THIRD-CHROMOSOME MUTAGEN-SENSITIVE MUTANTS OF *DROSOPHILA MELANOGASTER*

J. B. BOYD, M. D. GOLINO,[1] K. E. S. SHAW, C. J. OSGOOD AND M. M. GREEN

Department of Genetics, University of California, Davis, California 95616

Manuscript received September 20, 1980
Revised copy received January 22, 1981

ABSTRACT

A total of 34 third chromosomes of *Drosophila melanogaster* that render homozygous larvae hypersensitive to killing by chemical mutagens have been isolated. Genetic analyses have placed responsible mutations in more than eleven complementation groups. Mutants in three complementation groups are strongly sensitive to methyl methanesulfonate, those in one are sensitive to nitrogen mustard, and mutants in six groups are hypersensitive to both mutagens. Eight of the ten loci mapped fall within 15% of the genetic map that encompasses the centromere of chromosome 3. Mutants from four of the complementation groups are associated with moderate to strong meiotic effects in females. Preliminary biochemical analyses have implicated seven of these loci in DNA metabolism.

WITHIN the past few years, considerable effort has been devoted to the isolation of mutants in *Drosophila melanogaster* that are hypersensitive to chemical mutagens (for review, see SMITH, SNYDER and DUSENBERY 1980). A primary goal of these studies is the accumulation of a battery of strains that can be employed to study DNA metabolism in higher eukaryotes. The major effort thus far has centered on the *X* chromosome because of the relative ease with which *X*-linked mutants are recovered. At this point more than 60 mutants have been employed to define about 12 complementation groups on that chromosome. At the biochemical level, mutants mapping to two loci exhibit aberrant patterns of DNA synthesis in unmutagenized cells. One locus is essential for excision repair, and mutants at three loci are defective in post-replication repair (for review, see BOYD *et al.* 1980). Genetic analyses have demonstrated meiotic defects in mutants occurring at three loci, and mitotic chromosome instability is observed in mutants of six complementation groups (for review, see BAKER *et al.* 1980).

We report here an extension of that analysis in which similar selection procedures were employed to recover mutagen-sensitive mutants on chromosome 3. Recovery of this same class of mutants on the other major autosome of this species has been undertaken in Atlanta (SNYDER and SMITH, personal com-

Abbreviations used in text: MMS: methyl methanesulfonate. EMS: ethyl methanesulfonate. HN2: nitrogen mustard. AAF: 2-acetylaminofluorene. *mus*: a mutation that confers mutagen sensitivity on larvae.

[1] Current address: Department of Genetics, Development and Physiology, Cornell University, Ithaca, New York 14850.

munication). The genetic experiments reported here are focused on recovery of data complementary to the parallel biochemical effort. Thus, attention has been directed toward the identification of new complementation groups and the possible involvement of mutants within those groups in the meiotic process. Although more extensive genetic characterization of these mutants will undoubtedly prove profitable, the present state of their biochemical characterization necessitates a provisional description at this time. The biochemical studies are reported elsewhere (BOYD and SETLOW 1976; BOYD *et al.* 1980; BOYD and SHAW, in preparation; BOYD and HARRIS 1981).

MATERIALS AND METHODS

Mutants: Complete terminology for the new mutants identified in this study follows. For convenience, designations of visible markers and the chromosome identification are omitted from most references to the mutants in the text. Thus, *st mus(3)301^{D2}* is referred to as *mus301^{D2}*. The six mutants marked with an asterisk are associated with a second unidentified *mus* mutant.

st mus(3)301^{D1} .... *st mus(3)301^{D5}*
st mus(3)302^{D1} .... *st mus(3)302^{D3}*, *mus(3)302^{D4}* .... *mus(3)302^{D6}*
st mus(3)304^{D1} .... *st mus(3)304^{D3}*
st mus(3)305^{D1}, *st mus(3)305^{D2}*, *ry mus(3)305^{D3}**
mus(3)306^{D1}
*mus(3)307^{D1}**
st mus(3)308^{D1}, *mus(3)308^{D2}*, *st mus(3)308^{D3}*,*ry mus(3)308^{D4}**, *ry mus(3)308^{D5}**, *ry mus(3)308^{D6}**
st mus(3)309^{D1}, *st mus(3)309^{D2}*, *ry mus(3)309^{D3}**
st mus(3)310^{D1}
st mus(3)311^{D1} .... *st mus(3)311^{D3}*
mus(3)312^{D1}, *st mus(3)312^{D2}*.

D. T. SUZUKI generously provided the *Sb Ubx/Xa* stock that was employed in mutant selection scheme A. Stocks generated in this laboratory include *TM2, Ubx^{130} ry^{x1}* and *T(2:3) Cy; Ubx^{130} ry^{x1}/Sb ry*. Additional stocks are described in LINDSLEY and GRELL (1968).

Conditions for culture and selection: With the following exceptions, procedures and materials are described in BOYD *et al.* (1976). Vials contained 10 ml of medium and bottles contained 50 ml. The medium was prepared by boiling rather than under pressure. A stock is defined as sensitive in the initial screening when less than 10% of the expected homozygotes eclose. Most crosses were done at 23–25°.

Mutant selection: Three independent approaches were employed to recover mutagen-sensitive mutants on the third chromosome.

(1) Selection Scheme A: The approach depicted in Figure 1a was adapted from that of TASAKA and SUZUKI (1973). In that screen, mutagenized third chromosomes were produced by feeding 0.003 M EMS (LEWIS and BACHER 1968) to 3- to 4-day-old, unmated males homozygous for a chromosome 3 marked with *st*. Treated males were mass-mated to virgin *Sb Ubx/Xa* females for 5 days. The F_1 *st/Sb Ubx* males were individually mated to 3 or 4 virgin *ry Sb/Ubx ry* females in vials. F_1 parents were discarded after 6 days. The *st/Ubx ry* males and their nonvirgin female siblings were selected from the F_2 generation and crossed. After two days, the F_2 parents were transferred to a second (control) vial and 0.25 ml of 0.06% MMS (V/V) was added to the first vial. Since the mutagenized chromosome 3 is homozygous in the F_3 generation, induced recessive lethals were recognized by the absence of *st/st* flies in both vials. Putative mutants were identified as stocks in which *st/st* flies were present at a frequency of 10% or less of the frequency observed in the untreated control vial. Individuals from the control vial of putative mutant stocks were retested in duplicate to verify that the homozygous *st* individuals were hypersensitive to MMS.

