# HIGH MUTABILITY IN MALE HYBRIDS OF *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

The frequencies of sex-linked lethal mutations arising in hybrid male offspring from various crosses and in nonhybrid controls were determined. The hybrids were produced by crossing representative strains of the *P-M* system of hybrid dysgenesis in all possible combinations. Males from the cross of *P* males × *M* females had a mutation rate about 15 times higher than that of nonhybrid males from the *P* strain. Genetically identical males from the reciprocal cross had a mutation rate 3 to 4 times that of the nonhybrids. For crosses involving a *Q* strain, a significant increase in the mutation rate was detected in males produced by matings of *Q* males with *M* females. No increase was observed in genetically identical males from the reciprocal mating. Crosses between *P* and *Q* strains gave male hybrids with mutation rates not different from those of nonhybrids. Many of the lethals that occurred in hybrids from the cross of *P* males × *M* females appeared to be unstable; fewer lethals that arose in hybrids from the cross of *Q* males × *M* females were unstable. The relationship between *P* and *Q* strains is discussed with respect to a model of mutation induction in dysgenic hybrids.

CASES of high mutability in Drosophila were observed many years ago by DEMEREC (1937), STURTEVANT (1939) and IVES (1950). More recently, BERG (1979), KIDWELL and IVES (1977), PICARD *et al.* (1978), ENGELS (1979a) and WOODRUFF, THOMPSON and LYMAN (1979) have presented evidence concerning this phenomenon. This renewed interest coincides with the identification of a condition that occurs nonreciprocally in the offspring of crosses between certain Drosophila strains. The condition is known as hybrid dysgenesis (KIDWELL and SVED 1977; BREGLIANO *et al.* 1980) and is characterized by several aberrant traits, including sterility, male recombination, segregation distortion and high mutability. Dysgenesis is brought about when males from a strain classified as "paternally contributing" are crossed with "maternally contributing" females. Hybrids from the reciprocal cross are normal, or nearly so.

There are at least two functionally independent systems of hybrid dysgenesis. In one (PICARD 1976), the maternally contributing strain is labeled "reactive"

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(*R*), while the paternally contributing strain is called "inducer" (*I*). In the other (KIDWELL, KIDWELL and SVED 1977), the maternally and paternally contributing strains are designated *M* and *P*, respectively. Both systems also include apparently neutral strains, designated *N* in the *I-R* system and *Q* in the *P-M* system. Published data (KIDWELL 1979) indicate that when a neutral strain is crossed with a maternally or paternally contributing strain from the same system, in whichever direction, fertile hybrids result. In the *P-M* system, hybrids from the cross of *P* males × M females are often sterile, especially if raised at high temperature (27 to 29° ). The sterility is due to the failure of the gonads to develop, a condition referred to as gonadal dysgenesis (ENGELS and PRESTON 1979; KIDWELL and NOVY 1979). In the *I-R* system, sterility occurs only in females derived from crosses between *I* males and *R* females and only when these females are placed at low temperature (18 to 21° ). In this case, however, the sterility is not due to gonadal dysgenesis, but to a lethal defect in the eggs laid by the dysgenic females.

In this paper, we report data concerning the occurrence of sex-linked lethals in the hybrid male offspring of *P*, *M* and *Q* strains and in nonhybrid controls. The data are somewhat better than those reported earlier, insofar as objective scoring methods were used, reciprocal hybrids were contrived to be genetically identical and nonindependent mutational events were given proper statistical treatment. Moreover, the data include results with a *Q* strain and its hybrids, none of which had been tested previously for mutability. All of the strains used for making hybrids were *I*, so that the only relevant maternal-paternal interaction was that of the *P-M* system. The results indicate that the *Q* strain resembles the *P* strain in its ability to produce highly mutable hybrids when crossed to females of an *M* strain. This implies that *Q* is more closely related to *P* than to *M*; in fact it may be a weakened or defective *P* strain, possessing modified *P* factors (ENGELS 1979b) on its chromosomes.

## MATERIALS AND METHODS

*Stocks*: Three inbred wild-type strains were used: CS (Canton S), $\pi_2$ and $\nu_6$. The first is a long-standing laboratory strain of the *M* type; the second and third are highly inbred strains obtained from W. R. ENGELS, who derived them from a natural population in Madison, Wisconsin. $\pi_2$ is a strong *P* strain; $\nu_6$ is a *Q* strain. All 3 wild-type strains are classified as inducers in the *I-R* system.

*C(1)DX, y f/Y* females × *y cin w* $f^s$ $su(f)^{ts67g}/Y$ males is an attached-*X M* strain in which the males carry a multiply marked free-*X* chromosome bearing the mutations *y* (yellow body), *cin* (cinnamon, a maternal-effect mutant described by BAKER 1973), *w* (white eyes), $su(f)^{ts67g}$ (a temperature-sensitive lethal described by DUDICK, WRIGHT and BROTHERS 1974) and $f^s$ (a suppressible allele of forked bristles). The lethal effect of $su(f)^{ts67g}$ is manifested at 29° but not at 25°. The free-*X* chromosome in this stock will be abbreviated *y cin w*. We should also note that the *Y* chromosome in the females of this stock is derived by descent from that in the males. The attached-*X* stock is an inducer in the *I-R* system.

