MALE-STERILIZING INTERACTIONS BETWEEN DUPLICATIONS AND DEFICIENCIES FOR PROXIMAL *X*-CHROMOSOME MATERIAL IN *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

The genetic limits of sixty-four deficiencies in the vicinity of the euchromatic-heterochromatic junction of the *X* chromosome were mapped with respect to a number of proximal recessive lethal mutations. They were also tested for male fertility in combination with three *Y* chromosomes carrying different amounts of proximal *X*-chromosome-derived material ($B^{S}Yy^{+}$, $y^{+}Ymal^{126}$ and $y^{+}Ymal^{+}$). All deficiencies that did not include the locus of *bb* and a few that did were male-fertile in all male-viable *Df(1)/Dp(1;Y)* combinations. Nineteen *bb* deficiencies fell into six different classes by virtue of their male-fertility phenotypes when combined with the duplicated *Y* chromosomes. The six categories of deficiencies are consistent with a formalism that invokes three factors or regions at the base of the *X*, one distal and two proximal to *bb*, which bind a substance critical for precocious inactivation of the *X* chromosome in the primary spermatocyte. Free duplications carrying these regions or factors compete for the substance in such a way that, in the presence of such duplications, proximally deficient *X* chromosomes are unable to command sufficient substance for proper control of *X*-chromosome gene activity preparatory to spermatogenesis. We conclude that there is no single factor at the base of the *X* that is required for the fertility of males whose genotype is otherwise normal.

IN 1972, LIFSCHYTZ and LINDSLEY postulated that precocious inactivation of the *X* chromosome *vis-à-vis* the autosomes in the primary spermatocyte is an important event in the regulation of spermatogenesis in animal species in which the male is the heterogametic sex. Among the phenomena that led them to this hypothesis was the observation in *Drosophila melanogaster* that

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approximately 80% of translocations between the *X* chromosome and either or both of the major autosomes are completely male sterile. They attributed this sterility to the removal of *X*-chromosome material from, and the placing of autosomal material under, the control of a postulated *cis*-acting *X*-inactivating element. They postulated the existence of such a controlling element in the proximal heterochromatic region of the *X* on the basis of an observed sterility of males carrying *X* chromosomes deficient for both *su(f)* and *bb*. No sooner had they enunciated this generalization than SCHALET (1972) and later SCHALET and LEFEVRE (1976) reported the recovery of fertile males bearing such deficiencies. Subsequently, LIFSCHYTZ and LINDSLEY (1974) reported that four such deficiencies induced in *In (1)sc*^{8} were male fertile.

There is no doubt that some deficiencies for *su(f)* and *bb* are male fertile, but just what distinguishes the fertile from the sterile ones is unclear. Accordingly, we undertook a systematic investigation of deficiencies at the base of the *X* chromosome in order to try to define the chromosomal concommitants of male sterility and thus to infer the prerequisites for male fertility. Our findings indicate that no proximal *X*-chromosome deficiency is by itself able to cause male sterility; however, certain deficiencies in combination with duplications for proximal regions of the *X* chromosome, as represented by duplication-bearing *Y* chromosomes used to compensate for deficiencies for *su(f)*, are indeed male sterile. Failure of earlier authors to recognize the contribution of the duplicated *Y* chromosome to the observed phenotype doubtlessly accounts for the discordance of the previous reports.

We examined the fertility of males carrying an array of proximal *X*-chromosome deletions in combination with several different *Y* chromosomes bearing compensating duplications of varying amounts of *X*-chromosome material. We find that the adverse effects on male fertility are correlated with the quantity of duplicated material carried by the *Y*, as well as with what we infer to be the quantity of material deleted from the *X*. Three contributing elements have been tentatively identified at the base of the *X* chromosome; it has not, however, been possible to implicate specific factors in the duplicated segments of the *Y* chromosomes.

Both the *Y*-linked duplications and the deleted *X* chromosomes support male fertility in combination with normal homologues; it is only in combination with one another that they impair it. The mechanism of the observed interaction remains a mystery.

MATERIALS AND METHODS

Induction of deficiencies at the base of the X *chromosome*: Suppressor of forked [*su(f)*] is the proximal-most euchromatic locus on the *X* chromosome; it has been placed in polytene chromosome subdivision 20F by LEFEVRE (SCHALET and LEFEVRE, 1976). The bobbed locus (*bb*), which is in the proximal heterochromatin, lies between *su(f)* and the centromere. At 25°, females

