the lethal frequency in the F_2 was .421 (48/114).

Würgler, F.E. and M. Kälin. Swiss Federal Institute of Technology, Zürich, Switzerland. A "storage" effect with X-rayed mature sperm of Drosophila melanogaster.

Graf and Würgler (this volume) found that the rate of apparent X/O males recorded after anoxic X-irradiation of mature sperm of ring-X males depends on the genotype of the females used for the test crosses. In screening tests, in which several other types of females were used in addition to the y sn^3 and Inscy;dp bw;st p^p flies, another unexpected result was obtained. Data obtained with XY/XY females illustrate this: Two to three-day-old ring-X males (R(1)2, y B/B^S Y y^+) were pretreated with N_2 for 20 min and X-rayed (50 keV, 520 R/min) in nitrogen. Nonirradiated controls were treated with nitrogen in the same way. After the treatment, the males were mated for 7 to 8 hours to 4-day-old virgin females in empty bottles, where the females did not deposit eggs. The females are homozygous XY/XY (Parker 110-8, y^2 su $(w^a)w^a$ KS.KL y^+). At the end of the mating period the males were discarded and the inseminated females transferred to standard culture vials. Every 24 hours the vials were changed until 4 successive broods had been obtained. The progeny from every vial were classified according to the phenotypes: normal B/+ females (F), normal B^S males (M), apparent X/O males (non-Bar, su$(w^a)w^a$) (L), and mosaics for sex chromosome loss (ML). The pooled data of two experiments, which gave very similar results, are given in the table. The percentage of sex chromosome loss is calculated as 100 x (L/F+M+L+ML).

brood (day)	control	2000 R	4000 R
1	2.2% (11/266+227+11+0)	8.7% (49/225+282+49+6)	13.7% (115/286+428+115+9)
2	1.3% (3/125+91+3+1)	3.5% (7/78+113+7+1)	4.5% (12/105+148+12+2)
3	1.9% (6/154+145+6+0)	3.7% (11/138+146+11+1)	8.0% (22/113+139+22+1)
4	1.7% (2/55+63+2+0)	2.2% (4/80+100+4+0)	6.6% (14/67+128+14+3)
2 - 4	1.7% (11/334+302+11+1)	3.2% (22/296+359+22+2)	6.4% (48/285+415+48+6)

The data show that the rates of sex chromosome losses are extremely high in the first brood. In broods 2, 3 and 4 the rates are low, but more or less constant. This finding, which looks like a "storage" effect, could have different causes:

a) Since most sex chromosome losses result from damaged ring-X chromosomes, a preferen-