ANALYSIS OF Y-LINKED MUTATIONS TO MALE STERILITY IN *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

The frequencies of newly induced male-sterilizing lesions on both the X and Y chromosomes of *Drosophila melanogaster* were determined after either 4000 r of γ-irradiation or adult feeding of ethyl methanesulfonate. The Y chromosome is approximately twice as sensitive as the X chromosome to newly induced male-sterilizing lesions after γ-irradiation, but slightly less sensitive after ethyl methanesulfonate treatment. A large proportion of the radiation-induced lesions are associated with Y-autosome or X-autosome translocations, with the Y chromosome recovered in translocations far in excess of the frequency expected from metaphase lengths. Although translocations between the X and Y chromosomes or between autosomes do not appear to sterilize heterozygous males, interchanges between sex chromosomes and autosomes often sterilize males carrying them in a dominant manner, suggesting that the organization of the genome is critical for normal spermatogenesis. Complementation tests between recessive Y-linked male-sterilizing mutants do not reveal the existence of any additional fertility loci beyond the six previously defined.

THE Y chromosome of *Drosophila melanogaster* accounts for 13% of the metaphase chromosome length in a normal diploid male (after BRIDGES, from DOBZHANSKY 1929; GOWEN and GAY 1933) but is dispensable in all cells except the germ line of the male; males lacking a Y chromosome are viable but sterile (BRIDGES 1916). In an earlier paper (KENNISON 1981) the organization of the Y chromosome of *D. melanogaster* into six restricted regions necessary for male fertility separated by large blocks of chromosomal material not necessary for male fertility was described. These fertility regions appear to be associated with the nonfluorescent blocks that are seen after Hoechst 33258 staining. All male-sterilizing X–Y translocations with genetic breakpoints in the same fertility region fail to complement, suggesting that each region contains only one functional unit.

The X chromosome, approximately the same metaphase length as the Y chromosome (after BRIDGES, from DOBZHANSKY 1929), is estimated to contain 100–150 genes that can mutate to male sterility (LINDSLEY and LIFSCHYTZ 1972), whereas only six male fertility loci on the Y chromosome have been identified.

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In spite of this, after irradiation with X-rays or γ-rays, the incidence of sterility among males receiving an irradiated Y chromosome is greater than twice that of males receiving an irradiated X chromosome (D. L. LINDSLEY, S. A. TOLEDO and C. C. MUSATTI, unpublished results; M. SCHWARTZ, unpublished results). In addition, translocations involving the Y chromosome appear to often inactivate one of the six fertility loci. Of translocations recovered in *T(X; Y)/Y* or *T(Y; A)/Y* males, approximately one-half sterilize *T(X; Y)/O* or *T(Y; A)/O* males (NICOLETTI and LINDSLEY 1960; B. NICOLETTI, unpublished data; LINDSLEY *et al.* 1972; KENNISON 1981).

To investigate the basis for the high sensitivity of the Y chromosome to the induction of male-sterilizing mutations, mutagen-treated X and Y chromosomes were recovered in daughters of treated males and subsequently examined for effects on male fertility and for chromosome aberrations. The majority of Y-linked male-sterilizing mutations recovered after irradiation are associated with chromosome aberrations. Approximately two-thirds are associated with Y-autosome translocations, more than one-half of which sterilize males even in combination with an XY chromosome and, thus, behave as dominant male-sterilizing mutations. The nontranslocated male-sterilizing Y chromosomes all behave as recessive male-sterilizing mutations and probably include for the most part intrachromosomal rearrangements, *i.e.*, deficiencies and inversions. These results, in addition to earlier studies on X–Y translocations (NICOLETTI and LINDSLEY 1960; B. NICOLETTI, unpublished results; KENNISON 1981), *T(2;3)s* and autosomal dominant male-sterilizing mutations (PRABHU 1941; J. A. KENNISON and L. S. B. GOLDSTEIN, unpublished results), and X-autosome translocations (LINDSLEY 1965; LINDSLEY and TOKUYASU 1980), suggest that the organization of the genome is critical for normal spermatogenesis. Although translocations between the X and Y chromosomes or between the autosomes do not appear to sterilize heterozygous males, interchanges between sex chromosomes and autosomes often sterilize males carrying them in a dominant manner.

MATERIALS AND METHODS

For simplicity, X or Y chromosomes that sterilize the males carrying them will often be referred to as "male sterile" X or "male sterile" Y chromosomes. Although the results will be discussed with respect to the X and Y chromosomes of *D. melanogaster* in general, it should be noted that all experiments presented here are with specifically marked X and Y chromosomes. With the exception of the Y-linked male-sterilizing mutations induced and characterized here, all chromosomes and mutations used are described in more detail in LINDSLEY and GRELL (1968), LINDSLEY *et al.* (1972), BROSSEAU (1958, 1960) or KENNISON (1981). Flies were raised at 25° unless otherwise noted. The scheme used for the recovery and selection of mutagen-treated X and Y chromosomes is shown in Figure 1. An asterisk (*) will be used throughout to denote a mutagen-treated X or Y chromosome. *In(1)sc*^{7}, *sc*^{7} *w*^{a}/*B*^{S}*Yy*$^{+}$ males were either irradiated with approximately 4000 r of γ-rays from a ^{60}Co source or fed ethyl methanesulfonate (EMS) for 24 hr according to the method of LEWIS and BACHER (1968). Treated males were mass mated to virgin *In(1)B*^{M1}, *y*^{2} *su(w*^{a}*)* *w*^{a} *v* *B*^{M1}/*In(1)sc*^{4L}*sc*^{8R}, *y sc*^{4} *sc*^{8} *w*^{a} *cv v f*/*w*$^{+}$*Y2* females and discarded 2 days after mating for the EMS-treated males or 4 days after mating for the irradiated males. The inseminated females were then transferred once to new cultures for 4 days before being discarded.