*FM7/sc*$^7$ (= *FM7*, $y^{s1d}$ $sc^8$ $sn^{x2}$ *B/Ins(1)* $sc^7$ + *AM*, $sc^7$ $w^a$ $ptg^4$ $Bx^{78h}$ $l(1)^{78h}$) is a balancer stock maintained by crossing *FM7/sc*$^7$ females with *FM7/Y* males. The *FM7* chromosome carries recombination-suppressing inversions (MERRIAM 1968) and a mutation at the singed bristle locus ($sn^{x2}$) that sterilizes homozygous females; the $sc^7$ chromosome (*sc* = scute bristles) also carries

inversions, but unlike *FM7*, has a recessive lethal mutation, *l(1)*$^{78h}$, induced with ethyl methane sulfonate (EMS). Both chromosomes carry dominant markers, although the *Bx* allele on the *sc*$^{7}$ chromosome has low penetrance; this mutation was induced with EMS. The other markers on these chromosomes, as well as the inversion breakpoints, are described by LINDSLEY and GRELL (1968).

All stock cultures were maintained at 25° in half-pint milk bottles supplied with cornmeal-molasses medium that was sprayed with a suspension of live baker's yeast.

*Hybrids*: Hybrid males were obtained by performing reciprocal crosses between each of the wild-type strains and the attached-*X* strain at 25°. Contemporaneous crosses within strains were also made to produce nonhybrid wild-type males that were then treated in the same way as the hybrids. Additional hybrids were obtained from reciprocal crosses between $\pi_2$ and $\nu_6$. In every case, males were collected within 24 hr of eclosion. Approximately half of these were stored at 25° for 2 days, then mated; the other half were stored at the same temperature for 9 days before mating.

*Sex-linked lethal tests*: Hybrid and nonhybrid males were mated individually in shell vials (95 mm × 25 mm) to four *FM7/sc*$^{7}$ females at 21°. The parents were removed after 6 days and then, beginning on the 17th day and continuing through the 20th, 10 *FM7/+* daughters from each vial were placed individually into 100 × 13 mm culture tubes supplied with a special medium and stoppered with disposable foam plugs. These females had already had the opportunity to mate with their *FM7/Y* brothers. The special medium was based on sugar and debittered brewer's yeast. About 2,000 tubes can be filled by mixing 4,320 ml water, 34.8 g agar, 180 g sugar, 300 g yeast and 16 ml propionic acid.

The tube cultures were incubated for 4 days at 18° and then moved to 25° to complete growth; the initial period at low temperature minimized gonadal dysgenesis in the offspring, which were needed for additional genetic tests. We found that the tube cultures did best if kept horizontal for five days and then put upright. This prevented egg-bound females from sticking in water droplets or crevices on the surface of the medium.

Hatching cultures were moved to 21° after 12 days of incubation and then checked for the presence of wild-type males on day 14. Those with wild-type males were scored as nonlethal cultures; those without were labeled as suspected lethals. The putative lethal chromosomes were tested further by allowing *FM7/+* females to mate with their *FM7/Y* brothers. For each suspected lethal, 1 or 2 vial cultures of this type were established and reared at 25°. The progeny were counted 14 and 17 days later. Sometimes additional cultures were established by transferring parents to fresh medium, increasing the number of progeny and thereby reducing the chance for a classification error.

A chromosome was classified as lethal only if 32 or more progeny were tallied in the tests. If no wild-type males appeared, the chromosome was classified as a *complete lethal* (*c*); if a few appeared, but these constituted less than 2.5% of the total, the chromosome was classified as an *incomplete lethal* (*i*). If the number of progeny was less than 32 and no wild-type males appeared, the chromosome was designated an *unsure* (*us*); this class included instances in which all the tests of a suspected lethal were sterile. When wild-type males appeared and constituted more than 2.5% of the total progeny, whatever the number, the chromosome was classified as a *nonlethal* (*nl*).

## RESULTS

The detailed results of the experiments are presented in Tables 1 and 2. Sperm from 22 groups of males were tested for newly arisen sex-linked lethals. The experiments numbered 1, 2 and 3 in the tables identify tests with the $\pi_2$, CS and $\nu_6$ strains, respectively. Within these numerical designations, the letters A and B identify tests with reciprocal, but genetically identical, hybrids created by crossing the appropriate wild strain with the attached-*X M* strain. The A hybrids were produced by crossing wild-type males with attached-*X* females, the B hy-

