SEX-LINKED RECESSIVE LETHALS IN DROSOPHILA WHOSE EXPRESSION IS SUPPRESSED BY THE Y CHROMOSOME

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THE expression of mutant phenotype is subject to genetically controlled suppression; in *Drosophila melanogaster* the presence of a Y chromosome may in certain instances eliminate or decrease the phenotypic consequences of an impairment of gene function. Two general modes of Y suppression may be postulated: the Y may perform the function of the altered gene or it may stimulate the genome to perform the same function or an alternative function whose end result is a return toward phenotypic normality. The first method is the way that any wild-type gene suppresses the expression of its recessive allele. An example of this type of action by the Y chromosome is seen in the case of the sex-linked recessive gene bobbed (*bb*). Normal alleles of *bb* are located in the chromocentral heterochromatin of the X and in the short arm of the Y chromosome; this sharing of loci is part of the evidence that these regions are homologous. Instances of lethal changes that are in the chromocentral heterochromatin and are therefore covered by the Y are known, but they are few and involve the *bb* locus (*Df(1)bb*, *Df(1)bb^l*, and *In(1)sc^{4L,8R}*). An example of the second type of Y action is seen in the suppression of variegated (V-type) position effects (GOWEN and GAY 1934), which, though a commonly observed phenomenon, is still not understood (for a discussion, see COOPER 1956). SCHULTZ 1941 has demonstrated the existence of lethal V-type position effects that can be made to survive by adding a Y to the genome. He showed that $T(1;4)w^{258-18}$, which survives as a homozygous female or as an X/Y male and is variegated for white, is inviable as an X/0 male and that X^c survives as a homozygous female or an X/Y male but not as an X/0 male. Similarly, he showed that N^{264-6} , N^{264-9} , and N^{264-10} , which are lethal as X/Y males, survive as X/Y/Y males (see BRIDGES and BREHME 1944).

It was our purpose in the experiments reported here to determine to what extent induced lethal changes are suppressed by a single Y chromosome and to determine which of the mentioned modes of suppression was operating in any recovered cases. Since the ordinary methods for detecting sex-linked lethals rely on expression of lethality in X/Y males, they overlook the class that we seek to demonstrate. The second aim was to accumulate a series of different chromocentral lethals to provide new material for further linear resolution of the proximal heterochromatin of the X chromosome. Finally, we hoped to gain

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some insight into the contribution of V-type position effect to X-ray-induced sex-linked recessive lethals.

In the first endeavor we were quite successful in finding that, at 3 and 4 kr, approximately one fifth (49/239) of all sex-linked recessive lethals are suppressed by a Y chromosome. In practically every case, these lethals were found to be associated with chromosome aberrations. It follows that the Muller-5 procedure overlooks an appreciable sample of lethals, preferentially aberration-associated lethals. Of the Y-suppressed lethals, however, only about one eighth (6/49) seem to be in the region that is homologous to the Y chromosome, and since all these are deficient for the same gene (i.e., the *bb* locus), it appears that we have not accumulated a very versatile array of proximal heterochromatic lethals. The remainder of the Y-suppressed lethals, i.e., 18 percent (43/239) of all sex-linked lethals, can be shown to be V-type position effects. To these can be added roughly another three percent that die as X/Y but not as X/Y/Y males to yield a minimum estimate of about 21 percent as the contribution of position-effect lethals to sex-linked lethals produced by 3 and 4 kr.

Methods for recovering Y-suppressed lethals

The initial attempts to recover Y-suppressed sex-linked recessive lethals consisted in simply crossing the F_1 females of an ordinary Muller-5 test ($= X^*/M-5$ where X^* designates the irradiated X, and M-5 designates *Ins(1) $sc^{SIL,SR}$, S, $sc^{S1+S}w^a B$*) to XY/0 males (where XY represents an attached XY chromosome, specifically $Y^S X \cdot Y^L$, *Ins(1)EN*, dl-49, $\gamma w v f$, following the terminology of LINDSLEY and NOVITSKI 1959) rather than M-5/Y males. In this way the irradiated X appeared in X/0 rather than X/Y sons; X^*/XY daughters from all lethal-bearing cultures were then testcrossed to M-5/Y males, and any producing X^*/Y sons were suspected of being X/0 lethal, but X/Y viable and were consequently retested. This method had the disadvantage of requiring subsequent crossing of every suspected lethal to check for X/Y viability. A crossing scheme was desired that would allow recovery of both X^*/Y and $X^*/0$ sons in the F_2 in such a way that their survival could be scored separately. E. NOVITSKI (personal communication) suggested that this might be accomplished by recovering the irradiated X in an F_1 female that was $X^*/M-5/Y$ in constitution and crossing to XY/0 males. Such females should produce six types of ova: M-5/ X^* ; Y; M-5; M-5/Y; X^* ; and X^*/Y . The latter two types should occur with equal frequencies and, when fertilized by a nullo X, nullo Y sperm should produce $X^*/0 = \gamma$ and $X^*/Y = \gamma^+$ males, respectively, if X^* is marked with γ and the Y is marked with γ^+ , i.e., $\gamma^+ Y (= sc^S \cdot Y$ of MULLER 1948). Scoring the F_2 culture for survival of both, one, or neither type of males should allow classification of the irradiated X's into nonlethal, Y-suppressed lethal, or orthodox lethal, respectively. In actual practice, however, the incidence of secondary nondisjunction in the $X^*/M-5/Y$ females was so high, owing to the extreme sequential heterozygosity of the two X chromosomes, that a sample of X^* -bearing sons sufficiently large to allow unambiguous classification was seldom produced; consequently, many cultures had to be retested. However, more than half the Y-suppressed

lethals described in this paper were recovered by this method. Owing to the ambiguity of the initial classification of cultures, this method was abandoned for one in which M-5/X^c females could be used. The S-5 test was used to recover the remaining lethals in this study (see also LINDSLEY and EDINGTON 1957). The chromosomes used in the S-5 method and their essential features are as follows:

(1.) *Ins(1)sc^{8L,81R}dl-49, γ^{31d} sc⁻ v f B*: This chromosome is doubly inverted and marked with the recessive mutants γ^{31d} , v , and f and the dominant marker B ; in addition, it is deficient for the locus of scute. Deficiency for sc is very inviable in the male (STURTEVANT and BEADLE 1936) and is not covered by γ^+Y . This chromosome is very similar to Binscy (MULLER 1952) except that it is sc deficient and is consequently designated Biny.

(2.) $\gamma l(1)J1^{259} w m f: l(1)259$ was recovered as a lethal suppressed by γ^+Y but not by a normal Y ; it has been shown by FRYE (1959) to be allelic to $l(1)J1$.

(3.) *Ins(1)sc^{4L,8R}, S, γ sc⁴⁺ B w^a*: This chromosome is of essentially the same sequential and marker composition as M-5, but it differs from M-5 in that the left end of *In(1)sc⁸* has been replaced by that of *In(1)sc⁴* (i.e., it is γsc^4 M-5 designated S-5). This exchange of tips has resulted in the deletion of the majority of the proximal heterochromatin of the X (most of hA, all of hB, NO, hC, and the proximal one third of hD; COOPER 1959) including bb^+ , and the replacement of γ^+ with γ . Owing to its heterochromatic deficiency, this chromosome fails to pair with the Y chromosome regularly during spermatogenesis, and consequently primary nondisjunction is frequent. From a cross of S-5/ γ^+Y males to γ females the number of progeny resulting from fertilization by X-, Y-, 0-, and X/Y-bearing sperm were 2509, 1670, 631, and 98, respectively. Thus, the four sperm classes are produced in approximately the following proportions: X:Y:0:X/Y = 51:34:13:2. SANDLER and BRAVER (1954) attributed the inequality in recovery of reciprocal types to meiotic loss of unpaired chromosomes.