homozygous for *su(f)* and hemizygous males are normal in phenotype; however, females that are hemizygous for *su(f)* [*Df(1)su(f)/ (su(f)*] exhibit a characteristic phenotype, which we designate Minute-like or *M*-like; it includes fine bristles, rough eyes, spread wings, Confluens-like wing veins, etched abdominal tergites, delayed emergence and reduced viability (LINDSLEY and GRELL 1968; SCHALET 1968). At 30°, *su(f)* flies exhibit the *M*-like phenotype; whereas, *Df(1)su(f)/ su(f)* flies die (DUDICK, WRIGHT and BROTHERS 1974). This phenotype provides a method for the selection of newly induced deficiencies for the *su(f)* locus. Accordingly, two-to-four-day-old males of various genotypes (*+/ Y , y/ Y , 1(1)Q56/ Ymal*$^+$ or *y^2 v f 1(1)Q464/ Ymal*$^+$) were irradiated with 4000R of X-rays (administered by a TORR X-ray machine at 120 KV and 4mA with 0.25 mm aluminum filtration) and crossed to three-to-six-day-old females, whose genetic constitution was *Dp(1;1)sc^{V1}, y lz f car su(f).y$^+$/FM7*. *FM7* is a multiply inverted *X* chromosome marked with *y^{31d} sc^8 w^a sn^{X2} v^{Of} g^4 B* (MERRIAM 1968; MERRIAM and DUFFY 1972); for detailed descriptions of other mutant genes and rearranged chromosomes used, see LINDSLEY and GRELL (1968). All crosses were cultured at 25° in quarter-pint bottles on standard corn-meal-molasses medium; parents were removed after 7 days. *M*-like daughters began emerging on the fourteenth and continued until the twenty-second day of the culture. Non-*B M*-like females were selected, crossed to *FM7* males, and *y^{31d} B* daughters were recovered; since the centromere region of *Dp(1;1)sc^{V1}* is marked by *y$^+$*, these *y^{31d}* daughters carry the *su(f)* region derived from the irradiated chromosome. Some of the *M*-like *y^{31d} B* granddaughters of the irradiated males produced yellow sons indicating that their phenotype was not caused by an induced deficiency for *su(f)*, which is male lethal; they were, therefore, of no further interest.

Complementation mapping of deletions: The extent of each proximal *X*-chromosome deficiency was determined by testing its survival in combination with a number of proximal *X*-chromosome lethals and a sample of duplications extending different distances from the proximal heterochromatin into the proximal euchromatin. The proximal lethals utilized and their relative positions are diagrammed in Figure 1 (LIFSCHYTZ and FALK 1968; SCHALET and LEFEVRE 1976). Also depicted in Figure 1 are the duplications, which in order of increasing euchromatic content are *B^{S}Y$^+$* (BROSSEAU *et al.* 1961), *Dp(1;f)3* (LINDSLEY and SANDLER 1958), *y$^+$Ymal^{126}* (SCHALET and FINNERTY 1968), and *y$^+$Ymal$^+$* (SCHALET 1963). *y$^+$Ymal^{126}* is an X-ray-induced derivative of *y$^+$Ymal$^+$* from which a substantial portion of the duplicated *X* chromosome material, including *mal$^+$*, has been deleted (SCHALET and FINNERTY 1968). Although deficiencies were not tested in combination with lethals *A112, A7* or *114*, it was possible to infer that a deficiency included one of these loci on the basis of its failure to survive in combination with a duplication extending up to but not including the locus. For example, if *B^{S}Y$^+$* fails to cover a deficiency, we infer that the deficiency extends to the left at least as far as to include *l(1)114*. Similarly, deficiencies covered by *y$^+$Ymal^{126}* but not *Df(1;f)3* must include *l(1)A7*, and those not covered by *y$^+$Ymal^{126}* must extend to the left of *l(1)A112*.

Fertility testing: In order to test the fertility of males carrying the deficient *X* chromosomes, it is necessary to compensate for the absence of *su(f)$^+$* and adjacent euchromatic loci removed by the deletion. This is accomplished by recovering males carrying *Df(1)su(f)* in combination with *Y* chromosomes that are duplicated for proximal regions of the *X*. The three different duplicated *Y* chromosomes shown in Figure 1 were utilized for this purpose. Males carrying deficient *X* chromosomes in combination with various duplicated *Y* chromosomes were produced by crossing *Df(1)su(f)/FM7* females to males carrying the appropriate *Y*. Fifteen F_1 males were crossed

individually to single virgin females homozygous for *FM7* marked with y^{31d} sc^{8} w^{a} v^{Of} *B* and their fertility and fecundity ascertained. Fertile pairs were removed on the ninth day and the total numbers of progeny determined; sterile pairs were kept for fifteen to twenty days before being so scored and discarded.

Detection of X-autosome translocations by cytological and genetic means: Since it is known that many translocations between the *X* and either chromosome *2* or *3* are dominant male steriles (LINDSLEY, EDINGTON and VON HALLE 1960; LIFSCHYTZ and LINDSLEY 1972; LINDSLEY and TOKUYASU 1980), it was necessary to determine which, if any, of the deletions that are male sterile in all tested genotypes are associated with *T(X;A)*'s. Two criteria of *X*-autosome translocation were employed: (1) Sterile males carrying *X*-autosome translocations exhibit a failure in sperm-head elongation. Accordingly, testes of males carrying dominant sterile deletions were dissected in 0.7% saline, stained with 70% acetic orcein (2% orcein) and sperm-head morphology observed under Zeiss phase optics. Sperm bundles of each sterile male were examined and any abnormality photographed; (2) The presence of *X*-autosome translocations was also ascertained genetically. *Df(1)su(f)/FM7* females were crossed to *y/Y; SM5/+; TM6/+* males and their *Cy Ubx* daughters (*y/Df; SM5/+; TM6/+*) outcrossed to wild type males. Since all *Df(1)*-bearing sons die, failure to recover Cy^{+} sons indicates that the deficiency in the *X* is also involved in a *T(1;2)*; failure to recover Ubx^{+} sons is diagnostic of a *T(1;3)*.

RESULTS

This study involves the analysis of 64 deficiencies for *su(f)*; among these, 49 were induced in the present experiments, 12 were obtained from A. SCHALET, two from G. LEFEVRE and one from J. KENNISON.