TABLE 1

*Basic data from experiments to detect sex-linked lethals occurring in hybrid and nonhybrid males*

| Experiment | Male age | No. males | $\bar{f}$ | $\sigma_f$ | $\tilde{f}$ | $N$ | No. males producing at least one lethal | | | | | | | $l$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | *c* | *i* | *cc* | *ii* | *ci* | Other | Total | |
| 1A | 2 | 496 | 4.37 | 2.80 | 2.46 | 2,168 | 29 | 18 | 3 | 0 | 1 | 0 | 51 | 55 |
| ($\pi_2$♂♂ × *C(1)DX, y f/Y*♀♀) | 9 | 581 | 5.79 | 2.74 | 3.74 | 3,366 | 31 | 13 | 2 | 4 | 4 | 1(6*c*; 2*i*) | 55 | 72 |
| 1B | 2 | 842 | 6.92 | 2.42 | 5.33 | 5,831 | 21 | 4 | 3 | 0 | 0 | 0 | 28 | 31 |
| (*y cin w*♂♂ × $\pi_2$♀♀) | 9 | 762 | 7.08 | 2.49 | 5.30 | 5,395 | 18 | 8 | 1 | 0 | 0 | 0 | 27 | 28 |
| 1C | 2 | 854 | 6.70 | 2.45 | 5.04 | 5,726 | 5 | 0 | 0 | 0 | 0 | 1(1*c*; 3*i*) | 6 | 9 |
| ($\pi_2$♂♂ × $\pi_2$♀♀) | 9 | 752 | 6.66 | 2.34 | 5.14 | 5,010 | 6 | 1 | 0 | 0 | 0 | 0 | 7 | 7 |
| 2A | 2 | 719 | 7.28 | 2.34 | 5.70 | 5,238 | 6 | 1 | 0 | 0 | 0 | 0 | 7 | 7 |
| (CS♂♂ × *C(1)DX, y f/Y*♀♀) | 9 | 657 | 7.62 | 2.22 | 6.15 | 5,006 | 8 | 1 | 0 | 0 | 0 | 0 | 9 | 9 |
| 2B | 2 | 839 | 7.28 | 2.41 | 5.67 | 6,110 | 10 | 1 | 0 | 0 | 0 | 0 | 11 | 11 |
| (*y cin w*♂♂ × CS♀♀) | 9 | 779 | 7.27 | 2.35 | 5.66 | 5,664 | 7 | 4 | 0 | 0 | 0 | 0 | 11 | 11 |
| 2C | 2 | 814 | 6.97 | 2.34 | 5.46 | 5,675 | 8 | 1 | 0 | 0 | 0 | 0 | 9 | 9 |
| (CS♂♂ × CS♀♀) | 9 | 707 | 7.14 | 2.35 | 5.44 | 5,049 | 7 | 2 | 0 | 0 | 0 | 0 | 9 | 9 |
| 3A | 2 | 714 | 6.77 | 2.52 | 5.15 | 4,836 | 65 | 9 | 3 | 0 | 0 | 0 | 77 | 80 |
| ($\nu_6$♂♂ × *C(1)DX, y f/Y*♀♀) | 9 | 687 | 7.13 | 2.31 | 5.69 | 4,901 | 36 | 7 | 1 | 0 | 0 | 0 | 44 | 45 |
| 3B | 2 | 753 | 6.98 | 2.36 | 5.50 | 5,258 | 10 | 3 | 0 | 0 | 1 | 0 | 14 | 15 |
| (*y cin w*♂♂ × $\nu_6$♀♀) | 9 | 739 | 7.07 | 2.42 | 5.42 | 5,222 | 14 | 1 | 1 | 0 | 0 | 1(2*c*; 1*i*) | 17 | 20 |
| 3C | 2 | 777 | 5.51 | 2.55 | 3.79 | 4,282 | 11 | 1 | 0 | 0 | 0 | 0 | 12 | 12 |
| ($\nu_6$♂♂ × $\nu_6$♀♀) | 9 | 740 | 5.34 | 2.56 | 3.64 | 3,953 | 7 | 1 | 2 | 0 | 0 | 0 | 10 | 12 |
| 4 | 2 | 557 | 5.44 | 2.64 | 3.58 | 3,030 | 1 | 0 | 2 | 0 | 0 | 0 | 3 | 5 |
| ($\pi_2$♂♂ × $\nu_6$♀♀) | 9 | 538 | 5.97 | 2.60 | 4.13 | 3,212 | 7 | 1 | 0 | 0 | 0 | 0 | 8 | 8 |
| 5 | 2 | 569 | 5.54 | 2.77 | 3.47 | 3,155 | 5 | 3 | 0 | 0 | 0 | 0 | 8 | 8 |
| ($\nu_6$♂♂ × $\pi_2$♀♀) | 9 | 592 | 5.98 | 2.57 | 4.17 | 3,543 | 3 | 5 | 0 | 0 | 0 | 0 | 8 | 8 |

See text for a detailed explanation $\bar{f}$ = arithmetic mean number sperm sampled per male, $\tilde{f}$ = harmonic mean, $N$ = total sperm sampled. Numbers of males that produced one lethal chromosome are tallied under *c* (complete) and *i* (incomplete); numbers that produced two are under *cc* (two completes), *ii* (two incompletes) and *ci* (one of each). More complicated cases are given separately. $l$ = total number lethal chromosomes identified.