The crossing scheme of the S-5 test is presented in Figure 1. Yellow vermilion males are irradiated and crossed to Biny/ $\gamma l(1)J1^{259} w m f$ females (these females are obtained from a balanced stock containing Biny/ $\gamma l(1)J1^{259} w m f$ females and $\gamma l(1)J1^{259} w m f/\gamma^+Y$ males). The sons of this cross are inviable owing to the failure of a normal Y to cover sc^- or $l(1)J1^{259}$; consequently, only the γv /Biny and $\gamma v/\gamma l(1)J1^{259} w m f$ daughters survive, and they will be virgin if the parents have been removed. F₁ γv /Biny females are selected and crossed individually to S-5/ γ^+Y males. The progeny of this cross are indicated in the square in Figure 1. The only regular sons expected from this cross carry the original irradiated X chromosome; roughly 75 percent (34/47) of them carry the γ^+Y and are consequently $\gamma^+ v$, whereas 25 percent carry no Y and are γv . Cultures are scored for the presence of v (X/Y) and γv (X/0) males. The presence of both classes (Table 1, line 1) indicates a nonlethal culture; the absence of both classes of males (Table 1, line 2) indicates an orthodox lethal; the presence of v males but not γv males (Table 1, line 3) indicates a change that is viable as an X/Y male but inviable as an X/0 male. The reciprocal of this last class is possible, i.e., where X/0 survives and X/Y is lethal (Table 1, line 4). A few tentative cases have been encountered, but this class of lethal will not be

$$y\ l(1)Jl^{259} w\ m\ f/Biny\ ♀ \quad \times \quad y\ v/Y\ ♂$$

$$\downarrow$$

$$y\ v/Biny\ ♀ \quad \times \quad S-5/y^+Y\ ♂$$

$$\downarrow$$

		PATERNAL GAMETES				
		REGULAR		EXCEPTIONAL		
		S-5 = 0.51	y ⁺ Y = 0.34	0 = 0.13	S-5/y ⁺ Y = 0.02	
MATERNAL GAMETES	REGULAR	y v	y v/S-5 y B/+ ♀	y v/y ⁺ Y v ♂	y v/0 y v ♂	y v/S-5/y ⁺ Y B/+ ♀
		Biny	Biny/S-5 y ^{31d} _{sc} B/B ♀	Biny/y ⁺ Y dies	Biny/0 dies	Biny/S-5/y ⁺ Y sc B/B ♀
	EXCEPTIONAL	0	S-5/0 dies	y ⁺ Y/0 dies	0/0 dies	S-5/y ⁺ Y sc w ^a B ♂
		y v/Biny	y v/Biny/S-5 dies	y v/Biny/y ⁺ Y v B/B ♀	y v/Biny y ^{31d} _v B/B ♀	y v/Biny/S-5/y ⁺ Y dies

FIGURE 1.—Crossing scheme of the S-5 method for detecting sex-linked recessive lethals whose expression is affected by the Y chromosome and the genotypes and phenotypes expected in the F_2 culture. $S-5 = Ins(1)sc^{4L}, s^{1R}, Y, sc^4 w^a B$, and $Biny = Ins(1)sc^{4L}, s^{1R}, dl-49, y^{31d} sc^- v f B$. Orthodox lethal cultures lack both v and $y\ v$ males; lethals that are suppressed by the Y produce v but not $y\ v$ males; and nonlethal cultures produce both types of males. Exceptional ova are produced only by certain induced rearrangements.

considered further in this communication. Several additional features of this method should be pointed out: (1) Since $Biny$ males fail to survive, nonvirginity is not a problem encountered in retesting lethal cultures. The only putative lethal-bearing cultures in which $y\ v/S-5$ females might be nonvirgin are where X/Y males survive and $X/0$ males die. In these cases, the original diagnosis may be confirmed by outcrossing the $y^+ v$ sons to $XX/0$ virgins (where XX designates any compound X chromosome, see NOVITSKI 1954) and the stock may be maintained by crossing the $y\ v/S-5$ and $y\ v/y^+ Y$ progeny. (2) Since the S-5 chromosome is deficient for the majority of the proximal heterochromatin, any lethal in the proximal heterochromatin should fail to survive in combination with S-5

TABLE 1

Survival of the diagnostic classes of progeny characteristic of the various types of lethals that can be distinguished in the F_2 of the S-5 test, and the ability of the $X^/S-5/Y$ females from the S-5 F_2 to produce $X^*/Y/Y$ sons*

Lethal type	Diagnostic classes among F_2 of S-5 test			Son of F_2 $X^*/S-5/Y\varphi$ from S-5 test
	$\gamma v \sigma = X^*/0$	$v \sigma = X^*/Y$	$\gamma B \varphi = X^*/S-5$	$X^*/Y/Y$
Nonlethal	+	+	+	+
Orthodox; not in proximal heterochromatin	—	—	+	—
Suppressed by one Y; not in proximal heterochromatin	—	+	+	+
Y-enhanced	+	—	+	+
Suppressed by one Y; in proximal heterochromatin	—	+	—	+
Orthodox; in proximal heterochromatin	—	—	—	+
Suppressed by two, but not by one, Y	—	—	+	+

(Table 1, line 5). Thus cultures carrying lethals covered by the γ^+Y can be checked for the survival of γB females, and it can thus be determined whether they carry a proximal heterochromatic lethal. Furthermore, it is possible that deficiencies are produced that include bb as well as regions not homologous with the Y; these would behave as orthodox lethals but would fail to survive in combination with S-5 (Table 1, line 6). (3) It is known that lethals may be produced that survive in the presence of two but not of one Y chromosome in the male. The S-5/ $\gamma v/\gamma^+Y$ females in orthodox lethal cultures can be crossed to normal males, and survival of their $\gamma v/\gamma^+Y/Y$ males scored (Table 1, line 7). This test was not systematically carried out in the experiments reported in this paper, but one such lethal was incidentally recovered. (4) Some lethals are associated with rearrangements characterized by high nondisjunction in the γv /Biny females. The exceptional females are γv /Biny/ γ^+Y or γv /Biny, and since Biny carries v , they are $v B$ or $\gamma^{sid} v B$, respectively, and can thereby be recognized. Irradiation of γ rather than γv males in the experiments reported here made the detection of nondisjunction considerably more difficult.

In the initial design we intended to balance the Y-suppressed lethals by crossing $\gamma v l/\gamma^+Y$ males to XX/γ^+Y females. It was discovered however that a large fraction of the Y-suppressed lethal X's were male sterile, and they had to be carried balanced to S-5 (or M-5 in the initial experiments). On the basis of absence or presence of the γB females, the Y-suppressed lethals may be divided into those affecting chromocentral heterochromatic loci and those not; they may also be classified on the basis of fertility of the γ^+ males. So far, all proximal heterochromatic lethals (henceforth referred to as bb^- lethals) have proved to be male fertile; consequently, the Y-suppressed lethals fall initially into three classes (bb^- , bb^+ male fertile, and bb^+ male sterile), and these classes will be treated separately in the following pages.