Individual virgin F_1 females with either a mutagen-treated X or Y chromosome (*i.e.*, X/X* or X/X/Y*) were mated to three males of the appropriate genotype and transferred to new vials every 6 days. F_1 females that gave no progeny were classified as sterile if they survived at least 12 days after the initial mating, and otherwise as having died too soon for a valid test. Mutagen-treated X

chromosomes were recovered in single F_1 $In(1)B^{M1}/In(1)sc^{7*}$ or $In(1)sc^{4L}sc^{8R}/In(1)sc^{7*}$ females and mated to $In(1)B^{M1}$, y^2 $su(w^a)$ w^a B^{M1}/Y males. F_1 females were scored as heterozygous for a newly induced sex-linked lethal or semilethal if they produced at least 20 sons, fewer than 5% of which carried the mutagen-treated X chromosome. Males carrying a mutagen-treated X chromosome that was viable in combination with a normal Y chromosome were tested for fertility; approximately five $In(1)sc^7$, sc^7 w^a/Y (X^*/Y) males were placed in a vial with five virgin $C(1)RM/Y$ females, and the vials were scored for the presence or absence of larvae between 4 days and 2 weeks after mating. Genotypes with fewer than five larvae per test vial were retested and classified as sterile if they continued to produce fewer than one larva per tested male.

Mutagen-treated Y chromosomes were recovered in single F_1 $In(1)B^{M1}/In(1)sc^{4L}sc^{8R}/B^SYy^{+*}$ females and mated to $XY^L \cdot Y^S$ (108-9), y^2 $su(w^a)$ w^a f/O males. Figure 2 gives the genotypes and corresponding phenotypes recovered among the progeny of this cross. Three types of sons with the mutagen-treated Y chromosome were recovered among the progeny: those with an X chromosome deficient for 80% of the proximal heterochromatin ($In(1)sc^{4L}sc^{8R}$), those with a normal X chromosome ($In(1)B^{M1}$), and those with an attached XY chromosome ($XY^L \cdot Y^S$). $In(1)B^{M1}$ was utilized because females carrying it in combination with $In(1)sc^{4L}sc^{8R}$ and a Y chromosome produced more nearly equivalent frequencies of sons of these three genotypes than did females carrying, instead, one of the other three small X chromosome inversions tested ($In(1)sc^7$, $In(1)AB$, and $In(1)B^{M2}$). Accordingly, this maximized the chances of recovering each Y chromosome in all three male genotypes. Each type of male was tested for fertility in the same manner as the males with a mutagen-treated X chromosome. Dominant sterile, Y-linked mutations are expected to sterilize all three classes of males. Recessive mutations are expected to sterilize males carrying $In(1)B^{M1}$ or $In(1)sc^{4L}sc^{8R}$, but not males carrying the XY chromosome. Both Y-autosome translocations and nontranslocated sterile Y chromosomes might be expected in these two classes of mutations. A third class of mutations, those that sterilize only males carrying $In(1)sc^{4L}sc^{8R}$, should include

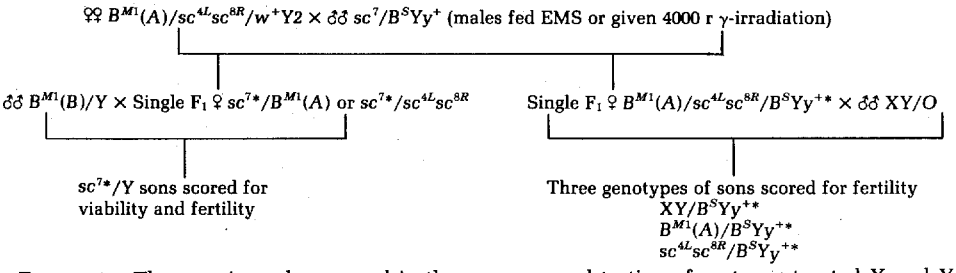

FIGURE 1.—The crossing scheme used in the recovery and testing of mutagen-treated X and Y chromosomes. Mutagen-treated X and Y chromosomes are noted with an asterisk (*). sc^7, $B^{M1}(A)$, $sc^{4L}sc^{8R}$, $B^{M1}(B)$, and XY refer to the following X chromosomes, respectively: $In(1)sc^7$, sc^7 w^a; $In(1)B^{M1}$, y^2 $su(w^a)$ w^a v B^{M1}; $In(1)sc^{4L}sc^{8R}$, y sc^4 sc^8 w^a cv v f; $In(1)B^{M1}$, y^2 $su(w^a)$ w^a B^{M1}; $XY^L \cdot Y^S$(108-9), y^2 $su(w^a)$ w^a f.

♀♀ $In(1)B^{M1}/In(1)sc^{4L}sc^{8R}/B^SYy^{+*}$ × ♂♂ $XY^L \cdot Y^S$(108–9)/O

	$XY^L \cdot Y^S$ (108–9)	O
$In(1)B^{M1}$	♀ y^2 $su(w^a)$ w^a	♂ y^2 $su(w^a)$ w^a v B^{M1}
$In(1)sc^{4L}sc^{8R}/B^SYy^{+*}$	♀ w^a f B^S	♂ sc^4 sc^8 w^a cv v f B^S
$In(1)B^{M1}/B^SYy^{+*}$	♀ $su(w^a)$ w^a B^S	♂ $su(w^a)$ w^a v B^S
$In(1)sc^{4L}sc^{8R}$	♀ y^2 w^a f	lethal
$In(1)B^{M1}/In(1)sc^{4L}sc^{8R}$	lethal	♀ y^2 w^a v
B^SYy^{+*}	♂ $su(w^a)$ w^a f B^S	lethal

FIGURE 2.—Genotypes and phenotypes of progeny from crosses of $In(1)B^{M1}$, y^2 $su(w^a)$ w^a v $B^{M1}/In(1)sc^{4L}sc^{8R}$, y sc^4 sc^8 w^a cv v f/B^SYy^{+*} females by $XY^L \cdot Y^S$(108-9), y^2 $su(w^a)$ w^a f/O males. The asterisk denotes the chromosome derived from the mutagen-treated father of the parental female.