(2) Selection Scheme B: The procedure shown in Figure 1b was developed to select for mutagen-sensitive mutants on chromosomes *2* and *3* simultaneously. Males homozygous for a chromosome *2* marked with cn^{55} and for a chromosome *3* marked with ry^{2} were fed EMS in concentrations ranging from 0.0005 M to 0.001 M (LEWIS and BACHER 1968). They were then mass-mated for 6 days to virgin females bearing a *2–3* translocation. F_1 *cn*; *ry/T(2;3) Cy*; *Ubx ry* males were mated individually in vials to 3–5 *T(2;3) Cy*; *Ubx/Sb ry* virgin females. After 3 days, the F_1 parents were removed and purine solution (0.125 to 0.15% W/V in water) was added to kill homozygous *ry* larvae. The surviving F_2 *T(2;3) Cy*; *Ubx/cn*; *ry* males and females were crossed to expand the stock and to identify recessive lethals (not shown in Figure 1b). The F_3 cultures with viable homozygotes were distributed to each of 3 glass vials and one plastic vial. After 3 days, the parents were discarded, and the vials were treated on the fourth day as follows: The cultures in plastic vials were treated with 0.25 ml of 0.2% AAF solution (W/V in 70% ethanol). One shell vial received 0.25 ml of MMS solution (0.06–0.1% V/V), another received 0.25 ml of HN2 solution (0.006–0.01% W/V), and the last was untreated. The F_4 progeny were scored for a reduction in the number of homozygous *cn*; *ry* flies to a frequency of 10% or less of prediction.

(3) Selection Scheme C: A total of 3067 cultures, carrying second or third chromosomes that had been exposed to EMS, were obtained from D. L. LINDSLEY. This collection was generated by R. HARDY in the laboratory of J. R. MERRIAM, employing several different selection schemes. Chromosomes bearing recessive lethals had been eliminated by that group. Of the 1761 stocks derived from a treated *bw*; *st* culture, 1672 had the third chromosome balanced with Ubx^{130} and 89 with the second chromosome balance *SM5*. Among 814 stocks derived from a treated Canton-S stock, 535 had the third chromosome balanced with either *TM3* or *Ubx*, and 279 had the second chromosome balanced with *SM5*. The remaining 474 stocks were apparently homozygous for a treated autosome since no balancers were identified. Each culture was transferred sequentially to 3 vials at 1-day intervals. Two days after transfer, one vial was treated with 0.25 ml of 0.008% HN2 and one with 0.25 ml of 0.08% MMS. The third vial served as an untreated control. Stocks were selected in the next generation from cultures in which the ratio of homozygotes to heterozygotes in the treated vial was $\leq 10\%$ of that in the control. In the case of homozygous stocks, a reduction in the total number of flies in the treated vial to 10% or less of that in the control vial was sought. Cultures that satisfied these criteria were retested.

Stock balancing: Each selected stock was outcrossed and its third chromosome balanced against *TM3*, as outlined in Figure 2. In this scheme, up to 5 single males were selected from the F_1 generation so that any chromosome carrying recessive lethal mutations could be discarded in the F_3 generation. Any chromosome *2* present during mutagenesis was eliminated, thereby providing a more uniform genetic background between stocks. Mutagenized *X* chromosomes were eliminated when a chromosome *3* was made homozygous both in our analysis (Figure 1) and in that of HARDY and MERRIAM (personal communication). All subsequent tests were made using balanced strains.

Analysis of larval sensitivity: Putative third-chromosomal mutagen-sensitive stocks were first analyzed for larval sensitivity as follows. Ten homozygous or heterozygous males were crossed to 20–25 heterozygous virgin females. Homozygous males were employed when fertile. After 1 or 2 days, the flies were transferred to medium in a ½ pint milk bottle. Females were allowed to lay for 1 day and then transferred to a second bottle for an additional 1-day egg-laying period. Two days after transfer, the first bottle received 1.25 ml of either MMS solution (0.1–0.12% V/V) or HN2 solution (0.008–0.012% W/V). The second bottle was similarly treated with 1.25 ml of water. Progeny were scored until day 17 or 18.

The most viable and sensitive stock was then selected to generate the detailed sensitivity data presented in Table 1. The mating scheme described in Figure 3 was used to determine the ratios for "Relative Survival" and "Dominance." Qualitative tests for cross-sensitivity to a second mutagen, also presented in Table 1, were performed as described above. Ratios in that test were accepted with as few as 50 control flies.

Complementation of mutagen sensitivity: Two forms of complementation testing were employed. A single-generation test was performed by crossing balanced heterozygous mutant individuals of one strain with either homozygous or heterozygous balanced individuals of a second

strain. At least 2 larval cultures of the F_1 generation were treated with mutagen at a dose appropriate to kill individuals of the less sensitive strain. Ratios of surviving progeny were normalized to the same ratios obtained in untreated control crosses. Normalized ratios that fell within the following ranges were scored as indicated: 0.00–0.15 = negative, 0.16–0.50 = ambiguous, > 0.50 = positive. The second form of complementation analysis is described in RESULTS.

Fertility analysis: To assay female fertility, 4 to 5 homozygous virgin females were crossed to 5 Oregon-R males. Male fertility was analyzed by crossing 4–5 homozygous mutant males to 5 virgin double-*X* females. The parents were discarded after 5–7 days and relative fertility was scored qualitatively on days 16–18.

Genetic mapping: The scheme outlined in Figure 4 was used to generate recombinants between the selected chromosomes and the multiply marked *rucuca* chromosome (see LINDSLEY and GRELL 1968 for the composition of this chromosome). Since all of the *rucuca* chromosomes examined in this laboratory were found to be hypersensitive to MMS, we used 0.008% HN2 (W/V) to map mutants sensitive to that mutagen. Subsequent analysis has shown that the MMS-sensitivity associated with the *rucuca* chromosome is due to the presence of a *mus(3)306* allele. An MMS-insensitive derivative of *rucuca*, which carries the *rucuca* markers except for *cu* and *sr*, was later recovered and used to map the MMS sensitivity of *mus306*^{D1}, *-307*^{D1}, *-310*^{D1} and *-311*^{D1}.

