THE GENETIC AND CYTOLOGICAL ORGANIZATION OF THE *Y* CHROMOSOME OF *DROSOPHILA MELANOGASTER*[1]

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Manuscript received November 26, 1980
Revised copy received May 7, 1981

ABSTRACT

Cytological and genetic analyses of 121 translocations between the *Y* chromosome and the centric heterochromatin of the *X* chromosome have been used to define and localize six regions on the *Y* chromosome of *Drosophila melanogaster* necessary for male fertility. These regions are associated with nonfluorescent blocks of the *Y* chromosome, as revealed using Hoechst 33258 or quinacrine staining. Each region appears to contain but one functional unit, as defined by failure of complementation among translocations with breakpoints within the same block. The distribution of translocation breakpoints examined appears to be nonrandom, in that breaks occur preferentially in the nonfluorescent blocks and not in the large fluorescent blocks.

THE *Y* chromosome of *Drosophila melanogaster* has many advantages as a system for the study of chromosome organization. It is relatively easy to manipulate genetically and appears to differ greatly from much of the rest of the genome, not only in molecular and genetic organizations, but also in function. It is dispensable in all cells except the germ line of the male. This paper provides a cytogenetic characterization of the organization of the *Y* chromosome with respect to those functional regions essential for normal spermatogenesis.

The *Y* chromosome of *Drosophila melanogaster* accounts for about 13% of the total length of all metaphase chromosomes in a normal diploid male (after BRIDGES, from DOBZHANSKY 1929; GOWEN and GAY 1933). It appears to be entirely heterochromatic at mitotic prophase (HEITZ 1933), contains more than 70% of highly repeated, simple-sequence satellite DNA (PEACOCK *et al.* 1977) and is necessary only for male fertility; males lacking a *Y* chromsome are viable, but completely sterile (BRIDGES 1916). In most cases, males and females with two *Y* chromosomes are viable and fertile, but a few combinations of *X* and *Y* chromosomes from different strains are lethal or sterile as *X/Y/Y* males (GRELL 1969); males with three *Y* chromosomes are sterile (COOPER 1956). The *Y* chromosome is submetacentric, with the long arm (Y^L) about 1.5 to 2 times the length of the short arm (Y^S) (COOPER 1959); the presence of both arms is necessary for male fertility (STERN 1929).

[1] The work reported here was submitted in partial fulfillment of the requirements for the Doctor of Philosophy, Department of Biology, University of California, San Diego.

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Despite its large size, the *Y* chromosome appears to contain little unique genetic information. It contains one locus that is necessary for viability, which is also present in the heterochromatin of the *X* chromosome. This is the bobbed (*bb*) locus on Y^S (COOPER 1959; LINDSLEY, EDINGTON and VON HALLE 1960), thought to be the site of the ribosomal RNA genes (RITOSSA *et al.* 1966). The *Y* chromosome also contains a number of genes necessary for male fertility. From previous genetic studies (NEUHAUS 1938, 1939; BROSSEAU 1960; WILLIAMSON 1970, 1972), the number of fertility loci is very small, probably fewer than ten.

In contrast to its low density of fertility loci as determined by complementation analysis, the *Y* chromosome is very sensitive to the induction of male sterility mutations (see LINDSLEY and TOKUYASU 1980 for review). Approximately one-half of all translocations involving the *Y* chromosome are male fertile if they also bear an extra *Y* chromosome, but are male sterile if they do not; this sterility has been attributed to the loss of function of specific male-fertility genes on the *Y* chromosome (NICOLETTI and LINDSLEY 1960; NICOLETTI, unpublished; LINDSLEY *et al.* 1972). After irradiation with X rays or gamma rays, the frequency of sterility of males receiving an irradiated *Y* chromosome is more than twice that of males receiving an irradiated *X* chromosome (LINDSLEY, TOLEDO and MUSATTI, unpublished; M. SCHWARTZ, unpublished). From the frequency of EMS-induced mutations, however, the *X* chromosome has been estimated to contain 100–150 genes that can mutate to male sterility (LINDSLEY and LIFSCHYTZ 1972), while the *Y* chromosome is thought to contain fewer than ten genes that can mutate to male sterility (WILLIAMSON 1970, 1972).