Recovery and analysis of Y-suppressed lethals

In Table 2 the lethals recovered from a series of exposures of γ males are broken down into the various classifications outlined in the preceding section; several points of interest emerge from this table. The first is that approximately 20 percent of the lethals recovered are suppressed by the Y chromosome, at least in the 3- and 4-kr experiments. There is then an appreciable fraction of sex-linked recessive lethals that are not recognized by the Muller-5 method. A second point of interest is the small proportion of the Y-suppressed lethals that are in the proximal heterochromatin of the X chromosome (6/52), and finally, although many Y-suppressed lethals are found, the majority of them are male sterile (35/52).

Tests for bb^+ : Those lethals that fail to survive in combination with S-5 are considered to be located between the right break points of $In(1)sc^4$ and $In(1)sc^{31}$. Although the only locus in this region that is known to be subject to lethal mutation is bb , we considered it likely that there are other such loci, including the nucleolus organizer, which BEERMAN (1960) has shown to be necessary for development in *Chironomus*. Consequently, the phenotype of each lethal that was in the proximal heterochromatin, according to the criterion that it was inviable in combination with S-5, was tested in combination with bb . For these tests two different alleles of bb were used; both were carried in homozygous stocks, but in one case $bb/S-5$ females were bb in phenotype, and in the other case they were lethal. This has been interpreted to indicate that the second allele, which fails to survive in combination with a known deficiency for the locus and which will be designated bb' , can differentiate between mutation and deletion of the bb locus whereas the first cannot. None of the six proximal heterochromatic lethals were bb^+ ; furthermore, each succumbed in combination with bb' , but the four tested in combination with bb were bobbed. Thus, the chromosomes tested provide no evidence of loci, other than bb , in the proximal heterochromatin that are capable of lethal mutation. Furthermore, all the bb lethals behave as deficiencies with respect to their survival in combination with bb' . Additional evidence that these lethals are associated with heterochromatic deficiencies is that, during

TABLE 2

Recovery and classification of Y-suppressed sex-linked recessive lethals

Dose (kr)	Chromosomes tested	Nonlethal	Orthodox	Lethal		
				Y-suppressed		
				bb^-	bb^+	
					Male fertile	Male sterile
1	323	318	5	0	0	0
2	416	387	26	0	1	2
3	255	235	18	0	0	2
3*	1,624	1,458	129	3	9	24
4	408	354	43	3	1	7

* Lethals recovered from $\gamma^*/\gamma^- ac^- M-5/\gamma^+ Y \times XY/0$ rather than from $\gamma^*/M-5 \times S-5/\gamma^+ Y$.

spermatogenesis, they exhibit varying degrees of primary nondisjunction from the Y chromosome.

Measurement of relative viability: In order to give some indication of the viability of the lethal-bearing chromosomes as X/Y and X/0 males, each was made heterozygous to a balancer chromosome (γ^2 M-5 in the case of the bb^- lethals and S-5 in the remainder), and the resulting females were crossed to XY/Y and to XY/0 males; the XY used was $Y^sX \cdot Y^L$, $In(1)EN, KS B f v \gamma \cdot KL \gamma^+$ where KS and KL refer to the fertility complexes of Y^s and Y^L , respectively (terminology of LINDSLEY and NOVITSKI 1959; BROUSSEAU 1960). The results for the bb^- lethals are presented in Table 3. The data in this table require several comments that are also applicable to the two subsequent tables. Ideally, there should be a regular class of progeny that is constant from one cross to the next with which the recovery of lethal bearing males could be compared. In Table 3 the logical reference genotype is S-5/XY but, owing to an ambiguity in the classification of $B/+$ and B/B in some of the crosses, the frequency of this class could not be counted reliably. Consequently, both classes of regular daughters were combined and used as the reference genotype. In a cross where the maternal normal sequence X does not carry a lethal, the number of sons carrying this X should be about half the number of regular daughters, and the viability ratio (γ males/regular females) should approximate 0.5. Any recessive inviability of the normal X would reduce the numerator of the viability ratio and, thus, reduce the ratio below 0.5. Dominant inviability, on the other hand, would reduce the denominator as well as the numerator and consequently cause relatively less decrease in the viability ratio than recessive mortality. This uncertainty would not have been encountered had the Bar classification been unambiguous. The difference in recovery of $\gamma w^a B$ males in the XY/Y and XY/0 crosses is attributable to the well-known reduced viability of $In(1)sc^s/0$ males. It is evident from the data presented in Table 2 that the inviability of the bb^- lethals as X/0 males is absolute, whereas X/Y males seem to show normal viability.

TABLE 3

Viability as X/Y and X/0 males of bb^- lethals from $\gamma^1/M-5$ females $\times$ XY/0 and XY/Y males

Parental constitution		Progeny				Exceptional		Viability ratio $\left[\frac{\gamma \sigma \sigma}{B/+ + B/B} \right]$
		Regular				$\gamma f B \sigma$	$\gamma B/+ \sigma$	
Maternal	Paternal	$\gamma \sigma$	$\gamma w^a B \sigma$	$B/+ \sigma$	$B/B \sigma$			
S-5/l(1)3a	XY/0	0	91	273	253	0	0	0
	XY/Y	165	133	155	189	1	1	0.48
S-5/l(1)74	XY/0	0	40	543	550	0	0	0
	XY/Y	536	436	474	509	0	0	0.55
S-5/l(1)158	XY/0	0	57	491	495	0	1	0
	XY/Y	387	296	362	391	0	0	0.52
S-5/l(1)452	XY/0	0	45	555	560	0	0	0
	XY/Y	358	299	344	323	0	0	0.54
S-5/l(1)481	XY/0	0	32	390	402	1	0	0
	XY/Y	305	237	338	359	2	1	0.44

TABLE 4

Viability as X/Y and X/0 males of bb⁺ male-fertile, Y-suppressed lethals from γ l/S-5 females $\times$ XY/0 and XY/Y males

Parental constitution		Progeny				Viability ratio		
		Regular				Exceptional		$\gamma\sigma$ [$B/+♀ + B/B♀$]
Maternal	Paternal	$\gamma\sigma$	$\gamma w^a B\sigma$	$B/+♀$	$B/B♀$	$\nu f B\sigma$	$\gamma B/+♀$	
S-5/+	{XY/0	741	0	757	715	6	0	0.50
	}XY/Y	550	506	598	588	4	0	0.46
S-5/l(1)11	{XY/0	9	0	148	309	22*	0	0.02
	}XY/Y	189	191	180	349	26*	0	0.36
S-5/l(1)47	{XY/0	0	0	166	292	1	2	0
	}XY/Y	101	279	194	339	1	3	0.19
S-5/l(1)59	{XY/0	0	0	299	299	0	1	0
	}XY/Y	167	230	279	295	1	1	0.29
S-5/l(1)132	{XY/0	2	0	191	220	2	1	<0.01
	}XY/Y	137	211	170	188	0	2	0.38
S-5/l(1)146	{XY/0	0	0	191	218	1	0	0
	}XY/Y	66	153	141	214	0	0	0.19
S-5/l(1)227	{XY/0	0	0	375	332	3	0	0
	}XY/Y	127	245	293	292	0	0	0.22
S-5/l(1)231	{XY/0	1	0	243	246	1	1	<0.01
	}XY/Y	99	97	122	103	0	0	0.44
S-5/l(1)306	{XY/0	0	0	217	193	2	1	0
	}XY/Y	158	174	195	239	0	0	0.36
S-5/l(1)459	{XY/0	0	0	69	78	3	3	0
	}XY/Y	2	39	56	86	1	0	0.01

* These males were *f*⁺, and showed an obviously aneuploid phenotype including nicked wings.