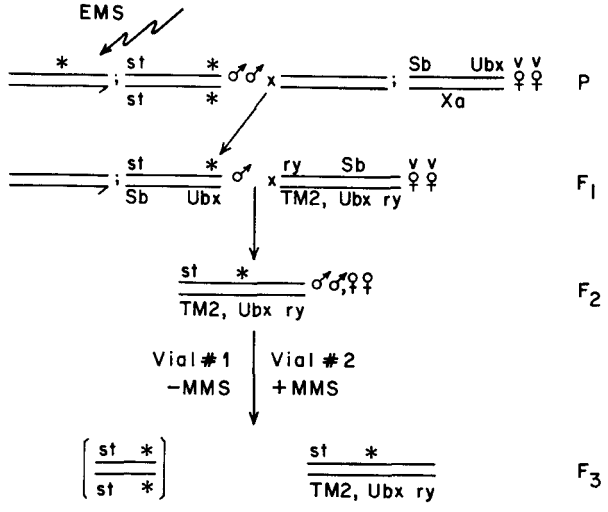
As shown in cross 3 of Figure 4, individual males bearing a recombinant chromosome *3* were balanced by *TM3*. In the next generation, 2–3 pairs of the genotype designated "recombinant chromosome/*TM3*" were crossed *inter se* to generate balanced stocks. After expansion of these stocks, heterozygous males and virgin females were mated and tested for mutagen sensitivity of the recombinant chromosome as detailed above. The ratios of homozygotes to heterozygotes were normalized relative to the corresponding control values. Treated vials giving adjusted survival ratios of less than or equal to 0.05 were scored as sensitive; whereas, values greater than 0.20 were scored as insensitive. Cultures containing fewer than 8 flies or producing normalized ratios between 0.05 and 0.20 were disregarded, as were crosses for which the expected number of homozygous mutant individuals was less than 2. Results obtained with occasional recombinant chromosomes that were inconsistent with the remainder of the data were attributed to undetected multiple recombinants. The 95% confidence intervals for the calculated map positions were computed as described by O'BRIEN and MACINTYRE (1978).

RESULTS

Mutant isolation: A total of 34 chromosomes were recovered with the selection programs presented above. The mutants *mus301*^{D1}, *-301*^{D2}, *-302*^{D1}, *-302*^{D2}, *-302*^{D3}, *-304*^{D1}, *-305*^{D1}, *-309*^{D1} and *-309*^{D2} were obtained among 2253 chromosomes screened for hypersensitivity to MMS in Selection Scheme A (Figure 1). A mutant previously identified as *mus303*^{D1} (BOYD *et al.* 1976) has since been lost. With the exception of *mus302*^{D2} and *mus302*^{D3}, all alleles were derived from different groups of mutagenized males and thus originated from independent mutational events.

Fifteen mutants were recovered after retesting 462 potentially sensitive stocks generated in Selection Scheme B from about 6400 F_1 crosses. The mutant phenotype of each of the 15 stocks was found to be associated with one of the two major autosomes by employing the multiply balanced stock *y; Pm/Cy Oster; Sb/TM6*. One of the two mutants identified as exhibiting AAF sensitivity, in fact, proved to be sensitive only to ethanol, although it exhibited normal levels of alcohol dehydrogenase (T. FLETCHER, personal communication). The second AAF-sensitive mutant mapped to chromosome *2* and was the only stock initially identified as being sensitive to all three agents. Characterization of that mutant

a. Scheme A. (Third chromosomal selection, after Tasaka and Suzuki, 1973).



b. Scheme B (Autosomal selection)

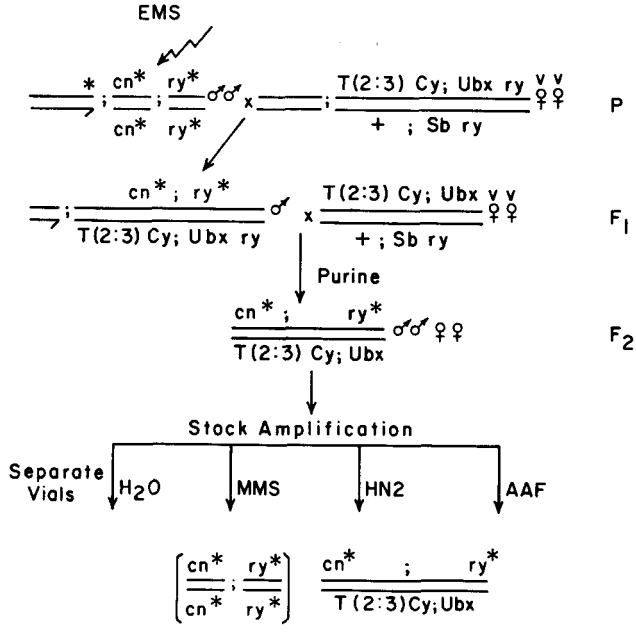


FIGURE 1.—Mating schemes employed for selection: A more complete description of the strains employed is presented in MATERIALS AND METHODS. *Identifies a potentially mutagenized chromosome.

[*mus(2)201*^{D1}] will be described elsewhere (BOYD *et al.*, in preparation). Subsequent to the recovery of the 13 chromosome *3* mutants, the original unmutagenized stock expressed sensitivity to MMS. We are unable to explain the failure of this chromosome to exhibit sensitivity during the early stages of that study. Its current expression, however, means that each of the selected third chromosomes may carry two *mus* mutants. As a result of this complication, only those four chromosomes that carry alleles of other mutants described here are included. Since all three chromosomes that carry *mus308* mutations were derived from one group of mutagenized males, the *mus308* component of those stocks may have originated from a single mutational event.

The remaining mutants described in this report were recovered from stocks donated by LINDSLEY and HARDY (Selection Scheme C). The initial screen of that collection produced 132 putative mutants, of which 92 were linked to chromosome *3*. The 40 mutants, potentially linked to chromosome *2*, were forwarded to R. D. SNYDER and P. D. SMITH. They ultimately isolated three strong mutants defining three complementation groups (personal communication).

Relative mutagen sensitivity: Prior to determining the optimum mutagen concentration for analyzing the individual mutants, each selected stock was rebalanced by the procedure outlined in Figure 2. This scheme produces cultures in which the only major mutagenized chromosome is the third. Five individual stocks for each mutant were retested in bottles, and one was selected for further analysis. The mutagen sensitivity of the resulting stocks was then evaluated with the protocol depicted in Figure 3. This procedure provides an estimate of the mutagen sensitivity of homozygous mutant larvae relative to control individuals of genotype *D/TM3*. Such flies provide a relatively mutagen-insensitive standard with which to compare mutant individuals that are generated in the same cross.

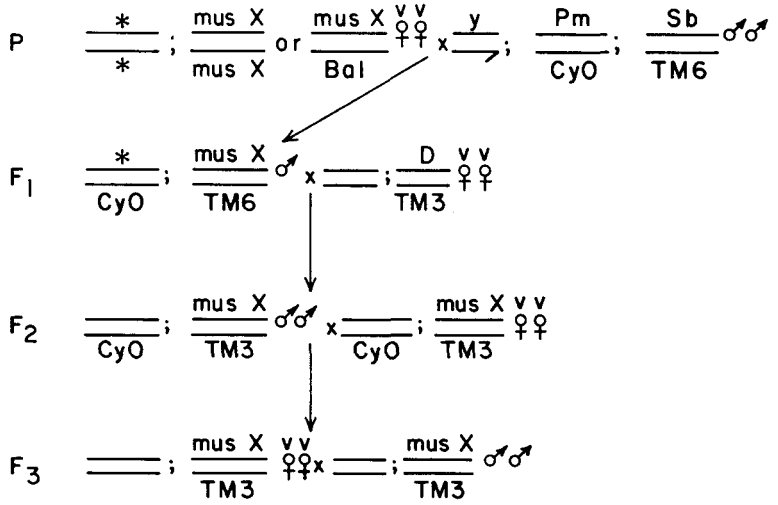


FIGURE 2.—Generation of balanced mutant stocks. *Denotes a potentially mutagenized chromosome *2*.