TABLE 2

*Frequencies of sex-linked lethals occurring in hybrid and nonhybrid males*

| Experiment | Male age | $u \pm$ s.e. | | $us$ | $v \pm$ s.e. | | $x$ | $u^* \pm$ s.e. | | Pooled $u^* \pm$ s.e. | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1A | 2 | 0.02537 | 0.00341 | 24 | 0.01107 | 0.00237 | 0.585 | 0.03185 | 0.00372 | | |
| ($\pi_2$♂♂ × *C(1)DX, y f/Y*♀♀) | 9 | 0.02139 | 0.00348 | 44 | 0.01337 | 0.00224 | 0.566 | 0.02897 | 0.00375 | 0.03042 | 0.00264 |
| 1B | 2 | 0.00532 | 0.00103 | 32 | 0.00549 | 0.00098 | 0.352 | 0.00725 | 0.00112 | | |
| (*y cin w*♂♂ × $\pi_2$♀♀) | 9 | 0.00519 | 0.00100 | 17 | 0.00315 | 0.00076 | 0.373 | 0.00637 | 0.00105 | 0.00678 | 0.00077 |
| 1C | 2 | 0.00157 | 0.00080 | 22 | 0.00384 | 0.00081 | 0.166 | 0.00221 | 0.00082 | | |
| ($\pi_2$♂♂ × $\pi_2$♀♀) | 9 | 0.00140 | 0.00052 | 11 | 0.00220 | 0.00066 | 0.132 | 0.00169 | 0.00054 | 0.00185 | 0.00045 |
| 2A | 2 | 0.00134 | 0.00050 | 1 | 0.00019 | 0.00019 | 0.170 | 0.00137 | 0.00050 | | |
| (CS♂♂ × *C(1)DX, y f/Y*♀♀) | 9 | 0.00180 | 0.00059 | 0 | 0 | 0 | 0.219 | 0.00180 | 0.00059 | 0.00155 | 0.00038 |
| 2B | 2 | 0.00180 | 0.00054 | 1 | 0.00016 | 0.00016 | 0.234 | 0.00183 | 0.00054 | | |
| (*y cin w*♂♂ × *CS*♀♀) | 9 | 0.00194 | 0.00058 | 0 | 0 | 0 | 0.255 | 0.00194 | 0.00058 | 0.00188 | 0.00039 |
| 2C | 2 | 0.00158 | 0.00052 | 2 | 0.00035 | 0.00025 | 0.163 | 0.00164 | 0.00052 | | |
| (CS♂♂ × CS♀♀) | 9 | 0.00178 | 0.00059 | 1 | 0.00020 | 0.00020 | 0.230 | 0.00183 | 0.00059 | 0.00172 | 0.00039 |
| 3A | 2 | 0.01654 | 0.00180 | 6 | 0.00124 | 0.00050 | 0.615 | 0.01730 | 0.00183 | | |
| ($v_6$♂♂ × *C(1)DX, y f/Y*♀♀) | 9 | 0.00918 | 0.00135 | 1 | 0.00020 | 0.00020 | 0.494 | 0.00928 | 0.00135 | | |
| 3B | 2 | 0.00285 | 0.00078 | 3 | 0.00057 | 0.00033 | 0.230 | 0.00298 | 0.00078 | | |
| (*y cin w*♂♂ × $v_6$♀♀) | 9 | 0.00383 | 0.00100 | 1 | 0.00019 | 0.00019 | 0.384 | 0.00390 | 0.00100 | 0.00333 | 0.00062 |
| 3C | 2 | 0.00280 | 0.00080 | 1 | 0.00023 | 0.00023 | 0.279 | 0.00286 | 0.00080 | | |
| ($v_6$♂♂ × $v_6$♀♀) | 9 | 0.00303 | 0.00100 | 5 | 0.00126 | 0.00056 | 0.285 | 0.00339 | 0.00102 | 0.00306 | 0.00063 |
| 4 | 2 | 0.00165 | 0.00099 | 1 | 0.00033 | 0.00033 | 0.294 | 0.00175 | 0.00099 | | |
| ($\pi_2$♂♂ × $v_6$♀♀) | 9 | 0.00249 | 0.00087 | 4 | 0.00124 | 0.00062 | 0.380 | 0.00296 | 0.00091 | 0.00241 | 0.00067 |
| 5 | 2 | 0.00254 | 0.00089 | 12 | 0.00380 | 0.00117 | 0.347 | 0.00386 | 0.00105 | | |
| ($v_6$♂♂ × $\pi_2$♀♀) | 9 | 0.00226 | 0.00079 | 9 | 0.00254 | 0.00084 | 0.333 | 0.00311 | 0.00087 | 0.00341 | 0.00067 |

$u$ = proportion of lethals among chromosomes tested; $us$ = total number unsure chromosomes; $v$ = proportion of unsures among chromosomes tested; $x$ = proportion unsure chromosomes expected to be lethal; $u^*$ = adjusted mutation rate.

brids by crossing wild-type females with males from the attached-*X* stock. The letter C designates tests with nonhybrids. The experiments numbered 4 and 5 identify tests with reciprocal hybrids between the $\pi_2$ and $v_6$ wild-type strains. In all experiments, tests were performed with males aged two and nine days from the time of collection. In some cases, slightly younger males were used in the latter group, but never less than seven days old. Altogether, 101,630 sperm were sampled from 15,468 hybrid and nonhybrid males; 471 of these sperm carried newly arisen lethal mutations on the *X* chromosome. However, it is apparent from the tables that the incidence of the lethals varied among experimental groups.