Similar data for the *bb*⁺ lethals are presented in Tables 4 and 5; however, in these cases S-5 was used in place of M-5. Consequently, the $\gamma w^a B$ class is completely absent in the crosses to XY/0 males owing to the inviability of S-5/0. Several points about these data are noteworthy. The *bb*⁺ male-fertile lethals tested uniformly exhibit complete or nearly complete inviability as X/0 males, but as X/Y males their viability ratio ranges from 0.19 in the case of 47 and 146 to 0.44 in the case of 231. The value of 0.01 for 459 is probably in error for some reason as a value of 0.36 was obtained from a $\gamma l(1)459/\gamma^2$ M-5 $\times$ XY/Y cross. These results indicate that many of the X/0 lethals have reduced viability as X/Y males. The *bb*⁺ male-sterile lethals also show a range of X/Y male viability, with viability ratios ranging from 0.07 for 150 and 216 to 0.51 for 219. This sample of lethals also shows a range of X/0 viability, with ratios ranging from 0 in many cases to as high as 0.13 in the case of 129. This apparent difference in X/0 viability between the male-fertile and the male-sterile lethals is not real. It is simply an apparent result of using male sterility as an additional criterion on which to base selection; it resulted in saving lethals that produced some X/0 males that would have been discarded in instances where the X/Y males were fertile. It seems quite clear that we are dealing with a continuous two-dimensional distribution of the viabilities of X/0 and X/Y males and that our sampling pro-

TABLE 5
Viability as X/Y and X/0 males of bb⁺ male-sterile, Y-suppressed lethals from y l/S-5 females × XY/0 and XY/Y males

Parental constitution		Number of progeny				Viability ratio		Proportion primary nondisjunction	
Maternal	Paternal	Regular		Exceptional		$y\delta$	$\frac{y\delta}{B/+q + B/Bq}$	$\frac{y\delta}{v/B\delta + yB/+q}$	$\left[\frac{v/B\delta + yB/+q}{v/B\delta + yB/+q + B/+q + B/Bq} \right]$
		$yw^a B\delta$	$B/+q$	v/Bq	$yB/+q$				
S-5/l(1)3	{XY/0 XY/Y	0 67	85 78	87 117	38 63	19	0	0.25	0.36
S-5/l(1)25	{XY/0 XY/Y	0 26	54 23	58 30	35 25	23	0	0.34	0.48
S-5/l(1)75	{XY/0 XY/Y	1 33	172 120	244 156	73 48	100	0.49	0.29	0.30
S-5/l(1)129	{XY/0 XY/Y	26 120	104 103	99 150	68 81	65	0.12	0.30	0.40
S-5/l(1)150	{XY/0 XY/Y	0 20	141 157	159 149	88 120	59	0.42	0.36	0.32
S-5/l(1)163	{XY/0 XY/Y	1 10	154 76	111 90	87 28	61	0.07	0.40	0.36
S-5/l(1)216	{XY/0 XY/Y	0 6	42 48	108 62	54 38	1	0.08	0.30	0.42*
S-5/l(1)219	{XY/0 XY/Y	0 244	268 240	298 235	156 90	124	0.07	0.33	0.27
S-5/l(1)223	{XY/0 XY/Y	0 43	33 95	43 133	0 2	0	0	0.51	0.02
S-5/l(1)252	{XY/0 XY/Y	2 121	93 81	104 182	58 60	45	0.19	0.34	0.28
S-5/l(1)361	{XY/0 XY/Y	3 96	142 90	159 109	124 76	88	0.46	0.41	0.42
S-5/l(1)451	{XY/0 XY/Y	6 71	176 125	200 121	0 1	3	0.01	0.02	0.01
S-5/l(1)453	{XY/0 XY/Y	0 61	122 55	125 82	62 32	82	0.29	0.37	0.36
S-5/l(1)454	{XY/0 XY/Y	0 14	44 28	54 34	20 12	12	0.45	0.25	0.22
S-5/l(1)463	{XY/0 XY/Y	15 17	75 32	117 42	48 19	60	0.23	0.09	0.36
						16	0.23	0.32	

* Owing to apparent inviability of exceptional females, $2(v/B\delta)$ was used in place of $v/B\delta + yB/+q$ in estimating primary nondisjunction.

cedures have selected for low X/0 viability and relatively higher X/Y viability. This is illustrated by Figure 2, where the correlation between the relative viability of X/0 males (viability ratio of $l/0 \div$ viability ratio of $+/0$) is plotted against the relative viability of XY males ($=$ viability ratio of $l/Y \div$ viability ratio of $+/Y$). Reductions in viability whose expression is unaffected by the Y should be represented by points scattered along the diagonal with slope 1; the sample selected for these studies can be seen to have a slope much below 1, but it seems reasonable to expect that, were the appropriate viability measurements made on a large random sample of irradiated X chromosomes, the range of slopes from 0 to 1 and probably beyond would be represented.

Among the bb^+ lethals it is possible to produce homozygous females in those cases that are male fertile. It has been incidentally recorded in six cases (11, 47, 59, 227, 231, and 306) that homozygous females survive in the absence of a Y chromosome; in two others (132 and 146) homozygous females survive only in the presence of a Y, and in one (459) homozygous females have not been observed.

A second point of interest regarding Tables 3-5 is the incidence of exceptional progeny apparently arising from maternal nondisjunction. Since the only Y chromosome in recent maternal ancestry is γ^+Y and its presence in the cross would have been detected, secondary nondisjunction is clearly not involved.

FIGURE 2.—The relative viability as X/0 males versus the relative viability as X/Y males of the lethals tabulated in Table 5. Δ , bb^- lethals; $\bigcirc$, bb^+ lethals; open symbols represent male-fertile and closed symbols male-sterile lethals.

When an extra Y is present, the lethal X and S-5 proceed to the poles at random. Nondisjunction of the X's in the primary oocyte results in an X/X gamete or a nullo X gamete; the former yields a scorable offspring when fertilized by a nullo X sperm and the latter when fertilized by an X-bearing sperm; consequently, only half the exceptional ova give rise to viable adults. Since the regular daughters constitute about half the products of regular disjunction, primary nondisjunction has been estimated by exceptions/(regular females + exceptions). Nondisjunction of the *bb*⁻ lethal chromosomes from γ^2 M-5 is extremely low, and with the possible exception of *l(1)459* the *bb*⁺ male-fertile lethal chromosomes disjoin regularly from S-5. An interesting observation from Table 4 is that, whereas the control crosses produced ten exceptional males and no exceptional females [in accord with the observations of MORGAN, BRIDGES, and STURTEVANT (1925) that exceptional sons resulting from maternal nondisjunction outnumber exceptional daughters], the lethals produced a total of 16 exceptional males and 15 exceptional females. The reason for this is not clear. With two exceptions (223 and 451), the *bb*⁺ male-sterile lethals exhibit an extremely high incidence of nondisjunction, averaging between 30 and 40 percent. The absence of γ^2 *w*^a *B* sons from the cross of γ *l(1)25*/S-5 $\times$ XY/Y is difficult to explain, but since these data are from a single pair, there is some doubt that this would be repeated with another S-5 chromosome. The absence of exceptional females among the progeny of γ *l(1)216*/S-5 females was repeatedly observed and is difficult to account for.