mutations in fertility loci on the Y chromosome that are also present in the proximal X heterochromatin between the sc^4 and sc^8 inversion breakpoints, as well as most male fertile Y-autosome translocations (BESMERTNAIA 1934; SCHULTZ 1947; LINDSLEY *et al.* 1979). All male fertile Y-autosome translocations tested by LINDSLEY *et al.*, except for insertional translocations in which a segment of autosomal material is inserted into the Y chromosome and reciprocal translocations with nearly terminal breakpoints in the autosome, were sterile or nearly sterile in combination with $Df(1)bb^{l158}$ (a deficiency for a majority of the X chromosome heterochromatin). Insertional translocations would not be detected in this screen. However, male fertile reciprocal Y-autosome translocations with nearly terminal autosomal breakpoints are recognizable since they produce viable and phenotypically distinct aneuploid progeny. All mutagen-treated Y chromosomes that sterilized males carrying the XY chromosome were kept by selecting $X/XY/Y^{\cdot}$ females and XY/O males each generation. Mutagen-treated Y chromosomes that did not sterilize males carrying a normal X chromosome, but either gave viable aneuploid progeny or sterilized males carrying $In(1)sc^{4L}sc^{8R}$, were retained as balanced stocks of $C(1)A$, $y/B^{S}Yy^{+\cdot}$ females and $In(1)B^{M1}/B^{S}Yy^{+\cdot}$ males. The remaining mutagen-treated Y chromosomes of interest, those that sterilized males carrying a normal X chromosome but not males carrying the XY chromosome, were retained as balanced stocks of $C(1)A$, $y/B^{S}Yy^{+\cdot}$ females and $XY/B^{S}Yy^{+\cdot}$ males.

All mutagen-treated Y chromosomes that sterilized one or more of the three classes of males tested, as well as those that were fertile in all three genotypes but gave viable aneuploid progeny, were tested for pseudolinkage of the Y chromosome and chromosomes 2, 3, and 4 among the progeny of either $y/y/B^{S}Yy^{+\cdot}$; $In(2LR)O$, $Cy\ dp^{lvl}pr\ cn^{2}/+$; $In(3LR)TM6$, $Hn^{P}\ ss^{P88}\ bx^{34e}\ Ubx^{P15}\ e/+$; $spa^{pol}/+$ females or $Y^{S}X\cdot Y^{L}$, $In(1)EN$, $y/B^{S}Yy^{+\cdot}$; $dp\ bw/+$; $st\ p^{p}/+$; $spa^{pol}/+$ males.

Sterile Y chromosomes not involved in Y-autosome translocations and a sample of the Y-autosome translocations were retested for effects on male fertility in combination with a normal sequence X chromosome marked with the mutations *y w f*. At least 20 males were raised and tested for fertility at both 25° and 18°. The arm of the Y chromosome involved was determined by fertility tests of males of the genotypes $X\cdot Y^{L}$, $y\ v/B^{S}Yy^{+\cdot}$ or $X\cdot Y^{S}$, $y\ w/B^{S}Yy^{+\cdot}$.

For the sterile Y chromosomes not involved in Y-autosome translocations, the fertility regions mutant or deficient in each were determined by complementation with synthetic Y chromosome deficiencies generated from X–Y translocations (KENNISON 1981) as follows: $X^{D}Y^{P}$ refers to one translocation element, the centric portion of the Y chromosome capped by the acentric distal portion of the X chromosome. $Y^{D}X^{P}$ refers to another translocation element, the distal acentric portion of the Y chromosome appended to the centric proximal portion of the X chromosome. If the $X^{D}Y^{P}$ element is derived from a translocation with a Y chromosome breakpoint proximal to a fertility region, and the $Y^{D}X^{P}$ element is derived from a translocation with a Y chromosome breakpoint distal to that fertility region, then males of the genotype $X^{D}Y^{P}/Y^{D}X^{P}$ will carry a synthetic deficiency for that fertility region. For each fertility region, males carrying the synthetic deficiency and a $w^{+}Y2$ chromosome (genotype $X^{D}Y^{P}/Y^{D}X^{P}/w^{+}Y2$) were mated to $C(1)A$, $y/ms(Y)$ females, and the $X^{D}Y^{P}/Y^{D}X^{P}/ms(Y)$ sons were tested for fertility as described earlier. The X–Y translocations used to generate the synthetic deficiencies were $T(X;Y)F12$, $T(X;Y)E15$, $T(X;Y)W27$, $T(X;Y)V24$, $T(X;Y)W19$ and $T(X;Y)V8$ (KENNISON 1981). Pairwise complementation tests between selected mutant chromosomes were performed by mating $y\ w\ f/y\ w\ f/ms(Y)A$ females to $y\ w\ f/w^{+}Y2/ms(Y)B$ males ($ms(Y)A$ and $ms(Y)B$ refer to two different mutant Y chromosomes). The white-eyed $y\ w\ f/ms(Y)A/ms(Y)B$ sons, distinguished either by the presence of extra hairs in the second posterior cell of the wing resulting from two doses of the sc^{8}-derived y^{+} marker (BROSSEAU 1960; after SCHULTZ, from LINDSLEY and GRELL 1968), very narrow eyes resulting from two doses of the B^{S} marker (WILLIAMSON 1968), or both, were then tested for fertility.

RESULTS

The results from three separate experiments are given in Table 1. In the first experiment, males were exposed to approximately 4000 r of γ-irradiation. In the second and third experiments males were fed a 0.24% solution of EMS for 24 hours. m_l and m_s are the mean numbers of newly induced male-lethal and male-sterile mutations per mutagen-treated chromosome, respectively. The induction

TABLE 1

Data on the recovery and fertility of mutagen-treated X and Y chromosomes

	Mutagen-treated chromosomes					
	γ-Irradiation		EMS-1		EMS-2	
	X*	Y*	X*	Y*	X*	Y*
Individual F_1 females						
Died	0	1	6	10	0	0
Sterile	3	11	4	10	8	20
Fertile	203	556	112	253	199	655
F_2 males						
In(1)sc^{7*}/Y						
Lethal	29		59		74	
Sterile	20				8	
Fertile	154				117	
In(1)B^{M1}/B^{S}Yy^{+*}						
Sterile		132		21		26
Fertile		423		232		628
Not tested		1		0		1
m_l	0.15		0.75		0.46	
m_s	0.12	0.27		0.09	0.07	0.04