The mutation rate for sex-linked lethals occurring in the sperm and sperm cell precursors of the tested males was estimated according to the weighted procedure prescribed by ENGELS (1979c). This takes proper account of mutant clusters, which were found in the experiments. Complete and incomplete lethals were lumped into a single category ($l$) and the proportion of these among all chromosomes tested was calculated; this is given, along with its empirical standard error, in the column headed "$u$" in Table 2. The value of $u$ was then adjusted upward by incorporating the fraction of chromosomes in the unsure category that were probably lethal. This quantity was estimated by obtaining the proportion of suspected lethals that turned out to be lethal, relative to those that could be classified as either lethal or nonlethal according to the criteria presented earlier. This estimate, designated $x$ in Table 2, was then multiplied by the frequency of unsures among all chromosomes ($v$ in Table 2), and the result was added to $u$ to obtain the adjusted mutation rate, $u^*$. The variance of $u^*$ was calculated as

$$V(u^*) = V(u) + x^2\,V(v) + v^2\,V(x),$$

where $V(x)$ was estimated from the binomial, and $V(u)$ and $V(v)$ were obtained empirically. (Again, ENGELS' procedure for reckoning with clusters was followed.) This formula neglects a covariance between $u$ and $v$, which is necessarily negative, so that the estimate for $V(u^*)$ is conservative.

The adjusted rates for the two- and nine-day groups were tested for consistency and, where no significant difference was found, the rates were pooled into a single estimate. Pooling was accomplished by weighing each rate by the reciprocal of its variance. The pooled values are given in the last column of Table 2, and are shown along with their confidence intervals in Figure 1. The only significant difference between two- and nine-day groups occurred in the $v_6$-A hybrids. For these, the males aged two days before mating had a higher mutation rate than their counterparts aged nine days ($P < 0.001$).

It is evident from the data that all the CS experimental groups had similar mutation rates, ranging from 0.00155 to 0.00188 lethals per *X* chromosome. These rates were also quite similar to the rate for the $\pi_2$ nonhybrids, which was 0.00185. The $v_6$ nonhybrids showed a slightly elevated rate, 0.00306, but this was not significantly greater than even the lowest of the previously mentioned values. Moreover, the rates for the $v_6$-B hybrids and for the reciprocal hybrids between

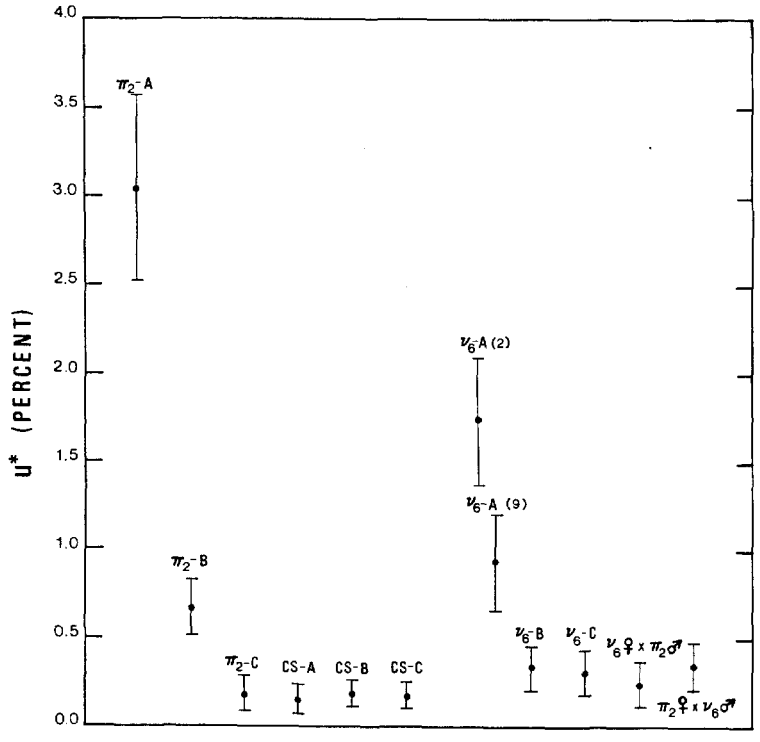


FIGURE 1.—Pooled sex-linked lethal rates ± 2 s.e., after adjusting for the unsure chromosomes. See text and Table 1 for an explanation of the symbols.

$\pi_2$ and $\nu_6$ were not significantly different from one another or from any of the previously mentioned values. The only groups that had significantly elevated mutation rates were $\pi_2$-A, $\pi_2$-B and $\nu_6$-A.

The first of these had a sex-linked lethal mutation rate of about 0.03; this is more than 15 times the control rate measured in the $\pi_2$ nonhybrids. Hybrid males from the reciprocal cross, $\pi_2$-B, also exhibited an increased mutability, but to a lesser extent; for these, the rate was between three and four times the control. The smaller increase in the B hybrids is reminiscent of ENGELS' (1979d) results with male recombination and segregation distortion, both of which were found to occur at lower frequencies in the B than in the A hybrids. There is also evidence that the B hybrids are intermediate between the nonhybrids and A hybrids when the frequencies of certain visible mutations are measured (ENGELS 1979a). However, the surprising result concerned the $\nu_6$-A hybrids, where the males aged two days were about half as mutable as the $\pi_2$-A hybrids, and those aged nine days were about a third so. This high mutability indicates that $\nu_6$ produces dysgenic hybrid males when crossed to *M* strain females. When the reciprocal cross was performed, there was no apparent increase in the hybrid male mutation rate.