Tests for linkage between the X chromosomes and the autosomes: It was likely that some of the Y-suppressed lethals were rearrangements, and so each was tested genetically for the presence of an X-autosome translocation. Because of the sterility of males carrying the majority of the chromosomes to be tested, it was necessary to carry out the tests in females carrying a dominantly marked and multiply inverted X, 2, and 3. γ *l*/S-5/ γ^+ Y females were crossed to *Ins* (2*L*+2*R*) *Cy/In*(2*LR*)*Pm*, *al*⁺ *ds*^{33k} *lt* *bw*^{v1}; *In*(3*LR*)*Dcx*F/*Sb* (= *Cy/Pm*; *D/Sb*) males and secondary exceptional γ *B* *Cy*, γ *B* *D*, and γ *B* *Cy* *D* daughters were recovered and outcrossed, usually to γ *w* males. The progeny were counted and the data examined for evidence of new linkage relations. The results of these crosses are summarized in column 6 of Table 6. Approximately half the chromosomes were tested for linkage with chromosome 4 (the *bb*⁻ lethals except 481; 11, 139, and 459 of the *bb*⁺ male-fertile lethals; and the *bb*⁺ male-sterile lethals except 3, 25, 75, 135, 184, 223, and 455); where linkage was encountered, the information is included in column 6 of Table 6. The striking feature of these data is the high incidence of X-autosome translocations among the male-sterile lethals (17/18) and the correspondingly low incidence among the male-fertile lethals (3/16).

Cytological analysis: An analysis of the salivary gland chromosomes of each *bb*⁺ lethal was undertaken, and with one exception, every lethal examined was found to carry a rearrangement involving the X chromosome. The nature of the rearrangement found, and its break points, are summarized in columns 7-9 of Table 6. The salivary configuration of lethals 25, 75, 361, and 463 appears normal; and we inferred, from linkage tests in which these lethals behave as X-autosome translocations, that both break points are in the chromocentral hetero-

TABLE 6

Summary of the cytological and genetic data from the chromosomes studied in this paper

Class	Number*	Relative viability of		Nondisjunction in γ l/S-5 ♀	X chromosome linked to	Cytological data		
		X/0	X/Y			Type of rearrangement involving X†	Break in	
							X	Autosome
<i>bb</i> ⁻	3	0	1.04	<0.01	.	n.t.		..
	74	0	1.20	<0.01	.	n.t.		..
	158	0	1.13	<0.01	.	n.t.		..
	452	0	1.17	<0.01	.	n.t.		..
	456	n.t.†	n.t.	<0.01	.	n.t.		..
	481	0	0.96	<0.01	.	In	12E/F-14B	..
<i>bb</i> ⁺ male-fertile	11	0.04	0.78	<0.01	4	Tr	15	101
	47	0	0.41	<0.01	2	Ti	8F-9B	?
	59	0	0.63	<0.01	.	In	3/4-19/20	..
	132	<0.01	0.83	<0.01	.	In	4/5-19/20	..
	139	0	0	<0.01	.	Inp	3C-XR	..
	146	0	0.41	<0.01	.	In	5/6-19/20	..
	227	0	0.48	<0.01	.	In	1/2-19/20	..
	231	<0.01	0.96	<0.01	.	In	1C/D-19/20	..
	306	0	0.78	<0.01	.	Ti	1B/E	?
	459	0	0.02	0.02	2;3	Ring	3D/F-XR	50
<i>bb</i> ⁺ male-sterile	3	0	0.74	0.31	3	Tr	4A	81
	25	0	1.06	0.39	2	Tr	19/20	40/41
	75	<0.01	0.26	0.30	2	Tr	19/20	41
	129	0.26	0.91	0.37	2	Tr	18B	41
	150	0	0.15	0.36	2	Tr	16/17	40
	163	<0.01	0.17	0.34	3	Tr	17A/B	80/81
	216	0	0.15	0.44	2;3	n.t.		..
	219	0	1.11	0.30	2	Tr	10A	40
	223	0	0.41	0.02	2	Ti	14F	41-50E
	252	0.02	1.00	0.30	3	n.t.		..
	361	0.02	1.04	0.42	3	Tr	19/20	80/81
	451	0.04	0.63	<0.01	.	..		..
	453	0	0.98	0.37	3	Tr	12D	80/81
	454	0	0.50	0.23	2;3;4	Tr	12B	80/81
	455	n.t.	n.t.	n.t.	n.t.	Tr	3C	81
	463	0.18	0.50	0.35	3	Tr	19/20	80/81
Other male-sterile	65	1.18	0.85	0.38	3	Tr	16/17	79D
	135	0	0	0.41	2;3	Tr	18/19	41
	184	0	0.01	0.40	n.t.	Tr	18A	81
	220	0.25	0.30	0.41	2;3	Tr	14A	50A;75C

* All chromosomes with numbers below 250 were recovered from $\gamma^- ac^-$ M-5/ γ/γ^+Y daughters of irradiated γ males and those with numbers above 250 from M-5/ γ daughters of irradiated γ males.

† Not tested.

Tr=reciprocal translocation.

Ti=insertional translocation.

In=paracentric inversion.

Inp=pericentric inversion.

chromatin. Every lethal-associated rearrangement in which all breaks have been localized has at least one break point in the proximal heterochromatin; and, in reciprocal translocations between the X and chromosome 2 or 3, the autosomal break point is chromocentral, whereas the X chromosome break point may be either euchromatic or heterochromatic. This non random distribution of break points is illustrated in Figure 3.

Several of the lethals require additional comment, especially those associated with rearrangements involving more than two break points; in the more complex cases, only the break points of the rearrangements involving the X chromosome and inferred to be lethal associated are presented in Table 6 and Figure 3. Lethal 481 carries, in addition to a *bb* deficiency, a small inversion from 12E-F to 14B that is unassociated with the lethal effect. This can be shown by the recovery of a recombinant that carries the inversion without the lethal, but not its reciprocal, in an X/0 male. Lethal 139 is characterized by its having the portion of the X to the left of 3C7 associated with the chromocenter. Since the proximal portion of X is not capped with any recognizable element, and the X chromosome behaves as a unit in meiosis, *l(1)139* must be a pericentric inversion with a break between 3C5-6 and 3C7. Lethal 139 requires two Y's to survive, and the X/Y/Y males are characterized by white variegation and a phenotype similar to *rst*². The combination *l(1)139/γ w spl sn* variegates for *w* but not *spl*, which is in accord with the transfer of the loci *w* and *rst*, but not *spl*, into chromocentral heterochromatin.

FIGURE 3.—The salivary localizations of the break points of the inversion, ring, or translocation associated with each Y-suppressed lethal. Each such rearrangement is characterized by two break points and consequently may be completely represented on Cartesian coordinates. Δ , *In(1)* and *X*^c; $\bullet$, *T(1;2)*; $\circ$, *T(1;3)*; $\triangle$, *T(1;4)*.