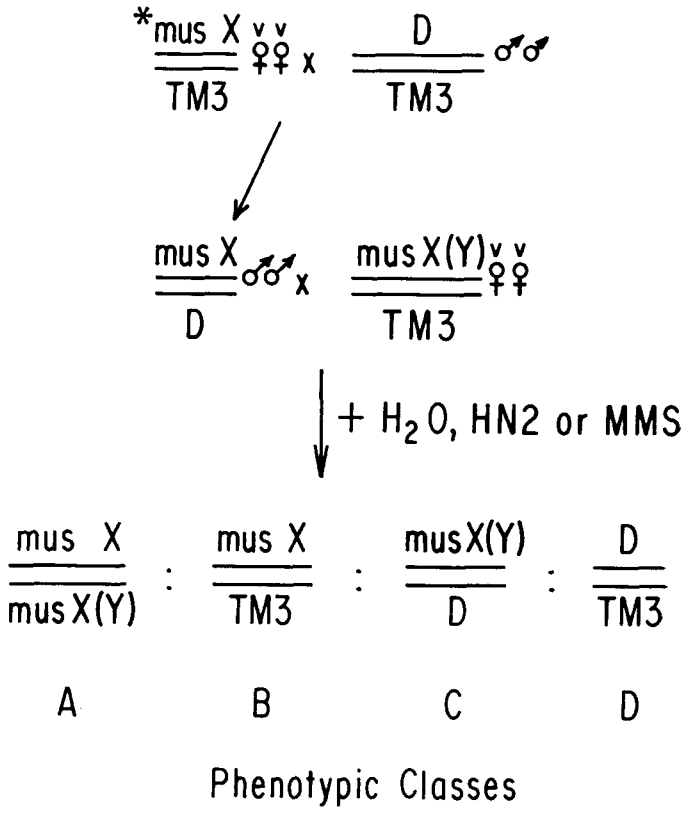


FIGURE 3.—Determination of larval sensitivity to chemical mutagens. *The symbols X and Y refer to independently isolated mutants. For determinations of relative strain sensitivity and dominance, only one strain (X) was used. Relative strain sensitivity was evaluated from the ratio of phenotypic class A to class D. Dominance was determined by measuring the relative sensitivity of phenotypic class B to class D. Complementation was monitored in a similar cross in which two strains (X and Y) were used, and the ratio of classes A to D was recorded. Data obtained from three identically treated bottles were pooled and retained only if the sample contained an excess of 90 flies of the insensitive control genotype.

Thus, *D/TM3* flies exhibit 87% survival relative to individuals homozygous for Oregon-R-derived third chromosomes after treatment with either 0.1% MMS or 0.008% HN2.

An analogous study of *X*-linked mutants had previously indicated the appropriate mutagen concentrations and dose responses that were likely to be encountered (BOYD *et al.* 1976). The current study was therefore condensed from the earlier approach to provide only that information on sensitivity necessary for further genetic analysis. Where possible, the minimum dose of MMS required to kill more than 10% of the expected homozygotes was determined (Table 1). Subsequently, the cross sensitivity of those mutants to HN2 was evaluated qualitatively at a single high dose. Mutants assigned to the complementation groups *mus308* and *mus312* were analyzed at several HN2 concentrations be-

TABLE 1

Relative sensitivity of larvae to chemical mutagens

Mutant	Relative survival				Dominance†	
	1. Sensitivity to MMS					Sensitivity to 0.008% HN2*
	MMS conc. (%)					
	0.125	0.075	0.050	0.025		
mus301^{D1}			0.01	0.31	1.28	+
mus301^{D2}	0.00		0.00	0.18	0.83	+
mus301^{D3}	0.01		0.06	0.57	1.00	+
mus301^{D4}	0.00		0.09	0.31	1.06	+
mus301^{D5}			0.08	0.35	1.45	+
mus302^{D1}	0.01		0.00	0.00	1.01	+
mus302^{D2}	0.00		0.00	0.00	0.83	+
mus302^{D3}	0.00		0.00	0.00	0.76	+
mus302^{D4}	0.00		0.00	0.00	0.77	+
mus302^{D5}	0.00		0.00	0.02	0.83	+
mus302^{D6}	0.00		0.01	0.01	0.94	+Δ
mus304^{D1}	0.01	0.00	0.00	0.00	0.91	+
mus304^{D2}	0.00		0.00	0.00	0.97	+
mus304^{D3}	0.01		0.00	0.39	1.26	—
mus305^{D1}	0.00	0.00	0.17		1.05	+
mus305^{D2}	0.00		0.00	0.08	1.17	—
mus306^{D1}	0.06	0.23	0.68		0.72	—
mus307^{D1}	0.06		0.03	0.79	1.31	+
mus309^{D1}	0.02		0.00	0.02	1.17	+Δ
mus309^{D2}	0.00		0.02	0.08	0.80	+
mus310^{D1}	0.00		0.01	0.08	1.01	—
mus311^{D1}	0.03	0.94	0.98		1.08	—
mus311^{D2}	0.04	0.15	1.16		1.38	—
mus311^{D3}	0.03	0.41	0.72		0.88	—
	2. Sensitivity to HN2					Sensitivity to 0.125% MMS*
	HN2 conc. (%)					
	0.008	0.004		0.002		
mus308^{D1}	0.03	0.01		0.10	1.16	—Δ
mus308^{D2}	0.00	0.02		0.02	1.06	—
mus308^{D3}	0.02	0.05		0.42	1.16	—
mus312^{D1}	0.00	0.01		0.42	1.07	+
mus312^{D2}	0.00	0.03			0.82	+Δ

Relative survival values are normalized ratios of homozygous mutant individuals to D/TM3 control flies derived from the same culture (Figure 3). The ratio obtained from mutagen-treated cultures was divided by the same ratio found in cultures treated with water only. This normalization procedure places the range of relative survival between 0.00 and 1.00. The standard deviation of ratios derived from triplicate analysis of a single stock is 0.05. In this analysis the values of the ratio obtained in control (water-treated) cultures varied between 0.46 and 1.20. Further details are provided in the text.