Induction of su(f) *deficiencies:* Table 1 presents data on the recovery of Minute-like phenotypes among the progeny of irradiated males carrying different *X* chromosomes. It can be seen that the majority of *M*-like females recovered either died without producing progenies or could not be attributed to

TABLE 1

The recovery of Minute-like daughters from crosses of males irradiated with 4000 r of X rays and su(f) *females.*

Experiment	Genotype of irradiated males	Total F_1 females	Minute-like females					
			Total	Dead or sterile	$su(f)^{-}bb^{+}$	$su(f)^{-}bb^{-}$	(%)*	Other
1+2	+/Y	11,364	63	22	10	3	(.31)	28
3	y/Y	11,794	60	21	7	2	(.26)	30
4+5+6	*1(1)Q56/Ymal*$^{+}$	11,462	106	70	4	5	(.69)	27
5+7+8	y^{2} *v f 1(1)Q464/Ymal*$^{+}$	6,936	26	9	8	9	(.37)	0

*% = sum of Minute-like females in columns 2,3 and 4/total F_1 females

deficiencies for *su(f)*. The data are not completely consistent from experiment to experiment, but we can propose explanations for some of the inconsistencies. We note that the presence of *f* in the irradiated y^2 *v f l(1)Q464* chromosome allowed immediate selection of *su(f)* deficiencies, since the Minute-like phenotype was accompanied by suppression of forked. The fact that only *su(f)*-deficient females were scored reduces the incidence of Minute-like females recovered and probably accounts for the lower incidence of dead and sterile females in this case; it suggests that many females that failed to reproduce in the other crosses were in fact not deficient for *su(f)*.

The fraction of *su(f)* deficiencies that are also *bb* deficient is significantly lower in experiments 1-3 (5/22) than in experiments 4-8 (14/26). We attribute this to an undefined difference in the irradiated *X* chromosomes. Experiments 1-3 involved standard laboratory strains, and they agree with those of LIFSCHYTZ (1978) who found 20% of the *su(f)* deficiencies induced in a laboratory strain are also *bb* deficient. *l(1)Q464* and *l(1)Q56*, on the other hand, were induced in a Quyrat Anavim wild stock collected in Israel. Irradiation of the Quyrat Anavim *X* chromosome by LIFSCHYTZ and FALK (1968 and subsequent work) yielded 16 deficiencies for *su(f)*, of which seven were also deficient for *bb*.

Distribution of breakpoints on deficient chromosomes: The genetic limits of the 64 deficiencies, which were determined following the complementation mapping technique described under MATERIALS AND METHODS, are presented in Figure 1. Sixty of these deficiencies are viable with at least one of the *Y*-linked duplications. Three [*Df(1)R-1, R-16* and *R-39*] are lethal with all *Y*-linked duplications, as well as with all proximal lethals tested; they are likely deficiencies extending to the left of the limit of the $Ymal^+$ duplication. One [$Df(1)y^{x5}$] is lethal with all *Y*-linked duplications, but viable in combination with *l(1)R10-10* and other proximal lethals; therefore, $Df(1)y^{x5}$ most likely carries an independent lethal beyond the left limit of the y^+Ymal^+ duplication (SCHALET, personal communication). The nonuniform distribution of the left breakpoints of the 49 deficiencies induced in these experiments (those with prefix *R-*) is concordant with that reported by LIFSCHYTZ (1978) in a sample of 130 X-ray-induced *su(f)* deficiencies.

The 64 *su(f)* deficiencies illustrated in Figure 1 are divided into three groups with respect to their right breakpoints, based on their phenotypes in heterozygotes with w^e bb^1: 35 are bb^+ and therefore broken to the left of the majority of the ribosomal cistrons; three are bobbed and therefore the right breakpoint is judged to be within the ribosomal cistrons; the remaining 26 do not survive in combination with bb^1 and therefore lack the majority of the ribosomal DNA.

Fertility of the su(f)*-deficient males:* Sixty *su(f)* deletions were combined with B^SYy^+, y^+Ymal^{126}, and y^+Ymal^+; when viable, the resulting males were assayed for fertility and fecundity. Among 34 deficiencies for *su(f)* that are bb^+, all but two are male fertile in all viable combinations with duplicated *Y* chromosomes. The two exceptions, as will be discussed later, are *T(X;A)*'s. A