The single recombinant between $T(1;4)w^{ms}$ and $Inp(1)139$ loses the roughest and lethal characteristics of $l(1)139$, but retains the mottled phenotype; this crossover replaces the tip of X^R , which is distal to 3C7, with bands 3C4, 5, and 6 as well as most of chromosome 4 from $T(1;4)w^{ms}$. Lethal 459 is a four-break rearrangement with euchromatic break points at 3D in the X and around 50 in 2R. The proximal part of 2R has been capped by the tip of the X, and both the proximal part of the X and the distal piece of 2R have become attached to the chromocenter. Since, according to linkage tests, chromosome 3 is involved in this rearrangement, it is presumably participating in these chromocentral attachments. The proximal part of the X consistently appears to be a ring chromosome; this is confirmed by neuroblast prophases which reveal a ring as well as five large autosomal elements, three V's, and two rods. It seems that chromosome 3 has been broken into two centric fragments, one of which has captured the tip of 2R and the other of which may have captured X^R . A sperm with a normal complement of arms but with an extra centromere may arise by the splitting of one centromere into two functional fragments (McCLINTOCK 1938) in a normal haploid sperm or by the conversion of a disomic sperm into a haploid sperm with persisting disomy for the centromere region; e.g., in a disomic for 3, one chromosome 3 could be broken immediately to the right and one immediately to the left of the centromere. The acentric fragments could rejoin and, if somewhere else in the complement a ring or a dicentric had been produced, two free acentric fragments would be available for capture by the centric portions of the two chromosomes 3 (i.e., 3L and 3R). It is surprising to note that the preceding lethals represent two cases of breakage in the short arm of the X from a relatively small sample of rearrangements. Lethals 223 and 451 are the only male-sterile lethals not characterized by high primary nondisjunction; they are also the only ones not associated with a reciprocal translocation. Lethal 223 is associated with an insertional translocation of a segment of 2R from 40-50E into 14A of the X; the order of the inserted segment has not been determined. Lethal 451 is not associated with any rearrangement recognizable by either linkage tests or salivary analysis. It does, however, cause an appreciable reduction in recombination in the proximal half of the X; e.g., the $v-f$ region is reduced from the standard value of 0.237 to 0.120. By recombination measurements the locus of $l(1)451$ is inseparable from the locus of f . Lethal 454 is a five-break rearrangement with breaks in 12B, 22-23, 44F, 81, and 101; the new order is tip of X to 12B/81 through centromere of 3 to tip of 3L; tip of 2L to 22-23/12B to centromere of X; tip of 2R to 44F/101 to centromere of 4; and tip of 3R to 81/22-23 through centromere of 2 to 44F/101 to tip of 4.

At the end of Table 6, four translocations are included whose expression is not convincingly affected by the Y chromosome. They are included because (1) in other respects they resemble the bb^+ male-sterile lethals and (2) they demonstrate that (a) most of the properties of this class of lethals are correlated with their translocation nature and (b) Y-suppressed lethality is not a necessary correlate of male sterility or high primary nondisjunction. $T(1;3)65$ is nonlethal and carries, in addition to the translocation, a large inversion in the X from 1C

to 10B. It is male sterile and associated with high primary nondisjunction. This particular X, when heterozygous to $\gamma^2 cv v f$, produced no recombinants among 725 progeny; it is a very useful balancer for the X. T(1;2;3)135 and T(1;3)184 are orthodox lethals that are characterized by high primary nondisjunction of the X's; there are indications of excessive nondisjunction in about one eighth (8/65) of the orthodox lethals recovered at 3 and 4 kr by the S-5 method. In addition to the T(1;2) indicated in Table 5, *l(1)135* has a reciprocal T(2;3) with break points in 37 and 85A. T(1;2;3)220 is a semilethal characterized by male sterility and high primary nondisjunction; the distribution of the elements in the translocation is X^{D2P} , 2^{D3P} , and 3^{DXP} .

Tests of effect of enhancers of variegation: A number of observations made it seem likely that the bb^+ , Y-suppressed lethals were variegated position effects. To test this suspicion, we measured the viability of each lethal in combination with two different dominant enhancers of variegation, *M(2)S10* (see SCHULTZ in MORGAN, SCHULTZ, and CURRY 1940) and *Evar 7*, that were kindly furnished by DR. JACK SCHULTZ. Females heterozygous for S-5 and the lethal-bearing X were crossed to *In(1)w^m*; *Ins(2L+2R)Cy/Evar 7* or *Ins(2L+2R)Cy/M(2)S10* males and the proportion of *Cy* to non-*Cy* flies was scored among the lethal- and nonlethal-bearing sons. The data from these crosses are presented in Table 7. Before the lethal-bearing chromosomes were tested, both enhancers were tested against *In(1)w^m* and shown to still be very effective enhancers of the white variegation. Table 7 indicates that recovery of some of the lethals is reduced by *Evar 7* (i.e., 75 and 463), some by *M(2)S10* (i.e., 11, 3, 216, 219, and 453), both enhancers in some cases (i.e., 59, 146, 227, 454, and 455), and neither in still other cases (i.e., 306, 132, 231, 25, 129, 150, 220, 223, 252, and 361). These observations are in accord with those made earlier by SCHULTZ (1950), who found with visible phenotypes that enhancers of variegation may be specific in their action in that particular enhancers are characterized by enhancing a particular array of V-type position effects. The absence of S-5 sons from the *M(2)S10* cross is unexplained as yet, although a plausible explanation is the existence of a *Ybb⁻* in the *M(2)S10/Cy* stock. A further indication that we are dealing with position effects in these cases is that, in several cases where the X/Y males have a distinct phenotype, the phenotype was more extreme in the *Evar 7* than in the *Cy* flies.

The morphology of male sterility: Examination of testis preparations of a number of the male-sterile genotypes revealed that the responsible lesion was spermiogenic. Meiosis appeared to be normal; there was evidence of quadrivalent formation, as would be expected from translocation heterozygotes and of frequent nondisjunction, but there were no other meiotic abnormalities noted. The abnormal formation of sperm was first evident at the beginning of sperm head elongation. Whereas normal spermiogenesis produces a progressive elongation and narrowing of the spermatid nucleus with a concomitant loss of cytoplasm resulting in an extremely long, narrow heavily staining structure, condensation of the abnormal structure takes place in the absence of elongation. The mature sperm head is therefore a small, highly chromatic, spherical or ellipsoidal ele-

TABLE 7

Comparison of survival of bb⁺ Y-covered lethal-bearing chromosome in the presence and absence of specific dominant enhancers of variegation

	X/Y sons from γ l/S-5♀ crossed to									
	<i>ln(1)w^m/Y; Evar 7/lns(2L+2R)Cy</i>						<i>+Y; M(2)S-10/lns(2L+2R)Cy</i>			
	Regular				Exceptional		Regular		Exceptional	
	γ	γ Cy	γ w ^a B	γ w ^a B Cy	+	Cy	γ	γ Cy	+	Cy
<i>l(1)11</i>	43	32	24	42	3	5	14	38	1	22
<i>l(1)59</i>	16	73	98	66	0	0	10	109	1	1
<i>l(1)132</i>	38	32	27	35	0	1	39	50	2	0
<i>l(1)146</i>	28	45	52	47	0	0	23	40	0	1
<i>l(1)227</i>	45	88	118	104	0	1	1	49	5	0
<i>l(1)231</i>	60	63	66	58	1	1	21	33	0	0
<i>l(1)306</i>	32	48	32	48	0	1	8	16	0	0
<i>l(1)459</i>	0	1	8	9	0	0	0	0	1	0
<i>l(1)3</i>	23	34	30	46	20	12	13	29	10	16
<i>l(1)25</i>	37	39	36	46	20	24	8	4	19	25
<i>l(1)75</i>	8	22	32	27	13	19	26	31	31	28
<i>l(1)129</i>	47	42	27	35	20	30	30	38	21	21
<i>l(1)150</i>	5	4	21	23	18	16	6	11	40	57
<i>l(1)163</i>	0	8	51	51	19	25	0	1	29	27
<i>l(1)216</i>	0	0	7	9	1	6	1	13	11	17
<i>l(1)219</i>	58	49	35	51	10	21	12	49	23	44
<i>l(1)220</i>	2	5	8	18	15	20	4	9	17	31
<i>l(1)223</i>	11	30	29	41	2	5	15	27	1	5
<i>l(1)252</i>	19	18	17	20	9	12	6	13	1	7
<i>l(1)361</i>	42	53	65	78	26	37	34	54	27	51
<i>l(1)451</i>	0	0	71	76	5	9	0	0	2	2
<i>l(1)453</i>	30	24	28	28	24	32	14	52	22	36
<i>l(1)454</i>	7	30	24	23	5	11	0	5	5	8
<i>l(1)455</i>	4	14	69	46	45	33	1	4	23	31
<i>l(1)463</i>	3	95	61	66	24	30	30	44	40	28

ment. In many cases, there is evidence of disassociation of the spermatid nucleus from the nebenkern such that, when the sperm were mature, the heads were no longer associated with the tails.