An asterisk (*) designates a mutagen-treated X or Y chromosome. F_1 females were either *In(1)sc*^{7}, *sc*^{7} w^{a*}/*In(1)*B^{M1}, y^2 *su(w*^{a}*)* w^a *v* B^{M1} or *In(1)sc*^{7}, *sc*$^7 w^{a*}$/*In(1)sc*^{4L}*sc*^{8R}, *y sc*^{4} *sc*^{8} w^a *cv v f* for mutagen-treated X chromosomes and *In(1)*B^{M1}, y^2 *su(w*^{a}*)* w^a *v* B^{M1}/*In(1)sc*^{4L}*sc*^{8R}, *y sc*^{4} *sc*^{8} w^a *cv v f*/B^{S}Yy^{+*} for mutagen-treated Y chromosomes. F_1 females were scored as sterile only if they survived at least 12 days and gave no progeny and as having died if they gave no progeny but survived less than 12 days. *In(1)sc*^{7} and *In(1)*B^{M1} refer to the chromosomes *In(1)sc*^{7}, *sc*^{7} w^a *and In(1)*B^{M1}, y^2 *su(w*^{a}*)* w^a *v* B^{M1}, respectively. m_l and m_s, the mean numbers of newly induced male-lethal and male-sterile mutations per mutagen-treated chromosome, respectively, were calculated as given in the text.

of mutations was assumed to follow a Poisson distribution, and m was calculated from the frequency of mutation-free chromosomes that equaled e^{-m}.

The results in Table 1 for the chromosomes recovered after irradiation can be compared with previous results. For chromosomes recovered and tested in F_1 males (D. L. LINDSLEY, C. C. MUSATTI and S. A.TOLEDO, unpublished results; M. SCHWARTZ, unpublished results) or for chromosomes recovered in F_1 females and tested in sons (M. SCHWARTZ, unpublished results) the mean numbers of newly induced male-sterile mutations (m_s) vary from 0.08 to 0.13 for the X chromosome and from 0.16 to 0.30 for the Y chromosome. In the experiment presented here, the mean numbers of male-sterile mutations for the X and Y chromosomes, respectively, are 0.12 and 0.27. Both of these values are within the range of previous values. Although the m_s values vary among experiments, within one experiment, m_s for the Y chromosome is always approximately twice m_s for the X chromosome.

The comparative frequencies of X- and Y-linked sterility after EMS treatment are given in the second EMS experiment. Although the frequency of Y-linked male sterility is slightly lower than the frequency of X-linked male sterility, the two are not significantly different by a χ^2 test for homogeneity ($P = 0.5$). The ratio of m_s to m_l for the X chromosome is 0.15, in agreement with the average value of 0.15 given by LINDSLEY and LIFSCHYTZ (1972).

To characterize the Y-linked male sterile mutations each treated Y chromosome was tested for effects on male fertility in combination with several different X chromosomes. In addition, many of the treated Y chromosomes were also tested for the presence of Y-autosome translocations by pseudolinkage of Y-linked and autosomal marker mutations. The results from these tests are given in Table 2. Using these results, I divided the mutations into several classes with respect to the probable cause of male infertility.

The first possible class of mutations I will discuss are in fertility loci common to the X and Y chromosomes, referred to as "sc^4 sc^8 sterile mutations" in Table 2. Only loci in the X heterochromatin between the sc^4 and sc^8 inversion breakpoints would be detected in the mutant screen employed. This region includes about 80% of the centric X heterochromatin (COOPER 1959). Y-linked mutations in these loci should sterilize males carrying $In(1)sc^{4L}sc^{8R}$, but not males carrying $In(1)B^{M1}$ or $XY^L \cdot Y^S$. Forty-nine such mutagen-treated Y chromosomes were recovered. The majority of these (39) were associated with Y-autosome translocations. These probably do not represent mutations in fertility loci common to the X and Y chromosomes, as the sterility is dominant and cannot be rescued by the addition of either an extra Y chromosome or a duplication of the entire proximal X heterochromatin to the genotype (LINDSLEY *et al.* 1979). The remaining ten chromosomes sterilized $In(1)sc^{4L}sc^{8R}$-bearing males in the first two generations after mutagen treatment (at least five males were tested each generation) but failed to sterilize either $In(1)sc^{4L}sc^{8R}$- or $Df(1)bb^{l158}$-bearing males when retested several generations later. The cause of this transient sterility in combination with $In(1)sc^{4L}sc^{8R}$ is not known. Mutations in the *bb* locus, which is the only known gene necessary for viability found on both the X and Y chromosomes, are unstable under some conditions and can revert to wild type by a process known as magnification (RITOSSA 1968; TARTOFF 1973). It is possible that the transient sterility is due to lesions in genes necessary for fertility that also exhibit the phenomenon of magnification.

A large proportion of the male sterile mutants is associated with Y-autosome translocations. This is shown in Table 3. After irradiation, 67% (88 of 132) of the Y-autosome translocations recovered sterilize males carrying them in combination with a normal X chromosome ($In(1)B^{M1}$). Of the remaining Y chromosomes that do not appear to involve Y-autosome translocations, only 10% (44 of 423) sterilize males in combination with $In(1)B^{M1}$. For the chromosomes recovered after EMS treatment, there is an even greater correlation between male sterility and the presence of a Y-autosome translocation. Eighty-three percent (15 of 18) of the Y-autosome translocations, but only 3.5% (31 of 888) of the remaining Y chromosomes, sterilized $In(1)B^{M1}$-bearing males. The sterility of males bearing Y-autosome translocations and a normal X chromosome has previously been assumed to result from inactivation of specific fertility loci by the translocation breakpoint (NICOLETTI and LINDSLEY 1960; LINDSLEY *et al.* 1972; KENNISON 1981). This is probably true for Y-autosome translocations that sterilize males bearing them in combination with a normal X chromosome but not in combination with a compound XY chromosome (a chromosome that contains all of the X and Y chromosome material necessary for male fertility),

TABLE 2

Classification of mutagen-treated Y chromosomes with respect to the presence or absence of Y-autosome translocations and fertility of males of various sex chromosome genotypes