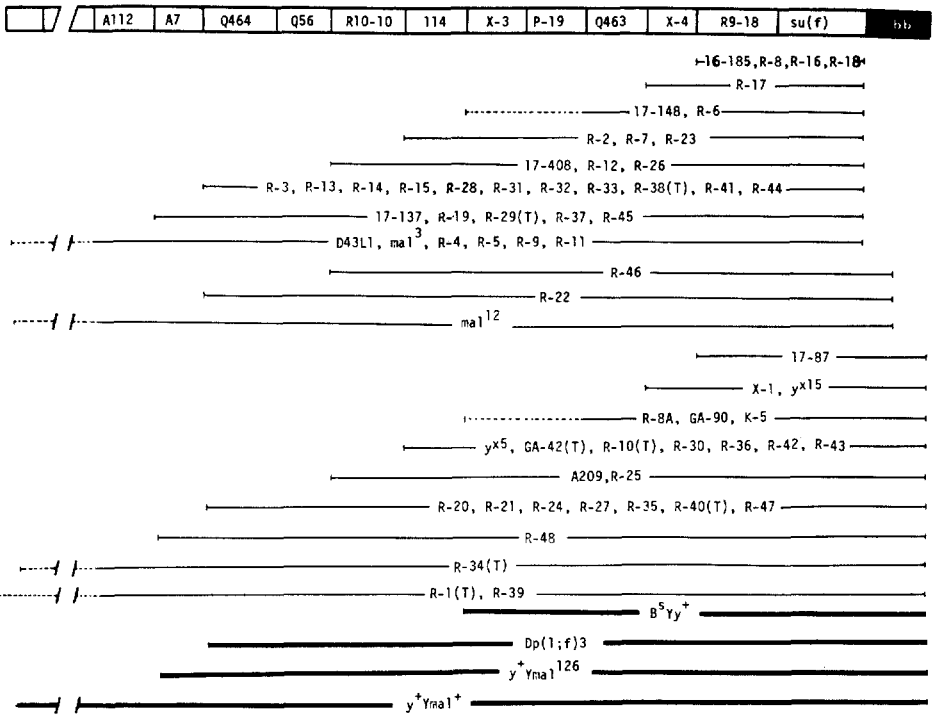

FIGURE 1.— Results of complementation mapping to determine the extents of the *su(f)* deletions with respect to an array of proximally located lethals. Narrow black lines indicate the extent of each deficiency and broken lines associated with some of them indicate untested loci that could also be included. Thick black lines below represent duplications for the proximal region of the *X* chromosome. (T) indicates that the deficiency is associated with an *X*-autosome translocation.

35th bb^+ deficiency was inviable in combination with all *Y* chromosomes. MUNOZ (personal communication) has noted additional male-sterile $su(f)^- bb^+$ deletions.

The remaining 26 deficiencies are subdivided into three groups, which are differentiated one from the other on the basis of the fertility of males carrying them in combination with the different duplicated *Y* chromosomes. These results, excluding those deficiencies subsequently demonstrated to be associated with *X*-autosome translocations, are presented in Figure 2. The nine deficiencies of the first group differ from the remainder in that they are male fertile in combination with y^+Ymal^+; they are also male fertile in all other male-viable combinations. Three of these deficiencies [*R-46* and *R-22*, as well as mal^{12} reported by SCHALET (1969) but not observed by us] are only partially deficient for *bb* as indicated by the bobbed phenotype of $Df(1)/bb^1$ females.

The second subset contains five deficiencies, all of which are male sterile in combination with y^+Ymal^{126} and male fertile, when viable, in combination with

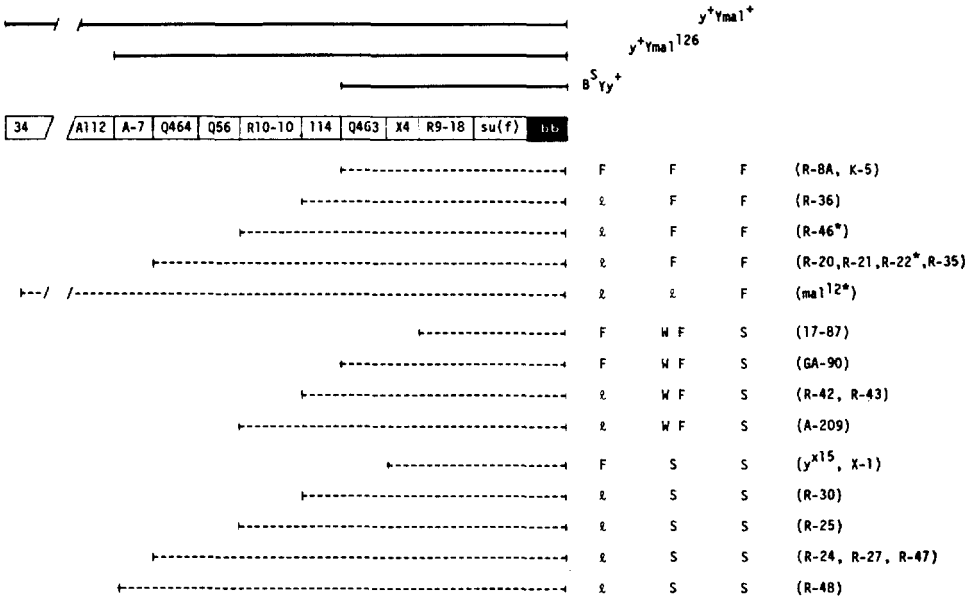

FIGURE 2.— Fertility of males carrying *su(f)-bb* deficiencies in combination with three different duplicated *Y* chromosomes. Three solid lines in the upper left quadrant and their designations on the right represent the different *Y* duplications. Broken lines in the lower left quadrant represent the extents of the *X* deficiencies. The phenotypes of various combinations are tabulated in the lower right quadrant. l = lethal; F = fertile with >10 progeny per male; WF = weakly fertile with <4 progeny per male; S = sterile. The three deficiencies marked with an asterisk do not include the entire *bb* locus, as indicated by their survival in combination with bb^1 as bobbed females. Deficiencies shown to be associated with either a *T(X;2)* or a *T(X;3)* are not included.

B^SYy^+. It should be emphasized that the distinction between sterile, weakly fertile, and fertile persists under repeated testing. The final subset is composed of twelve deficiencies that are male sterile in combination with both y^+Ymal^+ and y^+Ymal^{126}; the two deficiencies [*Df(1)X-1* and $Df(1)y^{x15}$] in the third group that survive with B^SYy^+ are male fertile in that combination. Table 2 summarizes the fecundity data from the various fertile genotypes.