DISCUSSION

It has been shown in the preceding pages that sex-linked recessive lethals whose expression is suppressed by the Y chromosome are a common consequence of radiation. In fact, they constitute approximately 20 percent of all sex-linked recessive lethals produced by 3 and 4 kr. The Y-suppressed lethals were immediately classifiable into two groups on the basis of their ability to survive in combination with *ln(1)sc^{4L,8R}*, which is deficient for the majority of Xh [the proximal third of hD, all of hC and hB, and nearly all of hA (see COOPER 1959, Figure 56)]. Irradiated chromosomes that fail to survive in this combination are presumed to carry lethals in the proximal heterochromatin of the X. Their suppression is

attributable to the fact that the Y carries normal loci homologous to the affected loci in the irradiated X. It is of interest to know the proportion of proximal heterochromatic lethals that can be covered by the Y chromosome. Although this question cannot be answered by the data presented in this report, we can surmise that there are loci in Xh for which there are no normal alleles in the Y. An example of such a locus is *su^w-f* (COOPER 1959). Two lethals were recorded in S-5 experiments that were not covered by the Y and failed to survive in combination with S-5. Presumably, they were deficient for a portion of the X that is homologous with the Y and a portion that is not. It was not determined whether the latter portion was heterochromatic or euchromatic, however. It might be of interest to screen a sample of orthodox sex-linked recessive lethals for survival in combination with a duplication for Xh. It is known that the *In(1)sc^{4L,8R}* deficiency is completely within the region of X-Y homology insofar as lethally mutating loci are concerned, since the deficiency is covered by the Y. There may be heterochromatic loci, however, that are carried by both *In(1)sc^{4L,8R}* and the Y chromosome. Lethal mutation at these loci would produce a mutant that is covered by the Y and would survive in combination with *In(1)sc^{4L,8R}*. Of the lethals studied in this report, six were judged to be in the proximal heterochromatin on the basis of their failure to survive in combination with *In(1)sc^{4L,8R}*. The remaining lethals, which survive in combination with *In(1)sc^{4L,8R}*, differ from these six in many respects and clearly fall into another category; consequently, we have no evidence of any proximal heterochromatic lethal that is covered by the Y and yet survives in combination with *sc^{4L,8R}*. A further point of interest is that all six proximal heterochromatic lethals seem to be deficient for *bb*, judging from their failure to survive in combination with an allele of *bb* that itself dies in combination with a known deficiency for *bb*. Further evidence of their deficient nature is the observation that none of them segregate from the Y chromosome with complete regularity. Thus, our data provide no evidence of loci capable of lethal mutation in the portion of Xh that is homologous to Y, other than *bb*. It seems quite likely that a deficiency for the nucleolus organizer, but not *bb⁺*, would be lethal as BEERMAN (1960) has shown to be the case in *Chironomus*, but as *bb⁺* and NO are very closely linked, the chances of separating them are small (COOPER 1959).

The other major class of Y-suppressed lethals is made up of those that survive in combination with *In(1)sc^{4L,8R}*. These lethals are not confined to the proximal portion of the X but are scattered over its entire length. They are further distinguished from the proximal heterochromatic lethals in that, with one exception, all are associated with gross rearrangements involving the X chromosome in which at least one break is in the chromocentral heterochromatin. These observations agree with the hypothesis that these are variegated position-effect lethals (LEWIS 1950). This explanation is strengthened by the observation that the expression of many of these lethals in XY males is enhanced by specific autosomal dominant enhancers of variegation. Finally, in several cases where the test was possible, we found that females homozygous for the lethal-bearing X survive in the absence of a Y chromosome. It is a common observation that the effect of Y chromosomes on the expression of variegation is related, not to the absolute

numbers of Y's present, but to the number of Y's in relation to a normal constitution; that is, it is a departure from the normal heterochromatin-euchromatin balance and should, as COOPER (1956) has postulated, affect the expression of V-type position effect, so that the phenotypic expression may roughly be seriated $X/0 > X/Y = X/X > X/Y/Y = X/X/Y$. The survival similarity of homozygous females to X/Y rather than X/0 males, then, accords well with phenotypic observations on variegated position effects. These arguments have led us to the conclusion that the second category of lethals are variegated position effects.

The location of the break points of the variegated lethals agrees well with previous experience with V-type position effects. The inversions have the distal break point in the euchromatic portion of the X (Xe) and the proximal break in chromocentral heterochromatin of the X (Xh). The translocations may have their X break in either Xe or Xh, but the autosomal break point is invariably in the chromocentral heterochromatin (Ah). We can probably generalize from these observations that the chromosomal realignments that are sex-linked variegated lethal compatible are Xe-Xh, Xe-Ah, and Xh-Ah, and those that are not are Xe-Xe, Xe-Ae, and Xh-Ae. Variegation for loci in the proximal heterochromatin, if indeed that is the explanation of lethality in the T(Xh;Ah) cases, is not a generally observed result of a wholly heterochromatic rearrangement in *D. melanogaster*. In a total of 193 *R(ci⁺)*'s, KHWOSTOVA (1939) observed translocation of chromosome 4 to Xh distal to *bb⁺* in only 12 cases and to the distal regions of the Y in 19 cases, but not to any other chromocentral region. In similar experiments with the light locus, HESSLER (1958) found among 35 *R(lt⁺)* variegated effects that each had one break in region 40 of 2L, but that in no case was the second break in chromocentral heterochromatin. In the present study, however, 4/17 of the postulated V-type position effect translocations have both break points in the chromocentral heterochromatin. These cases presumably provide additional examples of heterochromatic loci whose variegation is suppressed rather than enhanced by the Y chromosome (GRELL 1959).

The present experiments demonstrate that approximately 18 percent of the lethals produced by 3 and 4 kr are V-type position effects that can be made to survive by adding a single Y chromosome to the complement. Experiments currently in progress indicate that about 4 percent of the remainder can be made to survive by adding a second Y. This means that relatively few of the lethals sampled by orthodox procedures (i.e., as X/Y males) can be shown to be V-type position effects by suppression with an extra Y. We might question how closely these procedures have estimated the contribution of position effect lethals to all sex-linked recessive lethals and to inquire into the importance of position effect in semilethal and subvital changes. The observed proportion of about 21 percent position-effect lethals probably underestimates the importance of position effects in induced viability changes. Our methods have tended to select effects that can be converted from essentially complete inviability to some degree of viability by the addition of a Y chromosome and to ignore effects that can be changed from low viability to higher viability by addition of a Y (see, however, lethals 129 and 463). Also remaining undetected were any position effects sufficiently extreme

that the suppression afforded by two Y's was insufficient to impart viability to them. Furthermore, it is clear that stable (S-type) position effects have been entirely overlooked, since their expression is unaffected by Y chromosome constitution. MULLER (1948) has reported evidence for induced S-type position effect lethals, but since he presents no data, we have no way of estimating their contribution.