	XY/ $B^{S}Yy^{+*}$	B^{M1}/ $B^{S}Yy^{+*}$	$sc^{4L}sc^{8R}$/ $B^{S}Yy^{+*}$	T(Y;A)	γ	EMS-1	EMS-2
Dominant sterile	−	−	−	Yes	50	7	4
mutations	−	−	−	No	0	0	0
Recessive sterile	+	−	−	Yes	38	4	0
mutations	+	−	−	No	44	9	22
	+	−	−	NT	0	1	0
sc^{4} sc^{8} sterile	+	+	−	Yes	37	1	1
mutations	+	+	−	No	4	4	2
	+	+	+	Yes[a]	7	1	0
	+	+	+	NT	375	226	625
	NT	NT	−	Yes	1	0	0
	NT	NT	NT	NT	0	0	1
Total					556	253	655

+ denotes that the tested males are fertile and − that they are sterile. NT is not tested. γ refers to the sample after γ-irradiation. XY,B^{M1}, and $sc^{4L}sc^{8R}$ refer to $XY^{L}\cdot Y^{S}$ *(108-9)*, y^{2} *su(w^{a})* w^{a} *f*; *In(1)*B^{M1}, y^{2} *su(w^{a})* w^{a} *v* B^{M1} and *In(1)*$sc^{4L}sc^{8R}$, *y* sc^{4} sc^{8} w^{a} *cv v f*, respectively. * indicates mutagen-treated chromosome.

[a] Originally detected by the survival of one or more classes of aneuploid progeny.

TABLE 3

Relation between Y-linked male-sterility mutations and Y-autosome translocations

Treatment	Male fertility	No. of T(Y;A) Present	Absent[a]	Not tested	Total
γ-irradiation	Fertile	44	379	0	423
	Sterile	88	44	0	132
	Not tested	1	0	0	1
	Total	133	423	0	556
EMS	Fertile	3	857	0	860
	Sterile	15	31	1	47
	Total	18	888	1	907

Males tested were of the genotype *In(1)*B^{M1}, y^{2} *su(w^{a})* w^{a} *v* $B^{M1}/B^{S}Yy^{+*}$.

[a] Male-sterile chromosomes showed no autosomal linkage in genetic tests. Male-fertile chromosomes were completely fertile in combination with *In(1)*$sc^{4L}sc^{8R}$ and gave no viable aneuploid progeny.

since the sterility can be mapped to specific loci by complementation analysis (M. GATTI and S. PIMPINELLI, personal communication; J. A. KENNISON, this paper and unpublished results). In the majority of cases examined here (50 of 88), however, the sterility associated with the Y-autosome translocations would be described as dominant, since XY/*T(Y; A)* and X/*T(Y; A)*-bearing males are sterile. It should be noted that the majority of X-autosome translocations also sterilize males carrying them (after WARTERS, from LINDSLEY 1965), even in the

presence of a duplication covering the X chromosome breakpoint (LINDSLEY 1965; LIFSCHYTZ and FALK 1968).

Although many of the Y-autosome translocations have been classified as dominant male-sterile mutations for analysis, Y-autosome translocations do not fall into distinct classes with respect to fertility of *XY/T(Y; A)*-bearing males. Males bearing any of the Y-autosome translocations tested showed reduced fertility compared with males bearing the untreated $B^S Yy^+$ chromosome. In combination with the XY chromosome initially tested, only one or two progeny were recovered from a large number of *XY/T(Y; A)*-bearing males tested, whereas for other translocations the males were completely sterile. Several of the Y-autosome translocations were retested for effects on male fertility in combination with a compound XY chromosome of different sequence, $Y^S X \cdot Y^L$, *In(1)EN, y*. For most of the translocations tested, males carrying this XY chromosome exhibited increased fertility when compared with tests of males carrying the $XY^L \cdot Y^S$ chromosome initially used, but again a wide range of values was observed. Thus, males bearing either XY chromosome in combination with a Y-autosome translocation seem to have reduced fertility, with the severity of the effect greater for males bearing $XY^L \cdot Y^S$.

The remaining class of mutations sterilized X- but not XY-bearing males and probably represents inactivation of one or more Y-linked fertility loci. These will be referred to as recessive sterile mutations. In addition to some of the Y-autosome translocations previously noted, this class includes Y chromosomes that segregate independently of the autosomes. To better understand the Y chromosome fertility loci, these nontranslocated sterile Y chromosomes were analyzed in greater detail. For each chromosome, the arm of the Y involved (Table 4) and the individual fertility regions defective (Figure 3) were determined.

The first point examined is the contribution of rearrangement breakpoints to the inactivation of the fertility loci among the recessive sterile mutations. In addition to the Y-autosome translocations, many of the radiation-induced nontranslocated mutations are probably also associated with rearrangements, *i.e.*, deficiencies and inversions. Table 4 shows which arm of the Y is involved for Y-autosome translocations in which some aneuploid survival was recorded, as well as which arm or arms of the Y are lacking function in mutant Y chromosomes that segregate independently of the autosomes. The ratio of metaphase lengths of the two arms is approximately 2:1 (COOPER 1959), as is the ratio of numbers of fertility loci in each arm (4:2) and the number of radiation-induced Y-autosome (44:20 from this work) or X-Y translocations (97:57 from KENNISON 1981) in each arm. One would expect the same ratio for point mutations, but for deficiencies or inversions requiring two breakpoints in the same arm, the Y^L:Y^S ratio should be the square of the ratio of metaphase lengths. The observed ratio (36:10) for the nontranslocated mutant Y chromosomes is much closer to the square of the values (*i.e.*, 4:1). That many of the mutations not associated with Y-autosome translocations are associated with intrachromosomal rearrangements is also supported by the analysis of loci defective in the tested chromosomes. If mutations in the individual fertility loci are independent, five double

TABLE 4

The location of the Y-chromosome breakpoint of Y-autosome translocations as detected by surviving aneuploid progeny and of the genetic lesion in male-sterile Y chromosomes that did not show autosomal linkage in genetic tests

Treatment	Aberration	Y arm involved		
		Y^L	Y^S	Both
γ-irradiation	Male-fertile *T(Y;A)*	11	4	
	Male-sterile *T(Y;A)*	33	16	
	Male-sterile Y [not *T(Y;A)*]	31	5	5
EMS	Male-fertile *T(Y;A)*	1	1	
	Male-sterile *T(Y;A)*	5	4	
	Male-sterile Y [not *T(Y;A)*]	19	6	1

Male fertility was tested for each Y chromosome in combination with $In(1)B^{M1}$.

mutants would be expected among the 44 mutants in the irradiated sample; 22 apparent multiple-site mutants were observed among the 41 mutants analyzed. In contrast, with the exception of one double mutant in *kl-2* and *ks-2*, the EMS-induced mutants all map to single fertility regions.