Tests of su(f) *deficiencies for* T(X;A)*:* The majority of translocations between the *X* chromosome and either major autosome are known to be male sterile (LINDSLEY *et al.* 1968; LIFSCHYTZ and LINDSLEY 1972; LINDSLEY and TOKUYASU 1980), and no way of rescuing fertility in males carrying such translocations has been found. Thus, we considered the possibility that at least some of the deficiencies in the third subset are associated with *X*-autosome translocations. The two deficiencies surviving over B^SYy^+ are male fertile in that combination, indicating that they are not translocations between the *X* and either major autosome; y^{x15} has, however, been shown to involve a complex

TABLE 2

Number of progeny of su(f)-bb *and* bb *deficient males carrying different duplicated* Y *chromosomes when crossed to* FM7 *females in single pairs.*

	Proximal constitution		Mean number of progeny per male*		
Df(1)	*su(f)*	*bb*	$B^{S}Y_{y}+$	$y^{+}Ymal^{126}$	$y^{+}Ymal^{+}$
Canton S	+	+	28.1	54.1	63.1
R8A	–	–	62.3	73.7	79.2
K-5	–	–	70.2	29.6	16.7
R-36	–	–	lethal	56.0	22.1
R-35	–	–	lethal	74.0	42.1
R-21	–	–	lethal	51.3	97.9
R-20	–	–	lethal	75.2	76.3
R-22	–	bb	lethal	n.t.	57.8[a]
R-46	–	bb	lethal	71.6	60.6
mal^{12}	–	bb	lethal	lethal	76.9
$In(1)w^{m4L}\ w^{m51bR}$	+	–	61.4	n.t.	66.8
bb^{1}	+	–	66.4	95.6	9.6
$In(1)sc^{4L}\ sc^{8R}$	+	–	n.t.	n.t.	2.2
bb^{1-74}	+	–	79.4	68.7	0.6
bb^{1-158}	+	–	91.1	32.8	0
bb^{1-452}	+	–	93.7	20.6	0
17-87	–	–	43.9	1.4	0
GA-90	–	–	59.4	2.4	0
R-42	–	–	lethal	1.2	0
R-43	–	–	lethal	2.1	0
A209	–	–	lethal	3.1	0
X-1	–	–	37.2	0.06	0
y^{x15}	–	–	18.9	0	0

*Average from 15 pair matings; [a]Average from 5 pair matings; n.t. = not tested.

T(X;4) (SCHALET and LEFEVRE 1976). The spermiogenic lesion associated with *T(X;A)* male sterility is some degree of failure of sperm-head elongation (LINDSLEY *et al.* 1960). Of ten deficiencies that cause male sterility in all testable combinations, four produce highly condensed ellipsoidal heads in the most mature stages of spermiogenesis present and fail to produce motile sperm. The remaining six, on the other hand, show more nearly normal sperm-head morphology, although occasional bundles contain a few foreshortened sperm heads and variable levels of sperm motility. These observations were taken as suggestive evidence that four of the uniformly male-sterile deficiencies are

associated with *X*-autosome translocations. This suggestion was confirmed by genetic tests of the type described under MATERIALS AND METHODS. The deficient *X* was found to be linked to chromosome *2* in three *(GA-42, R-10* and *R-34)* and to chromosome *3* in one *(R-40)* of the ten cases discussed above. These four coincide with the four deficiencies exhibiting ellipsoidal sperm heads. Thus, *X*-autosome translocations account for some but not all of the uniformly sterile *X*-chromosome deficiencies. The translocated deficiencies are excluded from Figure 2. Also considered was the possibility that some of the six uniformly sterile translocations that were not associated with translocations involving the *X* and either major autosome were associated with *T(X;4)*'s. Since there are no genetic markers that allow the detection of an interchange of an *X* chromosome centromere free of bb^+ and a fourth chromosome centromere region, it was necessary to rely on a cytological analysis. GATTI *et al.* (1976) have shown that the centromere region of chromosome *4* fluoresces brightly when stained with quinacrine; whereas, the *X* centromere region fluoresces dully. The centromeres of the last six deficiencies listed in Figure 2 are all quinacrine dull; thus, there is no indication of fourth chromosome involvement.

Fertility of bb-*deficient males:* It seems quite probable that the disagreements between LIFSCHYTZ and LINDSLEY on one hand and SCHALET and his colleagues on the other stem from a failure of both to recognize the role of the duplication-bearing *Y* chromosome in causing male infertility. In general, these authors did not specify the *Y* chromosome utilized. The results reported here bear out the contention of LIFSCHYTZ and LINDSLEY (1972) that deficiencies that include *su(f)* but not bb^+ are male fertile; they do not, however, support the accompanying claim that all deficiencies including both loci are male sterile, since the first nine deficiencies listed in Figure 2 are deficient for $su(f)^+$ and bb^+ and yet are male fertile in all viable combinations [three of the nine (*R-46, R-22* and mal^{12}) retain sufficient ribosomal sequences to partially complement bb^1; others may retain lesser amounts]. They also state that deficiencies that include bb^+ but not $su(f)^+$ are invariably male fertile. Since the role of the *Y* chromosome was not recognized, it seems quite likely that this assertion was based on the well-known fertility of males carrying bobbed deficiencies in combination with a normal *Y*, a y^+Y or a B^SY. It therefore seemed appropriate to score the fertility of males carrying bobbed deficiencies in combination with other duplicated *Y*'s. Three ($bb^{l\text{-}74}$, $bb^{l\text{-}158}$ and $bb^{l\text{-}452}$) were recovered as *Y*-suppressed sex-linked lethals (LINDSLEY, EDINGTON and VON HALLE 1960). According to metaphase length measurements, they are deficient for 48, 82 and 32 percent of the centric heterochromatin, respectively (YAMAMOTO and MIKLOS 1978), and according to our determinations yield 13.9, 20.0 and 6.4 percent nondisjunction from the y^+Y chromosome in males. bb^1, which segregates regularly from a marked *Y* chromosome, removes 22% of the pericentric heterochromatin, according to YAMAMOTO and MIKLOS (1978), and judging from filter hybridization results of RITOSSA (1976) and PROCUNIER and TARTOF