The unidirectional mutation-inducing potential of $\nu_6$ is one characteristic that distinguishes it from $\pi_2$. Another is the inability of $\nu_6$ to produce sterile females when crossed to an *M* strain, even at high temperature. Daughters of the mating of $\pi_2$ males × *M* females are almost always sterile when raised at 27 to 29° (ENGELS and PRESTON 1979), but those from the mating of $\nu_6$ males × *M* females are almost always fertile. Although $\pi_2$ and $\nu_6$ clearly differ in this respect, they are more similar to one another than to the *M* strain. Both produce highly mutable male hybrids when crossed to *M* females, but when crossed *inter se*, they produce hybrids that are no more mutable than nonhybrid controls.

There is another difference between $\pi_2$ and $\nu_6$; this concerns the relative frequency of complete and incomplete lethals recovered from the A hybrids of each strain. When summed over two- and nine-day groups, $\pi_2$-A hybrids produced 60 single complete lethals and 31 single incomplete lethals (clusters were excluded from this analysis); for $\nu_6$, the comparable statistics were 101 and 16 (pooling across ages was appropriate in both cases). When compared by FISHER's exact test, there is a significant difference in the incidence of incomplete lethals in the two groups. This might be explained by supposing that a larger fraction of the lethals that occurred in the $\pi_2$-A hybrids were leaky, permitting occasional escapers to reach the adult stage; another possibility is that a larger fraction of the $\pi_2$-A lethals were unstable, producing occasional wild-type revertants. We attempted to distinguish between these possibilities by collecting wild-type males from the progeny of incomplete lethal cultures and mating them to attached-*X* females. If the males were escapers from some lethal effect, they would be expected to be weak and sterile or, if fertile, would not be expected to produce large broods. If they did produce large broods, the sex ratio would most likely be lopsided in favor of females. On the other hand, if the males were *bona fide* revertants, they would be expected to produce broods with a sex ratio more nearly equal to one.

Data bearing on the matter are presented in Table 3 and in Figure 2. These come from experiments in which several cultures for each of many lethal lines were established in order to detect rare wild-type males. We began these experiments about midway through the program to measure mutation rates in the various hybrids and nonhybrid controls, and we attempted to study every lethal identified from that point on. The procedure was the same as in the tests for the

TABLE 3

*Numbers of wild-type males recovered from incomplete lethal lines*

| | *c* | *i* | *n* | Total | Tested | Sterile | Poorly* fertile | Fertile |
|---|---|---|---|---|---|---|---|---|
| $\pi_2$-A | 31 | 26 | 509 | 142 | 91 | 31 | 10 | 50† |
| CS | 14 | 5 | 709 | 40 | 31 | 17 | 1 | 13‡ |
| $\nu_6$-A | 56 | 8 | 678 | 26 | 24 | 8 | 1 | 15§ |

See text for an explanation. $c$ = number complete lethals tested, $i$ = number incomplete lethals tested, $n$ = mean progeny size per lethal line.

* 10 progeny or less; †From 12 lines; ‡From 2 lines; §From 6 lines.

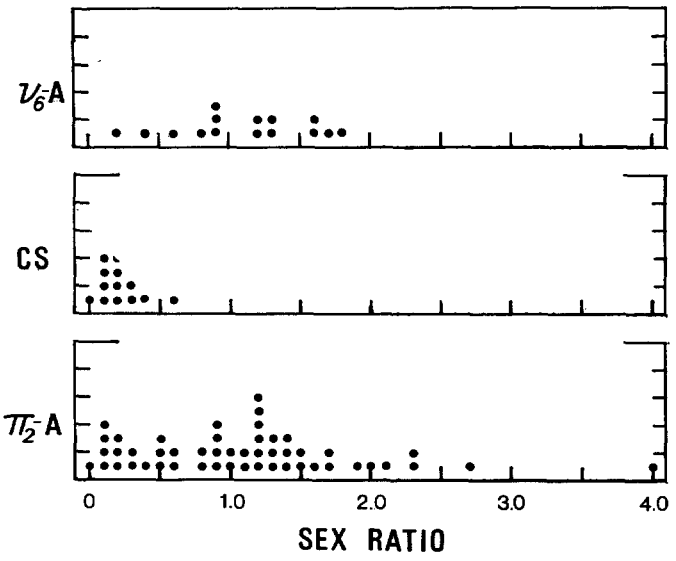


FIGURE 2.—Male:female ratio among the progeny of wild-type males recovered from various incomplete lethal lines. Each circle represents one male.

authenticity of suspected lethal chromosomes. *FM7/l* females were mated in vials to *FM7/Y* males at 25°; the progeny were scored on days 14 and 17 after mating. Wild-type males that emerged were mated individually to four *C(1)DX*, *y f/Y* females at 25°. The progeny of these cultures were scored as usual, and the ratio of males to females was calculated to distinguish between escapers and revertants.