Complementation tests between recessive mutants were performed for two reasons. The first was to determine whether any of the six regions previously identified (KENNISON 1981) contained more than one fertility locus. The second was to see whether the observations of WILLIAMSON (1972) of extensive complementation among EMS-induced mutations in the Y chromosome fertility loci were repeatable. Mutants that were defective for only one fertility region in Y^L, all mutants in Y^S, a sample of Y-autosome and X–Y translocations, and several mutants induced and characterized by BROSSEAU (1960) were used for the complementation tests. These chromosomes are listed in Table 5. All pairwise combinations involving mutants in Y^S were tested. All chromosomes defective in *ks-1* failed to complement other *ks-1* mutations. Similar behavior was seen for *ks-2* mutations. As expected, however, *ks-1* mutations complemented *ks-2* mutations. For Y^L only mutations within a given region were tested in pairwise combinations for complementation within that region. No complementation between mutants in the same region was observed. The sample of EMS-induced mutants recovered here differs greatly from the sample recovered and characterized by WILLIAMSON (1970, 1972). Whereas only one of 25 mutants tested here was defective for more than one fertility region, fewer than one-half of WILLIAMSON'S mutants were defective at only one of BROSSEAU'S fertility loci. In addition, among WILLIAMSON'S mutants there was often complementation between mutants within the same fertility locus. No complementation within a region was observed in the sample tested here. The reason for the difference in these two samples is not known. Both samples were induced in the same marked Y chromosome (B^SYy^+) but may differ in effective dose of EMS.

To test whether any of the mutations are temperature sensitive, 16 radiation-induced, male-sterile Y-autosome translocations, 41 radiation-induced and 24 EMS-induced nontranslocated male-sterile Y chromosomes were retested for effects on male fertility in combination with a normal sequence X chromosome

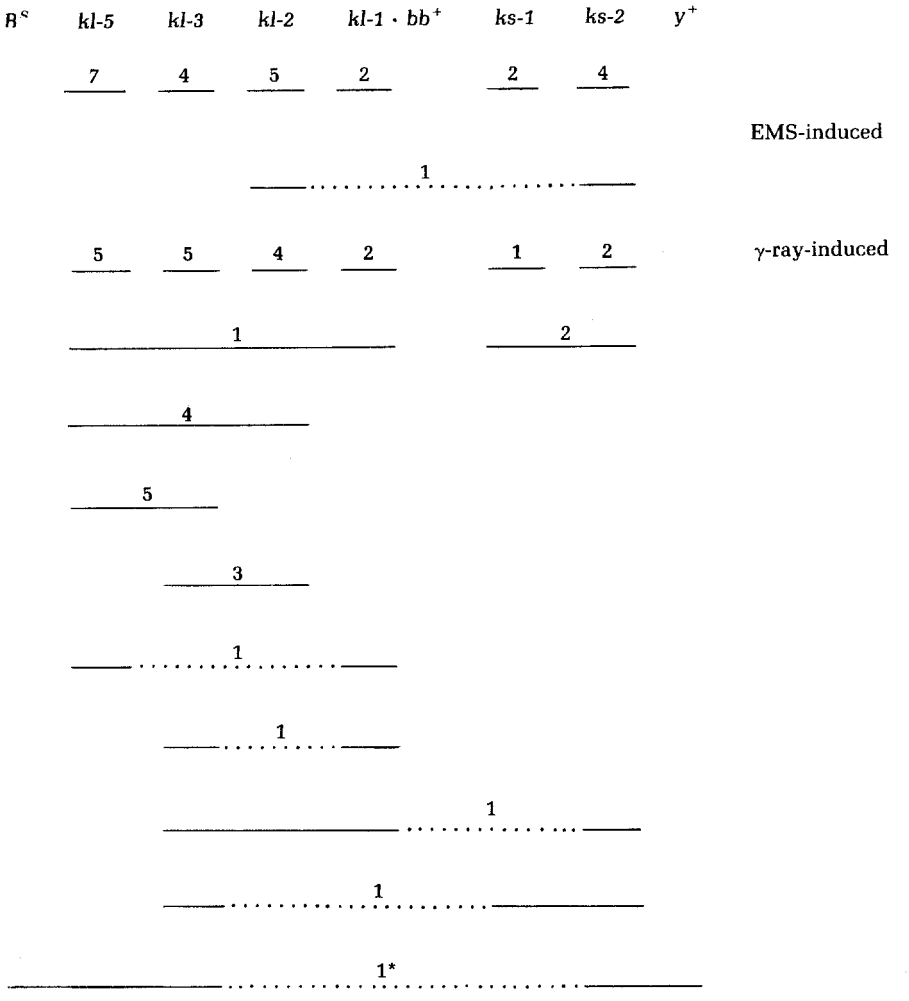

FIGURE 3.—Genetic analysis of fertility regions defective in male-sterile Y chromosomes that are not involved in Y-autosome translocations. The solid lines indicate the fertility regions defective; above each line is the number of mutant chromosomes of that type. A dotted line indicates the presence of functioning fertility regions between nonadjacent defective regions in the same mutant chromosome. The 25 mutant chromosomes in the upper half were EMS-induced, whereas the group of 39 mutants in the lower half were γ-ray induced. The chromosome indicated by an asterisk is a ring, recovered in a mosaic female, that has lost the terminal B^S and y^+ markers.