† Dominance relationships are defined by the normalized ratio of heterozygous mutant flies to control D/TM3 flies (Figure 3). The value reported for *mus301*^{D5} was derived from 65 control flies rather than the standard minimum.

cause of their strong sensitivity to that mutagen relative to MMS. Since the *mus307*^{D1} allele is associated with a second uncharacterized *mus* mutation, the data reflect a combined sensitivity of those two mutants. This analysis reveals that at least one allele of the following complementation groups can be analyzed with either mutagen: *mus301*, *-302*, *-304*, *-305*, *-309* and *-312*. All mutants falling within complementation groups *mus306*, *-310* and *-311* can be analyzed only with MMS, and the *mus308* mutants are sensitive only to HN2. Mutants of this latter class have not been previously identified in Drosophila. Although this analysis implies that the mutants fall into discrete sensitivity classes, in fact, mutants such as *mus304*^{D3}, *-308*^{D2} and *-311*^{D1} probably exhibit an intermediate sensitivity to the less well analyzed mutagen. The sensitivity is not strong enough, however, to permit the use of that mutagen in genetic tests.

As has been observed with *X*-linked mutants, the degree of mutagen sensitivity varies considerably among mutants falling into different complementation groups. Extreme examples of MMS sensitivity are found among the highly sensitive mutants of the *mus302* and *mus310* groups. By contrast, mutants at the *mus306* and *mus311* loci barely meet our criterion for sensitivity at the highest possible dose.

An evaluation of dominance effects on mutagen sensitivity was also derived from the genetic crosses described in Figure 3. In that study, the relative survival of heterozygous individuals was determined in cultures treated with a mutagen dose that was adequate to kill nearly all homozygous mutant larvae. A corrected ratio involving the number of heterozygous *mus/TM3* individuals over the number of *D/TM3* individuals is arbitrarily defined as reflecting a recessive condition if it exceeds 0.8. By this criterion, *mus302*^{D3}, *-302*^{D4}, *-306*^{D1} and *-309*^{D2} exhibit a semidominant effect (Table 1). This effect, which potentially complicates the complementation analyses, therefore appears to be somewhat less frequent than previously observed among *X*-linked mutants (BOYD *et al.* 1976).

Complementation for mutagen sensitivity: Studies of relative sensitivity and dominance described in the previous section, plus determination of fertility for each homozygous stock provided essential information for the design of complementation experiments (see Summary Table 3). Preliminary one-generation complementation tests were performed between selected pairs of mutants (see MATERIALS AND METHODS). From each of the tentative complementation groups, a single mutant was designated the *D1* allele and was employed as the "tester" for that group. Complementation analyses were then performed between tester stocks with the two-generation test described in Figure 3. This analysis remains incomplete for *mus308* mutants, which could not be tested against *mus306*^{D1}, *-310*^{D1} or *-311*^{D1} because the *mus308* alleles analyzed do not exhibit adequate MMS sensitivity. The complementation groups were then reorganized, and

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* Cross sensitivity to the alternate mutagens was estimated qualitatively as follows:
+ = sensitive — corrected ratios ≤ 0.10
— = insensitive — corrected ratios > 0.10.
Due to the presence of recessive lethals on chromosomes denoted by Δ, a direct sensitivity test could not be completed. Therefore, sensitivity to a second mutagen was investigated by complementation for mutagen sensitivity with an alternate allele of known sensitivity.

either one- or two-generation tests were performed between each tester strain and the members of its putative complementation group. The two-generation test was employed in all cases involving semidominance or intermediate mutant interaction. This approach should assure the absence of double mutants among the tester strains for any of the complementation groups that have been identified. It does not, however, permit the detection of double mutants among the subordinate members of each group. Mutants for which complementation data unambiguously meet the above criteria are listed in the Summary Table (Table 3). The 34 stocks listed define more than 11 complementation groups.

An unusual complication was encountered with *mus302*^{D3}. This mutant produces a strong negative complementation result in combination with *mus302*^{D2}. However, it interacts only weakly with *mus302*^{D1}, and also interacts with alleles of *mus310* and *mus311*. Such ambiguity, which probably arises from semidominance, has been resolved by biochemical analysis to be reported elsewhere (BOYD and SHAW, in preparation) and by mapping studies described in the next section.

Genetic mapping: With the exception of *mus309*, at least one mutant from each complementation group has been mapped genetically. Mutants sensitive to HN2 have been assigned map positions relative to *rucuca* markers as summarized in Table 3. Those mutants sensitive to MMS, but not to HN2, were mapped using the MMS-insensitive *ru h th st e*^{s} *ca* chromosome, as outlined in Figure 4. Included in this latter group were *mus306*^{D1}, *-310*^{D1} and *-311*^{D1}. The MMS-insensitive chromosome was also employed to map *mus307*^{D1}, because the *mus307*^{D1} chromosome confers partial hypersensitivity to heterozygotes after treatment with 0.008 and 0.006% HN2. A genetic map of chromosome 3 in which the *mus* loci are identified reveals a strong grouping of these mutants near the centromere (Figure 5). The chromosome carrying *mus307*^{D1} (map position 59) also possesses a second unnamed mutant that has not been separated from *st*$^{+}$.

X-chromosome loss and nondisjunction in female meiosis: The influence of the isolated third chromosomes on disjunction and/or loss of *X* chromosomes was monitored by crossing homozygous mutant females to *y Hw w/B*^{s}*Y y*^{s1d} males

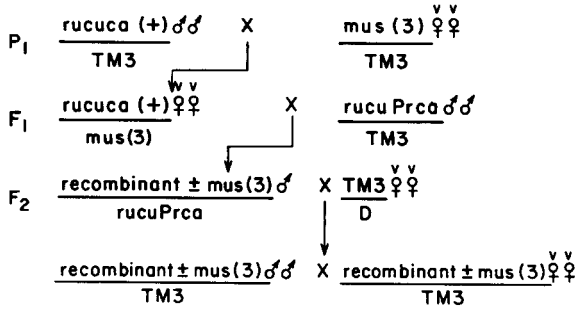

FIGURE 4.—Mapping Procedure. The indicated crosses were employed to generate balanced stocks of recombinant third chromosomes. The mutagen sensitivity of homozygotes relative to heterozygotes was subsequently assayed in order to identify those recombinants carrying a mutagen-sensitive mutation. Additional details are included in the text.

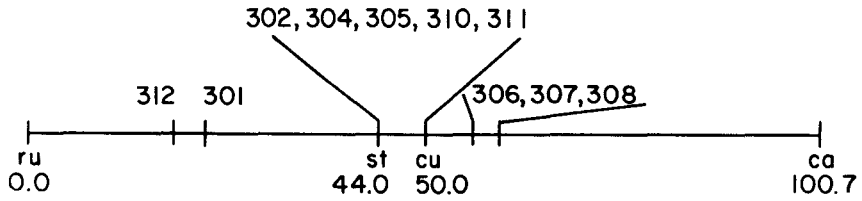

FIGURE 5.—Schematic representation of chromosome 3 showing approximate map positions of the *mus* mutations. Published map positions of the markers *ru*, *st*, *cu* and *ca* are included for reference.