(1978) with other lethal alleles of *bb*, removal of 80-85% of the ribosomal cistrons produces a bobbed lethal condition. *In(1)sc^{4L}sc^{8R}* eliminates approximately 80% of the proximal heterochromatin. *In(1)w^{m4L}w^{m51bR}*, which is a deficiency constructed by SPOFFORD and DESALLE (1978), has been shown by *in situ* hybridization studies of HILLIKER and APPELS (personal communication) to be deficient for more than 95% of the ribosomal sequences, but to retain a few cistrons at each end of the chromosome; thus the deficiency does not extend in either direction beyond the confines of the ribosomal-RNA-coding region of the *X* heterochromatin. The fertility of males carrying the *bb* deficiencies described above in combination with the three duplicated *Y* chromosomes was assessed (Table 2). The results are presented in Table 2, and it is evident that, except for the case of *In(1)w^{m4L}w^{m51bR}*, fertility is seriously diminished or abolished in males carrying simple *bb* deficiencies in combination with *y$^+$Ymal$^+$*. Thus, of the three postulates proposed by LIFSCHYTZ and LINDSLEY to describe the relation between proximal *X*-chromosome deficiencies and male infertility, only one is supported by the present observations. As indicated in Table 2, the fertility characteristics of the *bb* deficiencies place them in a position intermediate in severity between the first and second groups of *su(f)-bb* deficiencies listed in Figure 2 in that, although fertile in combination with *y$^+$Ymal^{126}*, they are sterile or nearly so in combination with *y$^+$Ymal$^+$*.

DISCUSSION

The results presented in Figure 2 demonstrate that the constitution of the duplication-bearing *Y* chromosome is an important variable in determining the fertility of deficiency-bearing males, since the same deficiency can be male fertile or sterile depending on the duplicated *Y* used. For example, *Df(1)GA-90* is fertile with *B^{S}Yy$^+$*, weakly fertile with *y$^+$Ymal^{126}* and sterile with *y$^+$Ymal$^+$*. Similarly some property of the deficiency is crucial since the fertility of males carrying the same duplicated *Y* chromosome, e.g., *y$^+$Ymal^{126}*, varies depending on the *X*-chromosome deficiency employed.

Relation between X-chromosome deficiency and male sterility: An examination of the euchromatic breakpoints of the three subsets of deficiencies for both *su(f)* and *bb* diagrammed in Figure 2 fails to reveal any correlation between the breakpoint and the male-fertility phenotype. Each fertility class contains deficiencies with broadly overlapping arrays of left breakpoints, and deficiencies with common left breakpoints exhibit different fertility phenotypes; for example, deficiencies *R-36, R-42* and *R-30* are broken between the same pair of adjacent lethals; yet, each falls into a different fertility subset. In two preliminary communications (RAHMAN and LINDSLEY 1979, 1980), we claim to have observed a correlation between the fertility phenotype and the euchromatic breakpoint of the *su(f)-bb* deficiency; however, when additional deficiencies were examined, the apparent correlation disappeared. MUNOZ (personal

communication) has also noted no correlation between male sterility and the complementation units to the left of *su(f)* involved in $su(f)^{-}\ bb^{-}$ deficiencies. Turning attention to the right-hand breakpoint, all of the deficiencies in Figure 2 are broken either within or proximal to the ribosomal cistrons. We postulate that it is the relative position of this breakpoint within the rather large heterochromatic region that results in the three classes of deficiencies. Deficiencies of the first class, which are male fertile in all viable combinations with duplicated *Y* chromosomes, are postulated to extend for the shortest distance to the right of *bb* and to uncouple the ribosomal cistrons from segments affecting male fertility. The observation that three of them still retain genetically detectable ribosomal cistrons as revealed by their *bb* phenotype when heterozygous with bb^{1}, is in agreement with this supposition. The deficiencies that are male sterile with both $y^{+}Ymal^{+}$ and $y^{+}Ymal^{126}$ are supposed to extend the farthest to the right of *bb*, and those that are weakly fertile in combination with $y^{+}Ymal^{126}$ are postulated to be broken at an intermediate position. These postulates imply two regions or factors to the right of *bb* that figure importantly in determining the male fertility of deficiencies for *su(f)* and *bb*. The fertility of deficiency-bearing males carrying $y^{+}Ymal^{126}$ provides a method for determining the constitution of the deficiency with respect to these factors. If the deficiency retains both factors, such males are fertile; if it lacks the more distal factor, they are weakly fertile; if it lacks both, they are sterile.