Table 3 shows the data for the $\pi_2$-A and $\nu_6$-A groups. For comparison, we have included data on complete and incomplete lethals identified in the three CS groups; the numbers in each of these were too small to be meaningful by themselves and were therefore combined. Notice that the average brood sizes were larger for the $\nu_6$-A group than for the $\pi_2$-A group. This means that, all other things being equal, the chance of detecting a wild-type male (and therefore of classifying the lethal as incomplete) should have been greater for the $\nu_6$-A group than for $\pi_2$-A group. In fact, it was less. Thus, the difference in the proportion of incomplete lethals between these two groups is even more significant than it first appears.

What is the nature of the wild-type males? The plots in Figure 2 indicate that probably both escapers and revertants occurred, but that the former predominated in the CS lines, while the latter did in the $\pi_2$ and $\nu_6$ lines. The sex ratio for the progeny of the wild-type males from the CS lines was lower than ½ in all cases but one. Unfortunately, these data come from only two incomplete lethal lines. The other incomplete lethals in this group produced wild-type males that were sterile or only poorly fertile. Although the information for the CS incomplete lethals is limited, it suggests that these lines included cases in which a few wild-type males escaped a lethal effect and reached adulthood.

The situation is different for the $\pi_2$-A incomplete lethals. The sex ratios for the progeny of 50 males derived from 12 different lines indicate that many reversions may have taken place. The frequency plot for the $\pi_2$ data is bimodal, with probability mass concentrated between 0 and ½, and also farther up on the scale. The mass at the lower end presumably represents the escapers, while that farther up represents the revertants.

The $\nu_6$ data are only slightly more extensive than the CS data. Sex ratios for 15 wild-type males derived from six different lines are plotted in Figure 2. In this case, there is a fairly even spread across the range, but with some concentration near 1.0. The implication is that some of the wild-type males from the $\nu_6$-A lines were revertants.

Although we cannot be certain, it seems that some of the lethal mutations that occurred in the $\pi_2$-A and $\nu_6$-A hybrids were unstable; moreover, the $\pi_2$-A mutations seemed to be unstable more frequently than their $\nu_6$-A counterparts.

## DISCUSSION

CROW and TEMIN (1964) summarized extensive data on the occurrence of *X*-linked lethal mutations in males. The overall rate was 0.25% per chromosome generation, regardless of whether the chromosomes came from nature or from laboratory stocks. The data presented here indicate that the mutation rate in male Drosophila can be much greater if the flies are hybrids. In particular, offspring from the crosses of *P* males × *M* females and *Q* males × *M* females are much more mutable than flies taken directly from either the *P* or *Q* strains. This may be understood in terms of an interaction between chromosomal factors from one strain and the cytoplasm of the other, the effect of which is to cause abnormalities in the germ line.

Evidence for high mutability in hybrid flies has been presented previously. KIDWELL, KIDWELL and IVES (1977) monitored the occurrence of *X*-linked lethals in reciprocal hybrids of *P* and *M* strains, but the data were for female hybrids only. WOODRUFF, THOMPSON and LYMAN (1979) also presented *X*-linked lethal data, but these were limited and did not involve reciprocal hybrids. The latter group also studied the occurrence of visible mutations in hybrid and nonhybrid flies, but their technique could be faulted for its subjective scoring method. This same criticism applies to the work of THOMPSON and WOODRUFF (1980), PICARD *et al.* (1978) and BERG (1979), all of whom scored visible mutations in uncoded cultures involving either hybrid or nonhybrid parents. The possibility of finding more visible mutants in cultures coming from hybrid parents simply because one expects to find them there is a real danger in any experiment in which the cultures are not coded. This problem is compounded when clusters of mutations occur, as they clearly do in dysgenic hybrids. The clusters indicate that some mutations are not independent and therefore require special statistical treatment (ENGELS 1979c).

ENGELS (1979a) published data from experiments to measure visible mutation rates at two *X*-linked loci in reciprocal, but genetically identical, hybrids

and in nonhybrid controls. In these experiments the cultures were coded and appropriate statistical tests were applied to the data. The results showed that the offspring of the cross of *P* males × *M* females are indeed more mutable than hybrids from the reciprocal cross, which are in turn more mutable than nonhybrids from the *P* strain. Our results parallel these.

ENGELS (1979a) has theorized that the high mutability of hybrids from the cross of *P* males × *M* females is due to the interaction of paternally contributed *P* factors with a maternally contributed cytoplasmic property called cytotype. The latter can exist in two states, *M* and *P*, and is ultimately determined by the genotype. The chromosomal *P* factors are capable of causing dysgenic traits in flies whose mothers were of the *M* cytotype. This condition arises whenever *P* males are crossed with *M* females, but not usually when the reciprocal cross is performed; nor does it occur in the nonhybrid progeny of either *M* or *P* strains, for the former lack *P* factors and the latter the *M* cytotype. In fact, *P* strains possess the *P* cytotype and this has been shown to suppress the dysgenic condition (ENGELS 1979a).