(Table 2). The recovered female exceptions arise from diplo-*X* female gametes and thus reflect the frequency of nondisjunction. Since the male exceptions arise from nullo-*X* gametes, their frequency reflects the combined levels of nondisjunction and chromosome loss. In this test, the *mus312*^{D1} chromosome exhibits by far the greatest meiotic influence. The equality of male and female exceptions produced by that mutant indicates that their production is probably due primarily to nondisjunction rather than to chromosome loss. This same conclusion applies to the moderately strong effect of the *mus311*^{D3} chromosome. On the other hand, the *mus301* and *mus305* chromosomes appear to have relatively little influence on nondisjunction, yet they significantly elevate the level of chromosome loss. The remainder of the tested mutants do not produce levels of male exceptions that differ significantly from the relatively high level of nondisjunction found in the stocks from which the mutants were isolated. Although several of the mutant chromosomes were recovered from an untested genetic background, the levels of exceptions they produce do not indicate the presence of significant meiotic effects. These data therefore identify six meiotic mutants associated with four complementation groups defined by mutagen sensitivity.

In the past, analysis of complementation for meiotic effects has proven particularly valuable in grouping mutagen-sensitive mutants, because expression of this phenotype is generally recessive. Data presented in Table 2, which demonstrate negative complementation between the five *mus301* alleles, considerably strengthen the identification of allelism between these mutants based on mutagen sensitivity. Analysis of heterozygotes between *mus312*^{D1} and *mus307*^{D1} or *mus312*^{D2} documents a completely recessive nature for the strong meiotic effect of the *mus312*^{D1} mutant. Analysis of the heterozygote *st/st mus311*^{D3}, however, suggests that this mutant expresses a slight semidominant influence on female meiosis. Finally, the frequencies of exceptions produced by *st/st mus310*^{D1} females further document the slightly elevated frequency of nondisjunction associated with *st/st* females in Table 4. This same chromosome has previously been identified as *st mu-1* (BOYD *et al.* 1976) to reflect its associated mutator activity.

DISCUSSION

Our search for chromosome 3 mutants that are hypersensitive to chemical mutagens has more than doubled the number of loci in this class that have previously been identified on the *X* chromosome (SMITH 1976; BOYD *et al.* 1976).

TABLE 2

Spontaneous nondisjunction and/or chromosome loss

Homozygous females parent	Frequency of exceptional: F_1 ♀♀	F_1 ♂♂	Heterozygous females parent	Frequency of exceptional: F_1 ♀♀	F_1 ♂♂
st/st	0.08% (14,844)	0.54% (13,248)	*st mus301^{D1}/st mus301^{D2}*	0.7% (147)	3.5% (171)
st mus301^{D1}	2.1% (577)	6.32% (569)	*st mus301^{D1}/st mus301^{D3}*	1.5% (724)	2.5% (681)
st mus301^{D3}	0.4% (249)	2.69% (260)	*st mus301^{D1}/st mus301^{D4}*	0.9% (439)	6.7% (299)
st mus302^{D1}	0.0% (280)	1.6% (250)	*st mus301^{D1}/st mus301^{D5}*	0.4% (486)	1.8% (441)
st mus302^{D3}	0.35% (285)	0.55% (183)	*st mus301^{D1}/mus312^{D1}*	0.2% (611)	0.0% (498)
mus302^{D5}	0.0% (775)	0.0% (575)	*mus307^{D1}/st mus312^{D2}*	0.0% (1,005)	0.1% (939)
mus302^{D6}	0.0% (881)	0.0% (715)	*mus312^{D1}/st mus312^{D2}*	0.0% (946)	0.0% (878)
st mus305^{D1}	0.3% (323)	3.05% (328)	*st mus311^{D2}/st mus311^{D3}*	0.0% (438)	0.5% (415)
st mus305^{D2}	1.1% (455)	1.92% (464)	*st mus311^{D1}/st mus311^{D3}*	0.4% (518)	0.6% (493)
mus306^{D1}	0.29% (2,412)	0.34% (2,022)	*st/st mus310^{D1}*	0.03% (3,782)	0.7% (3,518)
mus307^{D1}	0.11% (900)	0.38% (795)	*st/st mus311^{D3}*	0.6% (1,092)	1.5% (1,017)
st mus308^{D1}	0.58% (1,035)	1.19% (839)			
mus308^{D2}	0.07% (1,507)	0.64% (1,399)			
st mus310^{D1}	0.29% (2,414)	0.97% (2,069)			
st mus311^{D3}	7.3% (385)	7.3% (395)			
mus312^{D1}	16.8% (322)	14.8% (311)			
st mus312^{D2}	0.25% (403)	0.63% (317)			

Either homozygous or heterozygous mutant females were crossed with *y Hw w/B^{S}Yy^{+d}* males. Nondisjunction and loss of *X* chromosomes is recorded as the percentage of exceptions for a given sex, divided by the total for that sex. Total progeny scored is indicated in brackets by sex. Additional details are provided in the text.

Eleven new complementation groups have been characterized, of which eight are represented by two or more alleles. Complementary studies of SMITH, SNYDER and DUSENBERY (1980) and MASON *et al.* (1981), who have analyzed the second and *X* chromosomes, bring the total number of genetic loci associated with mutagen sensitivity in Drosophila to 30. Properties of the new chromosome *3* mutants reported here are summarized in Table 3.

Mutagen sensitivity: The majority of loci defined in this study are identified by mutants that are hypersensitive to both MMS and HN2. Mutants in three complementation groups (*mus306*, *-310* and *-311*) are hypersensitive to MMS, but relatively insensitive to HN2. Conversely, the *mus308* group represents a new class of Drosophila mutants that can be adequately analyzed only with HN2.

The spectrum of sensitivities within the individual complementation groups is relatively uniform. One apparent exception is the $mus304^{D3}$ mutant, which does in fact exhibit detectable but unexploitable sensitivity to HN2. This anomalous behavior can be explained by the fact that this is the least sensitive of the three mutants in this complementation group. The insensitivity of $mus305^{D2}$ to HN2 relative to its allelic partners, however, cannot be explained in this manner.