The above scheme is formally consistant with the data derived from the deficiencies for *su(f)* and *bb*; the results from deficiencies that have lost bb^{+} but not $su(f)^{+}$, however, cannot be reconciled with a two-factor model. Their fertility characteristics do not fall into any of the three categories illustrated in Figure 2; as pointed out previously, they comprise a group with fertility characteristics intermediate between those of groups 1 and 2 in Figure 2 (see Table 2). The one respect in which the *bb* deficiencies clearly differ from the deficiencies represented in Figure 2 is that they do not include *su(f)* or any locus to its left. One might postulate that this difference in behavior between the *bb* deficiencies and the *su(f)-bb* deficiencies lies in the retention of a factor distal to *bb* by the former that is missing in the latter. The *bb* deficiencies can be interpreted as falling into three male-fertility classes in much the same way as the *su(f)-bb* deficiencies. $In(1)w^{m4L}\ w^{m51bR}$ is male fertile in combination with $y^{+}\ Ymal^{+}$, and, although it has not been tested, it is presumably male fertile in combination with other *Y* chromosomes. The remaining *bb* deficiencies presumably extend into the region between the ribosomal DNA and the centromere, with $bb^{l\text{-}158}$ and $bb^{l\text{-}452}$, which are male sterile in combination with $y^{+}\ Ymal^{+}$, extending further to the right than bb^{1}, $In(1)sc^{4L}\ sc^{8R}$ or $bb^{l\text{-}74}$, which are weakly fertile.

The following formal model, which is summarized in Table 3, seems to be consistent with most of the observations: There are three regions at the base of the *X* involved in the phenomenon under consideration, one to the left and two to the right of *bb*; we term these factors, from left to right, *1*, *2* and *3*. A

TABLE 3

Model postulating three factors at the base of the X *chromosome illustrating the relations between the proposed genotypes and observed phenotypes of* Df(1)/Dp(1;Y) *males.*

Constitution of deficiency				Male fertility* in combination with		
[su(f) #1]	bb^+	#2	#3	B^SYy^+	y^+Ymal^{126}	y^+Ymal^+
−	+	+	+	F	F	F
−	−	+	+	F	F	F
−	−	−	+	F	WF	S
−	−	−	−	F	S	S
+	−	+	+	F	F	F
+	−	−	+	F	F	WF
+	−	−	−	F	F	S

*F = fertile; WF = weakly fertile; S = sterile.

deficiency for factor *1* alone is male fertile in combination with all three duplicated *Y* chromosomes (*e.g.*, Group 1, Figure 2); deficiencies that include both *1* and *2* are less able to support fertility of duplication-bearing males in that they are sterile in combination with y^+Ymal^+ and weakly fertile with y^+Ymal^{126}. Deficiencies for *1, 2* and *3* have even more drastic effects on male fertility in that they are male-sterile in combination with both y^+Ymal^+ and y^+Ymal^{126}, but not with B^SYy^+. Deficiencies for *2* alone are weakly fertile with y^+Ymal^+ but otherwise fertile; whereas, the loss of both *2* and *3* sterilizes y^+Ymal^+-bearing males but not y^+Ymal^{126}-bearing males. Factor *3* and the right breakpoints of $Df(1)bb^{l\text{-}158}$ and $Df(1)bb^{l\text{-}452}$ are all, according to this hypothesis, to the right of the right breakpoint of $In(1)sc^8$, which has heretofore been considered to be very close to the centromere based on mitotic cytology. In the same way that y^+Ymal^{126} distinguishes three classes of deficiencies among those that have lost factor *1*, y^+Ymal^+ resolves deficiencies that have retained factor *1* into three groups: fertile (presumably retaining both *2* and *3*), weakly fertile (lacking *2*, retaining *3*), and sterile (lacking both *2* and *3*).

It must be pointed out that, although the foregoing scheme roughly describes the results, it is not possible to force all the observations into a model in which the factors postulated are absolutely discrete and all the rearrangements utilized simple. For example $Df(1)y^{x15}$, which is a deficiency in the region removed distally by $In(1)sc^8$, contains as much or more of the centric heterochromatin normally proximal to the ribosomal genes as does $In(1)sc^{4L}sc^{8R}$; yet in accordance with the model $Df(1)y^{x15}$ has lost factor *3*, which is retained by $In(1)sc^{4L}sc^{8R}$. Similarly, cytological and *X-Y* nondisjunctional determinations suggest that $Df(1)bb^{l\text{-}452}$ is too short to include both the majority of the ribosomal cistrons and proximal

heterochromatic factors *2* and *3* as necessitated by the model.

Relation between X-*chromosome duplication and male sterility:* We now turn our attention to the features of the duplication-bearing *Y* chromosome that appear to be pertinent to the interaction. $y^+ Ymal^+$ and $y^+ Ymal^{126}$ together define at least four classes of proximal *X*-chromosome deficiencies based on male fertility. $B^S Yy^+$ on the other hand gives no evidence of being discriminatory in that it appears to be fertile in combination with all classes of deficiencies. However, YAMAMOTO and MERRIAM (personal communication) report that two doses of $B^S Yy^+$ sterilize *bb*-deficiency-bearing males, although $+/B^S Yy^+/B^S Yy^+$ males are fertile. $y^+ Ymal^+$ was derived through a sequence of spontaneous and induced events so that its heterochromatic constitution is indeterminate. Tentatively, its constitution may be written as follows:

$$l(1)J1^+ --ac^+\ KL \cdot bb^+\ ot^+ --su(f)^+\ bb^+\ KS.$$

bb^+ is represented twice on the grounds that $Y^S X \cdot Y^L$, *In(1)EN*, the chromosome from which $y^+ Ymal^+$ was derived, carries bb^+ at both ends as demonstrated by our observation that reciprocal recombinants between $Y^S X \cdot Y^L$, *In(1)EN* and $In(1)sc^{4L} sc^{8R}$ carry sufficient ribosomal cistrons to exhibit survival and normal phenotype in combination with bb^l; furthermore according to filter hybridization results, $y^+ Ymal^+$ retains approximately 90% of the rDNA originally present in $Y^S X \cdot Y^L$, *In(1)EN* (TARTOF 1973; PROCUNIER and TARTOF 1978). In polytene preparations, however, LEFEVRE notes the organization of a nucleolus at the *ot* end, but not at the *su(f)* end of the duplication (SCHALET and LEFEVRE 1973). $y^+ Ymal^{126}$ was selected as an X-ray-induced deficiency for mal^+ in this chromosome. Thus, the more deleterious effect of $y^+ Ymal^+$ than $y^+ Ymal^{126}$ on the fertility of deficiency bearing males must be attributed to the presence of something on $y^+ Ymal^+$ that has been deleted from $y^+ Ymal^{126}$. An incidental observation that bears on this point is that $Ysu(f)^-$, a derivative of $Ymal^+ 2$ that is deficient for a segment of *X* euchromatin different from that deleted in $Y^+ Ymal^{126}$, also supports male fertility in combination with $bb^{l\text{-}158}$. $Ysu(f)^-$ arose as a spontaneous deficiency in $Ymal^+ 2$ extending from *l(1)R10-10* through *su(f)* (RAHMAN and LINDSLEY 1981). Although $y^+ Ymal^+$, from which $y^+ Ymal^{126}$ arose, and $Ymal^+ 2$, in which $Ysu(f)^-$ originated, are of independent origin, both were induced by deleting the majority of the *X* euchromatin from $Y^S X \cdot Y^L$, *In(1)EN*, and thus they are likely to be comparable in structure. The fact that the sterility of $Df(1)bb^{l\text{-}158}/Ymal^+$ can be reversed by deleting euchromatin and most probably adjacent heterochromatic material from either end of the duplicated segment suggests that a specific factor on $Ymal^+$ is not involved in its male-sterile interaction with $bb^{l\text{-}158}$ and other proximal *X*-chromosome deficiencies; rather the quantity of duplicated *X*-chromosome material seems to be the determining factor. This suggestion is reinforced by YAMAMOTO and MERRIAM's previously cited observation that two doses of $B^S Yy^+$ sterilize $Df(1)bb^{l\text{-}158}$-bearing males;

whereas, one dose does not. There appears to be a relation between the quantity of *X* euchromatin duplicated in the *Y* chromosome and the severity of the effect of the *Y* on the fertility of deficiency-bearing males. However, we have not succeeded in characterizing the heterochromatic constitutions of the various *Y* chromosomes employed, and X heterochromatin may be an important component of the phenomenon being investigated.

X-chromosome duplications can be achieved in other ways; preliminary crosses have been made utilizing *Dp(1;f)3*, which contains in addition to the entire proximal heterochromatic complement of the *X*, a proximal euchromatic duplication virtually identical to that carried by $y^{+}Ymal^{126}$ (Figure 1). The average number of progeny of $Df(1)bb^{l\text{-}158}/y^{+}Ymal^{126}$ males is 32.8, while the average for $Df(1)bb^{l\text{-}158}/Y/Dp(1;f)3$ males is 1.9. This comparison implicates the *X* heterochromatin as an important component of the effect of *X* duplications on male fertility. The fact that $Df(1)bb^{l\text{-}158}/y^{+}Ymal^{+}/Dp(1;f)3$ males are completely sterile, plus the above result, also indicates that the contribution of the deficiency at the base of the *X* to the male sterility under consideration is not complemented by the provision of a compensating free duplication. Said in another way, proximal heterochromatic elements would seem to have to be in coupling with the *X* euchromatin in order to overcome the sterilizing effects of duplicated *Y* chromosomes. It is as though the proximal elements of the *X* chromosome were competing for some factor required by the *X* for the assurance of male fertility. Decreasing the quantity of heterochromatin at the base of the *X* reduces its ability to compete; alternatively, increasing the size of the duplication enhances its competitive ability, thus depriving the *X* of the hypothetical substance.

It should be emphasized that although it is possible to categorize the *Df(1)/Dp(1;Y)* combinations with respect to their effects on male fertility, the results from combinations classified as male fertile, for example, vary from 16 to 98 progeny per male, and those of weakly fertile combinations from .06 to 9.6. In spite of the fact that an attempt has been made to test male fecundity in a uniform manner, some of this variability may be attributable to inconstancy of experimental procedure. Another component may reside in the background genotypes of the males tested. These possibilities notwithstanding, it seems likely that a significant amount of the observed variation is attributable to differences among the component deficiencies and that the categories that we have erected represent but a coarse resolution of chromosomal organization.

Three different classes of abnormal chromosome constitutions are now known to interfere with spermatogenesis in *Drosophila melanogaster*: (1) translocations between the *X* chromosome and either chromosome *2* or chromosome *3* (SCHULTZ 1947; LINDSLEY, EDINGTON and VON HALLE 1960); (2) translocations between the *Y* chromosome and either chromosome *2* or *3* in combination with an *X* chromosome deficient for proximal heterochromatic material (BESMERTNAIA 1934; LINDSLEY *et al.* 1979); and (3) duplications for material from the right end of the *X* chromosome in combination with an *X*

chromosome deficient for proximal heterochromatic material (present study). In each case, proximal heterochromatic elements are separated from some or all of the *X* euchromatin, but as demonstrated in the present study, such a separation is necessary but not sufficient for the impairment of male fertility. The other condition or conditions that must be satisfied to achieve male sterility remain to be defined. At present, it is difficult to assign a mechanism to any of these effects, and it is even difficult to provide a convincing argument that the underlying mechanism is the same in all three cases.

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