(*y w f*) at both 25° and 18°. Of these, one of 16 of the Y-autosome translocations, two of 41 of the radiation-induced mutants and four of 24 of the EMS-induced mutants did not completely sterilize males (an average of one progeny per 19 males tested for fertility) at 25° but completely sterilized males at 18°. Many of the mutant chromosomes that lack Y^L function did not completely sterilize males that carried $X \cdot Y^S$ (these averaged one progeny per six males tested for fertility). This included two of 15 Y-autosome translocations tested, ten of 36 radiation-induced mutants, and 11 of 19 EMS-induced mutants. Similar results

TABLE 5

Y chromosome mutants and aberrations tested for complementation

Mutant	Chromosome	Source	Reference[a]
ks-1$^-$			
T(X;Y)Z13	B^SYy^+	γ-ray	1
T(Y;3)J162	B^SYy^+	X-ray	2, 3
ms(Y)E1	B^SYy^+	EMS	
ms(Y)G1	B^SYy^+	γ-ray	
ks-2$^-$			
ms(Y)E2	B^SYy^+	EMS	
ms(Y)G2	B^SYy^+	γ-ray	
ms(Y)G3	B^SYy^+	γ-ray	
ks-1$^-$, *2*$^-$			
ms(Y)G4	B^SYy^+	γ-ray	
ms(Y)G5	B^SYy^+	γ-ray	
ms(Y)S5	y^+Y	X-ray	4
kl-1$^-$			
T(X;Y)Z6	B^SYy^+	γ-ray	1
T(Y;2)B242	B^SYy^+	X-ray	2, 3
ms(Y)L13	y^+Y	X-ray	4
ms(Y)E3	B^SYy^+	EMS	
ms(Y)E4	B^SYy^+	EMS	
ms(Y)G6	B^SYy^+	γ-ray	
kl-2$^-$			
T(X;Y)R19	B^SYy^+	γ-ray	1
T(Y;2)R155	B^SYy^+	X-ray	2, 3
ms(Y)L37	y^+Y	X-ray	4
ms(Y)E5	B^SYy^+	EMS	
ms(Y)E6	B^SYy^+	EMS	
ms(Y)E7	B^SYy^+	EMS	
ms(Y)E8	B^SYy^+	EMS	
ms(Y)G7	B^SYy^+	γ-ray	
ms(Y)G8	B^SYy^+	γ-ray	
kl-3$^-$			
T(X;Y)T13	B^SYy^+	γ-ray	1
T(Y;2)P57	B^SYy^+	X-ray	2, 3
ms(Y)L11	y^+Y	X-ray	4
ms(Y)E9	B^SYy^+	EMS	
ms(Y)E10	B^SYy^+	EMS	
ms(Y)E11	B^SYy^+	EMS	
ms(Y)G9	B^SYy^+	γ-ray	
ms(Y)G10	B^SYy^+	γ-ray	
ms(Y)G11	B^SYy^+	γ-ray	
kl-5$^-$			
T(Y;2)G53	B^SYy^+	X-ray	2, 3
ms(Y)kl-5$^-$*Br*	y^+Y	X-ray	4
ms(Y)E12	B^SYy^+	EMS	
ms(Y)E13	B^SYy^+	EMS	
ms(Y)E14	B^SYy^+	EMS	
ms(Y)E15	B^SYy^+	EMS	
ms(Y)E16	B^SYy^+	EMS	
ms(Y)E17	B^SYy^+	EMS	
ms(Y)G12	B^SYy^+	γ-ray	
ms(Y)G13	B^SYy^+	γ-ray	
ms(Y)G14	B^SYy^+	γ-ray	
ms(Y)G15	B^SYy^+	γ-ray	

[a] Reference refers to: 1, KENNISON (1981); 2, LINDSLEY *et al.* (1972); 3, M. GATTI and S. PIMPINELLI, personal communication; 4, BROSSEAU (1960).

TABLE 6

Proportion of Y chromosomes defective in kl-3, kl-5 or both that did not completely sterilize males with extensive testing

		No. not completely sterile/no. tested		
		$kl\text{-}3^-$	$kl\text{-}3^-,5^-$	$kl\text{-}5^-$
$ms(y^+Y)$	X-ray induced[a]	1/4	2/7	3/3
$T(X;Y)$	γ-ray induced[b]	4/6	0/3	8/11
$ms(B^SYy^+)$	γ-ray induced	4/5	2/6	5/5
$ms(B^SYy^+)$	EMS induced	4/4	0/0	7/7

[a] Data from BROSSEAU (1960).
[b] Data from KENNISON (1981).

have been reported for X-Y translocations (KENNISON 1981) and for nontranslocated mutant Y chromosomes (BROSSEAU 1960; FRANKEL 1973). These have been interpreted as some type of variegated position effect on the Y chromosome fertility loci, as traditional variegated position effects are enhanced at lower temperature and suppressed by the addition of extra heterochromatin (see SPOFFORD 1976 for a review). This putative variegated position effect only occurred in chromosomes or translocations defective for *kl-3*, *kl-5*, or both. Table 6 gives the proportion of mutant chromosomes and X-Y translocations defective for *kl-3*, *kl-5*, or both that have been observed to yield occasional progeny. Males carrying synthetic deficiencies for *kl-3*, or *kl-5* generated from X-Y translocations gave no progeny in fertility tests, although more than 1800 males were tested (KENNISON 1981).

DISCUSSION

The high sensitivity of both the X and Y chromosomes to newly induced F_1 male sterility after irradiation appears to be due largely to breakage and rearrangement. Of a sample of γ-ray-induced, X-linked male sterile mutations analyzed by D. L. LINDSLEY and C. C. MUSATTI (unpublished results), 100 of 124 tested (81%) were associated with X-autosome translocations. This sterility is dominant, male specific, and probably due to an exchange of chromosome material and not to breakpoints near specific loci (see LIFSCHYTZ and LINDSLEY 1972). Furthermore, WARTERS (from LINDSLEY 1965) showed that approximately 62% (85 of 137) of translocations between the X and either chromosome 2 or 3, as detected by pseudolinkage in progenies of daughters of males treated with 4000 r of X-rays, are male-sterile. In the present experiment 88 male-sterile Y-autosome translocations represent 67% both of the 132 male-sterile Y chromosomes and of the 133 *T(Y; A)*s observed. The majority of these male-sterile Y-autosome translocations also behaves as dominant mutations, *i.e.*, they sterilize males even in the presence of extra Y chromosome material.