Meiotic effects: Mutants associated with four of the nine tested complementation groups exhibit elevated frequencies of nondisjunction (*mus301*, *-305*, *-311*, *-312*) (Table 2). A preliminary suggestion that $mus302^{D1}$ might be a meiotic mutant (BOYD and SETLOW 1976) is not fully substantiated by the current analysis. Although that allele appears to exhibit a stronger effect than any of the other alleles tested from that group (Table 2), its influence on meiosis is slight. Mutants in two complementation groups (*mus301* and *mus305*) exhibit moderate meiotic effects that have been documented for two alleles of each group. Furthermore, the allelism of all five *mus301* mutants has been substantiated by complementation of meiotic effects (Table 2). Failure of selected alleles within complementation groups *mus311* and *mus312* to express meiotic effects, or to interact with alleles that do, raises the possibility that the meiotic effects observed in the mutants $mus311^{D3}$ and $mus312^{D1}$ may not be associated with the mutagen-sensitive sites under analysis. Although mapping studies employing the $mus312^{D1}$ chromosome place the origin of both the meiotic and mutagen-sensitive phenotype within the same chromosomal region (GREEN, 1981), further analysis is required to resolve this question fully. It therefore appears that at least two and perhaps four of the nine characterized loci can influence meiosis.

Assignment of allelism: The *D1* allele at each locus has been carefully tested with all other *D1* alleles for complementation of mutagen sensitivity. Separation of allelic groups based on that analysis is therefore relatively secure, particularly in view of the predominantly recessive nature of these mutants. Mapping studies, however, have revealed the presence of a second *mus* locus on the $mus307^{D1}$-bearing chromosome. This chromosome, therefore, presumably carries two unique mutants that are not allelic with others in the collection. Since allelism of the subordinate mutants within each complementation group was assigned on the basis of negative complementation with a single *D1* allele, those chromosomes

TABLE 3

Summary of properties of homozygous stocks

Mutant	Source or Selection Scheme*	Mutant map position† ± 95% CI	Non-disjunctionΔ	Relative sensitivity‡		Fertility§	
				MMS	HN2	♂	♀
mus301^{D1}	A	23 ± 7	++	+++	sens.	+++	+++
mus301^{D2}	A			+++	sens.	—	+++
mus301^{D3}	C		+	+++	sens.	—	+++
mus301^{D4}	C			+++	sens.	+++	—
mus301^{D5}	C			+++	sens.	++	++
mus302^{D1}	A	45 ± 2	—	++++	sens.	+++	+++
mus302^{D2}	A			++++	sens.	—	—
mus302^{D3}	A	46 ± 2	—	++++	sens.	+++	+++
mus302^{D4}	C			++++	sens.	+	+
mus302^{D5}	C	46 ± 2	—	++++	sens.	+++	+++
mus302^{D6}	C		—	++++	sens.	++	++
mus304^{D1}	A	46 ± 2		++++	sens.	+++	+
mus304^{D2}	C			++++	sens.	+++	—
mus304^{D3}	C			+++	insens.	+++	+++
mus305^{D1}	A	44 ± 1	+	++	sens.	+++	++
mus305^{D2}	C		+	++++	insens.	—	+
mus305^{D3}	B			sens.	sens.	+++	+++
mus306^{D1}	C	56 ± 8	—	+	insens.	+++	+++
mus307^{D1}**	C	59 ± 8	—	+++	sens.	+++	+++
mus308^{D1}	C		—	insens.	++	+++	+++
mus308^{D2}	C	55 ± 5	—	insens.	+++	+++	+++
mus308^{D3}	C			insens.	++	—	+++
mus308^{D4}	B						
mus308^{D5}	B						
mus308^{D6}	B						
mus309^{D1}	A			++++	sens.	—	—
mus309^{D2}	A			++++	sens.	++	—
mus309^{D3}	B						
mus310^{D1}	C	47 ± 5	—	++++	insens.	+++	+++
mus311^{D1}	C	47 ± 5		+	insens.	+++	—
mus311^{D2}	C			+	insens.	+	+++
mus311^{D3}	C		++	+	insens.	+++	+++
mus312^{D1}	C	18 ± 7	+++	sens.	++	+++	++
mus312^{D2}	C	16 ± 11	—	sens.	++	—	++

* Mutant source, see MATERIALS AND METHODS, Mutant selection.

Δ Meiotic effects, see Table 2

+++ male nondisjunction >14%

++ male nondisjunction >6% <14%

\+ male nondisjunction >1.5% <6%

— no significant meiotic effects.

† 95% confidence intervals (CI) determined as described by O'BRIEN and MACINTYRE (1978). *mus306*^{D1}, *307*^{D1}, *310*^{D1}, *311*^{D1} were mapped with the MMS-insensitive chromosome *ru h th st e*^{s} *ca*, as described in the text.

** The *mus307*^{D1} chromosome carries two MMS-sensitive sites, one close or to the left of *st*, the other proximal to *e*^{s}.

‡ Relative sensitivity—see Table 1

also potentially carry mutations at more than one of the characterized loci. The exceptionally heavy mutagen doses employed to produce mutants in Selection Scheme C considerably enhanced the likelihood of recovering double mutants and further suggests that much of the observed infertility may not be associated with the *mus* mutants.

Occasional unexplained interactions between supposed non-alleles, as in the past (BOYD *et al.* 1976), has stimulated the use of independent criteria to substantiate assignments of allelism. The *mus301* and *mus312* mutants occupy closely adjacent sites on the left arm of chromosome *3*. It is therefore necessary to turn to meiotic studies for independent verification of allelic assignments in this region. The evidence linking meiotic effects with the *mus301* locus is clear; the two tested mutants elevate nondisjunction as homozygotes and complementation analyses employing this property confirm the allelism among all five alleles. Although the chromosome bearing $mus312^{D1}$ is associated with a strong meiotic effect, absence of this property in a second allele ($mus312^{D2}$) raises the possibility that the $mus312^{D1}$ chromosome carries two independent mutations (Table 2). In any case, the meiotic failure in $mus301^{D1}$ is not equivalent to that of the $mus312^{D1}$ chromosome, as demonstrated by the positive complementation observed between them (Table 2). The meiotic analyses therefore support the separation of these mutants into two allelic groups, in agreement with complementation studies employing mutagen sensitivity.

The most striking observation to arise from the mapping studies is that eight of the ten identified loci occur between positions 45 and 59 on the genetic map (Figure 5). This distribution closely parallels that obtained by TASAKA and SUZUKI (1973) in their analysis of temperature-sensitive lethals induced by EMS. They found that 88% of such mutants mapped between *st* (44) and *Sb* (58), an interval that includes only 12.9% of the genetic length of the chromosome, but over 55% of its physical length. A further parallel to our observations is found in the distribution of insertion events exhibited by a particular transposing element. ISING and RAMEL (1976) report that 50% of such events observed on chromosome *3* occurs within 15% of the genetic map spanning the centromere. No such clustering was evident on chromosome *2*. The parallel distribution of these various classes of genetic events suggests that they may be related in some way.