Our data also include results from experiments with a *Q* strain. The cross of *Q* males × *M* females yields hybrids with a higher mutability than the nonhybrids, but the reciprocal cross does not. Moreover, hybrids from either of the crosses between the *Q* and *P* strains have mutation rates on a par with those of the nonhybrids. These results are consistent with the notion that the *Q* strain is a weakened or defective *P* strain, possessing the *P* cytotype and a variant of the *P* factor. Since the female offspring of crosses between *Q* and *M* strains are fertile, even when reared at high temperature, the chromosomal factor carried by the *Q* strain is not fully equivalent to the *P* factor. If it were, *Q* male × *M* female crosses would produce mostly sterile daughters. On the other hand, the *Q* strain does have the *P* cytotype, as judged by the fertility of daughters from the cross *P* male × *Q* female. Moreover, sons from this cross are no more mutable than males from the *Q* strain itself, indicating that the *Q* strain possesses a cytotype capable of suppressing the mutation-causing properties of the *P* factor.

The mutagenic power of the *P* factor carried by the *Q* strain is apparently less than that of the *P* strain. The mutation rate in *Q* × *M* hybrids is lower than that in *P* × *M* hybrids; moreover, the mutations that do occur exhibit greater stability. This indicates either a much lower frequency of *P* factors on the chromosomes of the *Q* strain or the *P* factors there are defective. In the latter case, it would be proper to designate the *P* factor carried by the *Q* strain as different, *i.e.*, $P^q$. For the present, it is impossible to discriminate between these two possibilities.

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## LITERATURE CITED

BAKER, B. S., 1973 The maternal and zygotic control of development by *cinnamon*, a new mutant in *Drosophila melanogaster*. Develop. Biol. **33**: 429–440.

BERG, R. L., 1979 Global patterns of mutability in natural populations of *Drosophila melanogaster*. Genetics **91**: s8–9.

BREGLIANO, J. C., G. PICARD, A. BUCHETON, A. PELISSON, J. M. LAVIGE and P. L'HERITIER, 1980 Hybrid dysgenesis in *Drosophila melanogaster*. Science **207**: 606–611.

CROW, J. F. and R. G. TEMIN, 1964 Evidence for the partial dominance of recessive lethal genes in natural populations of Drosophila. Am. Naturalist **98**: 21–33.

DEMEREC, M., 1937 Frequency of spontaneous mutations in certain stocks of *Drosophila melanogaster*. Genetics **22**: 469–478.

DUDICK, M. E., T. R. F. WRIGHT and L. L. BROTHERS, 1974 The developmental genetics of the temperature-sensitive lethal allele of the suppressor of forked, *1(1)su(f)$^{ts679}$*, in *Drosophila melanogaster*. Genetics **76**: 487–510.

ENGELS, W. R., 1979a Extrachromosomal control of mutability in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **76**: 4011–4015. ——, 1979b Hybrid dysgenesis in *Drosophila melanogaster*: rules of inheritance of female sterility. Genet. Res., Camb. **33**: 219–236. ——, 1979c The estimation of mutation rates when premeiotic events are involved. Environ. Mutagenesis **1**: 37–43. ——, 1979d Germ line aberrations associated with a case of hybrid dysgenesis in *Drosophila melanogaster* males. Genet. Res., Camb. **33**: 137–146.

ENGELS, W. R. and C. R. PRESTON, 1979 Hybrid dysgenesis in *Drosophila melanogaster*: the biology of female and male sterility. Genetics **92**: 161–174.

IVES, P. T., 1950 The importance of mutation rate genes in evolution. Evolution **4**: 236–252.

KIDWELL, M. G., 1979 Hybrid dysgenesis in *Drosophila melanogaster*: the relationship between the *P-M* and *I-R* interaction systems. Genet. Res., Camb. **33**: 205–217.

KIDWELL, M. G., J. F. KIDWELL and P. T. IVES, 1977 Spontaneous non-reciprocal mutation and sterility in strain crosses of *Drosophila melanogaster*. Mutation Res. **42**: 89–98.

KIDWELL, M. G., J. F. KIDWELL and J. A. SVED, 1977 Hybrid dysgenesis in *Drosophila melanogaster*: a syndrome of aberrant traits including mutation, sterility and male recombination. Genetics **86**: 813–833.

KIDWELL, M. G. and J. B. NOVY, 1979 Hybrid dysgenesis in *Drosophila melanogaster*: sterility resulting from gonadal dysgenesis in the *P-M* system. Genetics **92**: 1127–1140.

LINDSLEY, D. L. and E. H. GRELL, 1968 *Genetic variations of* Drosophila melanogaster. Carnegie Institution of Washington Publication No. 627.

MERRIAM, J., 1968 Drosophila Inform. Serv. **43**: 64.

PICARD, G., 1976 Non-Mendelian female sterility in *Drosophila melanogaster*: hereditary transmission of *I* factor. Genetics **83**: 107–123.

PICARD, G., J. C. BREGLIANO, A. BUCHETON, J. M. LAVIGE, A. PELISSON and M. G. KIDWELL, 1978 Non-Mendelian female sterility and hybrid dysgenesis in *Drosophila melanogaster*. Genet. Res., Camb. **32**: 275–287.

STURTEVANT, A. H., 1939 High mutation frequency induced by hybridization. Proc. Natl. Acad. Sci. U.S. **25**: 308–310.

THOMPSON, J. N., JR. and R. C. WOODRUFF, 1980 Increased mutation in crosses between geographically separated strains of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **77**: 1059–1062.

WOODRUFF, R. C., J. N. THOMPSON, JR., and R. F. LYMAN, 1979 Intraspecific hybridization and the release of mutator activity. Nature **278**: 277–279.

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