The fact that 20 percent of the chromosomes lethal as X/0 males survive as X/Y males, whereas only four percent of the lethals that are lethal as X/Y males survive as X/Y/Y males indicates that the first Y has a relatively much greater effect on the expression of variegated lethals than the second Y. This observation is understandable because the addition of a Y chromosome to an X/0 male causes a proportionately greater increase in the heterochromatic complement than the addition of a Y to an X/Y male.

The V-type position-effect lethals are further divisible into two groups on the basis of fertility of X/Y males. We believe that this subdivision is less related to the nature of lethality than to the type of rearrangement associated with lethality. A fairly accurate generalization from the observations is that the inversions and insertional translocations are male fertile, and the reciprocal translocations are male sterile. The association of sterility with homozygosis for reciprocal translocations has been observed since the early thirties (e.g., PATTERSON, STONE, BEDICHEK and SUCHE 1934), but to the best of our knowledge, SCHULTZ (1947) was the first to point out the strong correlation between male sterility and X-chromosome rearrangements, specifically reciprocal translocations between the X and the autosomes. He considered that sterility was the consequence of gene mutation near the point of breakage or, more probably, of position effect. The position effect explanation of this sterility is also invoked by LEWIS (1950). The present observations disagree with the position effect as well as the mutational hypothesis of male sterility. Our sample of variegating lethals includes inversions and reciprocal and insertional translocations, and, though all are V-type position effects with regard to their lethal phenotype, only the reciprocal translocations are associated with male sterility. Furthermore, we found that the addition of a second Y chromosome has no effect on fertility (lethals 361, 453, and 455 tested as X/Y/Y males). One attribute that is shared by the sterilizing rearrangements found in the present experiments is disruption of the continuity between the two ends of the X chromosomes, and it may be that the linear continuity of the X plays some role in normal spermiogenesis. The situation cannot actually be so simple since there is not a perfect correlation between disruption in the continuity of the X chromosome and loss of male fertility; there are four apparent exceptions in the present material. Lethal 11 is a T(X;4) that is male fertile; examination of the known T(X;4)'s listed in BRIDGES and BREHME (1944), however, reveals that none are listed as sterile and, although this may be an artifact of the method of recovering the T(X;4)'s, it is also possible that the centromeres of X and 4 are interchangeable, so that a T(X;4) does not constitute an effective disruption of continuity. This same reservation also seems to apply to T(X;Y)'s (NICOLETTI and LINDSLEY 1960) where the T(X;Y)'s that are T(X;Y)/Y sterile are nearly

all $T(X;Y;A)$'s, whereas no $T(X;Y)/Y$ fertile case is a $T(X;Y;A)$. Lethal 459 is a quasi-reciprocal translocation that results in a disruption of the continuity of the X chromosome but is male fertile; the portion of the X distal to region 3D is translocated to 2R, but the proximal portion of the X forms a ring rather than capturing the tip of 2R. $T(1;2)$ 223 is an insertional translocation; yet it is male sterile. Since the sterility of this rearrangement is caused by variegation for missing external genitalia rather than abortive spermiogenesis, in a sense it may be considered male fertile. Finally, 451 is male sterile and unassociated with any cytologically detectable rearrangement. This chromosome is a mystery; genetic tests show no evidence of a translocation, but there is some reduction of crossing over in the region of f and the lethal seems to be inseparable from f^+ . Spermatogenesis appears to be normal, but the mature sperm are not motile.

SCHULTZ (1947) has attempted to establish some generalizations describing the relation between break points of reciprocal $T(X;A)$'s and male fertility. He claimed that, when both the X and the autosomal break point are heterochromatic, the translocation is usually male fertile; whereas, when both breaks are euchromatic, the translocation is usually male sterile. The situation for heterochromatic-euchromatic translocations is simply described as being more complex. Because the current sample was selected on the basis of variegated lethality, the autosomal break of each reciprocal $T(X;A)$ is in the chromocentral heterochromatin; and furthermore, in one third ($4/12$) of the cases, the X break also is heterochromatic. The finding that all these cases are male sterile fails to support SCHULTZ's generalizations, but, since selection of translocations has been biased with respect to break points, the present material does not supply information that would be valuable in formulating new generalizations.

High primary nondisjunction of the lethal-bearing X from a balancer X (S-5) is another characteristic in addition to male sterility that is associated with the reciprocal $T(X;A)$'s studied here. The only male-sterile lethals not characterized by high primary nondisjunction were 223 and 451, and these are the two male steriles that are not associated with a reciprocal $T(X;A)$. Similar high nondisjunction frequency is observed when the S-5 chromosome is replaced with a normal sequence, but the incidence of exceptions is about half that observed with S-5. Since a variable proportion of nontransmissible products of meiosis results from translocation heterozygosis, it is not possible to estimate the incidence of nondisjunction of the X's (i.e., 3-1 disjunction) among all primary oocytes from the incidence of exceptions among the viable progeny. The qualification must be added to the estimates of nondisjunction in Table 4 that primary nondisjunction of the X chromosomes is being estimated for only those cells that produce orthoploid segregations for the autosome involved in the $T(X;A)$. It is possible that selecting $T(X;A)$'s with chromocentral autosomal break points has tended to minimize the orderly pairing and separation of the X's, although $T(1;3)65$, which does not have a chromocentral break point, is also characterized by a high incidence of exceptions. DOBZHANSKY (1932) observed a similar high incidence of primary exceptions for the sex chromosomes from attached-X females carrying $T(Y;2)$'s; and indeed, judging from the break points (WHITTINGHILL 1937), he

did find that a high incidence of exceptions was correlated with a chromocentral break in chromosome 2. Furthermore, CROUSE (1960) has recently reported on a reciprocal translocation between the X chromosome and chromosome 2 in *Sciara coprophila* that produces high primary nondisjunction of the X chromosomes in heterozygous females.

SUMMARY

A method has been devised that allows the detection and recovery of sex-linked recessive lethals whose lethal phenotype is suppressed by the Y chromosome, as well as orthodox lethals that are inviable with or without a Y. With doses of 3 and 4 kr, approximately 20 percent of all induced sex-linked recessive lethals survive in the presence of a Y chromosome and are consequently overlooked by currently used methods of lethal detection. The Y-suppressed lethals may be divided into two major classes. About one eighth of them are deficiencies for the proximal heterochromatin, specifically for bb^+ , and they are suppressed by the Y because the Y carries the homologous region. The other seven eighths are nearly all associated with gross rearrangements with at least one break point in the chromocentral heterochromatin and at least one in the X. These are concluded to be variegated position-effect lethals. The V-type position-effect lethals may be further subdivided into one group that is male fertile and associated with X chromosome inversions or insertional translocations and another group that is male sterile and associated with reciprocal translocations with a break in the X and a break in the chromocentral heterochromatin of chromosome 2 or 3. The sterility of the reciprocal translocations is attributable to failure of sperm head elongation. It is postulated that continuity of the X chromosome plays an important role in normal spermiogenesis and that this continuity is disrupted by reciprocal translocation.

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