— insensitive
+ relative survival ≤ 0.1 at 0.125% MMS
++ relative survival ≤ 0.1 at 0.075% MMS
+++ relative survival ≤ 0.1 at 0.050% MMS
++++ relative survival ≤ 0.1 at 0.025% MMS
+ relative survival ≤ 0.1 at 0.008% HN2
++ relative survival ≤ 0.1 at 0.004% HN2
+++ relative survival ≤ 0.1 at 0.002% HN2
"sens." indicates that relative survival is ≤ 0.1 at highest practical mutagen dose.
§ Fertility analysis (see MATERIALS AND METHODS)
+++ highly fertile, strong culture
++ moderate fertility, few flies and pupae
+ poor fertility—some pupae, very few flies
— total sterility—no progeny flies.

Within that relatively narrow region, mutants on the *mus306*, *mus307* and *mus308* chromosomes have been mapped to closely adjacent sites at about map position 57 (Figure 5). Not only are these mutants inseparable by mapping, but *mus306* and *mus308* have not been tested for complementation of mutagen sensitivity because they do not exhibit a common mutagen sensitivity. Their separation into two allelic groups is based on the insensitivity of *mus306*^{D1} to HN2 and the exclusive sensitivity of the *mus308* alleles to that mutagen.

The five remaining loci mapping near the centromere have been localized between the *st* and *cu* markers. The *mus310* and *mus311* mutants are distinguished from the other three by their exclusive hypersensitivity to MMS. Biochemical studies reported elsewhere (BOYD *et al.* 1980) have revealed a post-replication repair deficiency in *mus302*^{D1}. Other than these differences, assignment of allelism for these mutants rests primarily on complementation studies employing mutagen sensitivity.

Biochemical characterization: The mutants described here have also been exploited in an analysis of DNA metabolism (BOYD *et al.* 1980). Such studies have been facilitated by the fact that fertile homozygous stocks can be generated from at least one allele of each complementation group, with the exception of the *mus309* and *mus312* groups. Among the mutants tested to date, two (*mus302*^{D1} and *mus310*^{D1}) exhibit a strong deficiency in post-replication repair, and three (*mus304*^{D3}, *-308*^{D2}, *-311*^{D3}) express a modest but significant reduction in that capacity (BOYD and SHAW, in preparation). The rate of DNA chain growth is slightly reduced in *mus307*^{D1} and *mus308*^{D2}. In addition, *mus302*^{D1}, *-304*^{D3}, *-306*^{D1} and *-308*^{D2} are partially deficient in excision repair (BOYD and HARRIS, 1981). The genetic studies reported here provide the necessary foundation from which to extend the molecular biology of these mutants.

We are indebted to R. HARDY and D. L. LINDSLEY for making the mutagenized autosomal stocks available to us. J. M. MASON provided valuable counsel throughout much of this work. SANDRA L. BARBEL performed the initial characterization of mutants that are sensitive to HN2, but not to MMS. JAMES YAMASHITA has performed the studies of mutants recovered from Selection Scheme B. A portion of the recombination mapping was completed by ANGELA H. WAPNER and JEANNETTE P. ROBERTSON, who also assisted in preparing the manuscript. SUSIE F. BOYD and SABADRA AYYAGARI participated in selected aspects of this study.

This work was supported by Department of Energy contract At(04-3)-34 PA 210 and Public Health Service grants GM16298 and GM22221.

LITERATURE CITED

BAKER, B. S., M. GATTI, A. T. C. CARPENTER, S. PIMPINELLI and D. A. SMITH, 1980 Effects of recombination-deficient and repair-deficient loci on meiotic and mitotic chromosome behavior in *Drosophila melanogaaster*, pp. 189–208. In: *DNA Repair and Mutagenesis in Eukaryotes*. Edited by W. M. GENEROSO, M. D. SHELBY and F. J. DESERRES, Plenum Press, New York.

BOYD, J. B., M. D. GOLINO, T. D. NGUYEN and M. M. GREEN, 1976 Isolation and characterization of *X*-linked mutants of *Drosophila melanogaster* which are sensitive to mutagens. Genetics **84**: 485–508.

BOYD, J. B. and P. V. HARRIS, 1981 Mutants partially defective in excision repair at five autosomal loci in *Drosophila melanogaster*. Chromosoma (Berl.) **82**: 249–257.

BOYD, J. B., P. V. HARRIS, C. J. OSGOOD and K. E. SMITH, 1980 Biochemical characterization of repair-deficient mutants of Drosophila. pp. 209–221. In: *DNA Repair and Mutagenesis in Eukaryotes*. Edited by W. M. GENEROSO, M. D. SHELBY and F. J. DE SERRES. Plenum Press, New York.

BOYD, J. B. and R. B. SETLOW, 1976 Characterization of postreplication repair in mutagen-sensitive strains of *Drosophila melanogaster*. Genetics **84:** 507–526.

GREEN, M. M., 1981 *mus(3)312^{D1}*, a mutagen sensitive mutant with profound effects on female meiosis in *Drosophila melanogaster*. Chromosoma (Berl.) **82:** 259–266.

ISING, G. and C. RAMEL, 1976 The behaviour of a transposing element in *Drosophila melanogaster*. Appendix to Chapter 23. pp. 947–954. In: *Biology and Genetics of Drosophila*, Vol. 1b. Edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

LEWIS, E. B. and F. BACHER, 1968 Methods of feeding ethyl methanesulfonate (EMS) to *Drosophila* males. Drosophila Inform. Serv. **43:** 193.

LINDSLEY, D. L. and E. H. GRELL, 1968 *Genetic variations of* Drosophila melanogaster. Carnegie Inst. Wash. Publ. No. 627.

MASON, J. M., M. M. GREEN, K. E. S. SHAW and J. B. BOYD, 1981 Genetic analysis of X-linked mutagen-sensitive mutants of *Drosophila melanogaster*. Mutation Research **81:** 329–343.

O'BRIEN, S. J. and R. J. MACINTYRE, 1978 Genetics and biochemistry of enzymes and specific proteins of Drosophila. pp. 396–552. In: *The Genetics and Biology of Drosophila*, Vol. 2a. Edited by M. ASHBURNER and T. R. F. WRIGHT. Academic Press, London.

SMITH, P. D., 1976 Mutagen sensitivity of *Drosophila melanogaster*. III. X-linked loci governing sensitivity to methyl methanesulfonate. Molec. Gen. Genet. **149:** 73–85.

SMITH, P. D., R. D. SNYDER and R. L. DUSENBERY, 1980 Isolation and characterization of repair-deficient mutants of *Drosophila melanogaster*. pp. 175–188. In: *DNA Repair and Mutagenesis in Eukaryotes*. Edited by W. M. GENEROSO, M. D. SHELBY and F. J. DE SERRES. Plenum Press, New York.

TASAKA, S. E. and D. T. SUZUKI, 1973 Temperature-sensitive mutations in *Drosophila melanogaster*. XVII. Heat- and cold-sensitive lethals in chromosome 3. Genetics **74:** 509–520.

Corresponding editor: T. C. KAUFMAN