Although the Y chromosome is only slightly larger than the X chromosome at mitotic metaphase (after BRIDGES, from DOBZHANSKY 1929), it appears to be recovered in translocations at nearly two times the frequency. VALENCIA (1970), who recovered and selected in females translocations from irradiated X- and Y-

bearing sperm, observed 3% (six of 181) X-autosome translocations and 12% (16 of 129) Y-autosome translocations. Although the incidence of γ-ray-induced X-autosome translocations was not measured directly in the present work, the relations discussed in the previous paragraph provide the means for estimating this frequency. Accordingly, the 20 X-linked male-sterile mutants observed among 203 treated X chromosomes imply an incidence of 13% *T(X; A)*s. From the same irradiated fathers the incidence of *T(Y; A)*s was 24% (133 of 556). The samples that indicate Y-autosome translocations occur with the frequency expected from metaphase lengths (KIRSANOV 1946; LINDSLEY *et al.* 1972) were recovered in males and, therefore, underestimated the true incidence of such translocations owing to the male sterility of many Y-autosome translocations even in the presence of all of the Y chromosome necessary for male fertility. Had the irradiated Y chromosomes in this study been recovered in males carrying an XY chromosome, the measured incidence of Y-autosome translocations would only have been 16% (82 of 506), not strikingly different from the estimated frequency of 13% *T(X; A)*s. The difference in frequencies of X- and Y-autosome translocations recovered is probably due to a difference in the incidence of their formation and not to a difference in viability of the zygotes in which they are recovered. X- and Y-bearing sperm have approximately the same sensitivity to irradiation as measured by viability of progeny of irradiated fathers (LINDSLEY, EDINGTON and VON HALLE 1963).

Although the Y chromosome utilized in this study ($B^S Yy^+$) has a complex origin (Brosseau 1958), after Hoechst 33258 or quinacrine fluorescence staining it differs from an Oregon R unmarked Y chromosome only at the distal ends of the arms, where the B^S and y^+ markers have been appended (M. GATTI and S. PIMPINELLI, personal communication). This appended chromatin may have some effect on the mutability of tbe fertility loci. Whereas the $B^S Yy^+$ chromosome has X-chromosome material appended to both Y^S and Y^L, the y^+Y and $B^S Y$ chromosomes carry extra material appended only to Y^L. In the y^+Y and $B^S Y$ chromosomes, therefore, Y^S should behave the same as in an unmarked Y chromosome. Excluding translocations, 20 male-sterile mutations in Y^S have been characterized in a $B^S Yy^+$ chromosome (this study and HAZELRIGG, FORNILI and KAUFMAN 1982) and 18 in a y^+Y chromosome (BROSSEAU 1960; M. GATTI and S. PIMPINELLI, personal communication). Five of the mutations in the $B^S Yy^+$ chromosome and five in the y^+Y chromosome lacked both *ks-1* and *ks-2*. Of the single-site mutations in Y^S, there were five in *ks-1* and ten in *ks-2* in the $B^S Yy^+$ chromosome. In the y^+Y chromosome the sensitivity of these two loci is reversed, with 12 mutations recovered in *ks*-1 and only one mutation recovered in *ks-2*. The addition of the extra chromatin in the $B^S Yy^+$ chromosome seems to increase the mutability of *ks-2*, possibly by increasing the probability of breakage distal to *ks-2* allowing an increased incidence of deletions of *ks-2*. This is supported by the frequency of breakpoints distal to *ks-2* among translocations between the X heterochromatin and Y^S. NICOLETTI and LINDSLEY (1960) recovered translocations involving either the y^+Y or the $B^S Y$ chromosome. Of 16 translocations recovered between the X heterochromatin and Y^S that were fertile in the presence of an extra Y chromosome, 15 were sterile in its absence,

due to either inactivation of a fertility factor by the translocation breakpoint or loss of the unmarked Y^DX^P element. Thus, only one of the 16 recovered translocations could have had a breakpoint distal to *ks-2*. Of 22 translocations between the X heterochromatin and Y^S of the B^SYy^+ chromosome, six had breakpoints distal to *ks-2* (KENNISON 1981).

It is likely that the sterility of Y-autosome translocations, like that of X-autosome translocations, is caused by the interchange of genetic material between a sex chromosome and an autosome and is not a result of the breakpoint alone. Autosomal breakpoints alone do not sterilize males when heterozygous (the autosomal breakpoints of X- and Y-autosome translocations are heterozygous in translocation-bearing males). J. A. KENNISON and L. S. B. GOLDSTEIN (unpublished results) recovered *T(2;3)*s and autosomal dominant male-sterile mutations in daughters of males treated with 4000 r of γ-irradiation. All 40 *T(2;3)*s recovered were male fertile when heterozygous, and the only dominant male-sterile mutation recovered among 640 tested genomes was associated with a cytologically normal second chromosome. Y chromosome breakpoints also do not sterilize males in a dominant manner. Samples of radiation-induced recessive male-sterile Y chromosomes that are not associated with Y-autosome translocations have been shown cytologically to include a large proportion (between 50 and 100%) of intrachromosomal rearrangements, *i.e.*, deficiencies and inversions (HAZELRIGG, FORNILI and KAUFMAN 1982; M. GATTI and S. PIMPINELLI, personal communication). Although the present sample has not been examined cytologically for deficiencies and inversions of Y-chromosome material, all of the male-sterile Y chromosomes that were not associated with Y-autosome translocations behaved as recessive lesions. In addition, Y chromosome breakpoints of X–Y translocations seldom, if ever, sterilize males in a dominant manner. Of 140 male viable reciprocal X–Y translocations recovered in females (NICOLETTI and LINDSLEY 1960; B. NICOLETTI, unpublished results) only 35 were sterile as *T(X; Y)/Y* males. Of these 35 all but ten were shown to involve an autosome in addition to the X and Y. Some, or all, of these ten could be sterile due to recessive effects involving the X chromosome breakpoints (3% expected) or to independent X-linked male-sterile mutations (2% expected).

The effects of various types of chromosome aberrations on spermatogenesis indicate that the organization of the genome in *D. melanogaster* is very important for the normal regulation of spermatogenesis. LINDSLEY and LIFSCHYTZ (1972) discuss the evidence, based on rearrangements, that the X chromosome and the autosomes are differentially regulated during spermatogenesis. The present work suggests that the Y chromosome is also differentially regulated in the primary spermatocyte, in a manner similar to that of the X chromosome. Thus, translocations between the X and Y or between the autosomes do not interrupt this chromosomal control and are compatible with male fertility, whereas interchanges of genetic material between the X or Y and the autosomes do interfere with chromosomal control and disrupt normal spermatogenesis.

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