In order to examine this paradox of low genetic content but high mutability, we have investigated the cytological and genetic organization of the *Y* chromosome. The method employed involves the generation of segmental duplications and deficiencies for interstitial regions by combining elements of translocations with different *Y*-chromosome breakpoints. Using a large series of translocations that do not affect male fertility, it is possible to create segmental deficiencies for adjacent regions along the entire length of the chromosome in order to identify which regions are necessary for male fertility. Because the deficiencies are adjacent and generated from translocations that themselves do not sterilize males that carry them, they should include all regions that are essential for spermatogenesis. Each essential region identified, however, may contain two or more functional loci between which no male-fertile translocation breakpoints were recovered. Therefore, where possible, each region was further analyzed by complementation between translocations that sterilize males and have breakpoints within that region. A pair of translocations in the same region would be expected to show complementation if their breakpoints inactivate different functional loci within that defined region. The observations are consistent with the earlier conclusions of BROSSEAU (1960), based on complementation analysis of X-ray-induced *Y*-chromosome sterility mutations, except that one fertility factor inferred by BROSSEAU cannot be confirmed. Four regions necessary for fertility are identified on the long arm and two on the short arm of the *Y* chromosome. Cytological analyses of the translocations in Hoechst 33258 or quinacrine-stained mitotic figures reveals

that the breakpoints are concentrated within nonstaining blocks between blocks of brightly fluorescent material.

MATERIALS AND METHODS

All mutations and chromosome aberrations used are described in more detail in LINDSLEY and GRELL (1968), with the exception of the *X-Y* translocations induced and described here and *w+Y2*. *w+Y2* is a *Y* chromosome carrying the normal allele for white derived by irradiating *w+y+Y* (BROSSEAU *et al.* 1961) with 4000r of gamma rays and selecting for loss of *y+*. $B^S Yy+$ is a *Y* chromosome carrying the dominant marker Bar-Stone, derived from *T(1;4)*B^S, on the tip of the long arm and the normal allele of the mutant yellow on the tip of the short arm (BROSSEAU 1958). Despite its complex origin, the $B^S Yy+$ chromosome cytologically differs from an unmarked Oregon R *Y* chromosome only at its terminal regions (GATTI and PIMPINELLI, personal communication). *C(1)RM*, *C(1)A* and *C(1)DX* are compound-*X* chromosomes. *C(1)DX* has no nucleolus organizer region (*i.e.*, is *bb*$^-$) and is lethal in the absence of any other source of ribosomal cistrons.

The procedures used for selecting *X-Y* translocations are shown in Figure 1. Males of the genotype *y w f*/$B^S Yy+$/*w+Y2* (Series C) or *y w f*/$B^S Yy+$/*Y* (Series A and B) were irradiated with approximately 4000r of gamma rays from a ^{60}Co source and crossed to *C(1)RM*, y^2 *su(*w^a*)* w^a *bb* females either with (Series B and C) or without (Series A) a free unmarked *Y* chromosome. The irradiated P_1 males were discarded within four days of the time of treatment so that only postmeiotically irradiated cells were sampled. Individual F_1 males were crossed to *C(1)RM*, y^2 *su(*w^a*)* w^a *bb/Y* females and the progeny were scored for translocations between the treated *X* and *Y* chromosomes. In the absence of a translocation, B^S and *y+* remain completely linked and unlinked to the treated *X* chromosome. Linkage of either B^S, *y+*, or both with the treated *X* chromosome indicates a translocation between the treated *X* and *Y* chromosomes. One translocation element, the acentric distal portion of the *Y* chromosome (Y^D) appended to the centric proximate portion of the *X* chromosome (X^P), is referred to as $Y^D X^P$. The reciprocal element, referred to as $X^D Y^P$, is the centric portion of the *Y* chromosome capped by the distal acentric portion of the *X* chromosome.

The translocations can be classified according to the types of aneuploid progeny recovered. Translocations in which the aneuploid $X^D Y^P/Y$ males are inviable are assumed to have breakpoints in the *X* euchromatin (*Xe*). These translocations were not used in the experiments and are not considered further. Aneuploid males will survive only if the breakpoint in the *X* chromosome is either proximal or distal to all of the *X*-chromosome material required for the viability of *X/Y* males; such breakpoints are said to be in the *X* heterochromatin (*Xh*). Aneuploid $X^D Y^P/Y$ males from translocations with breakpoints in the *X* heterochromatin to the left of the centromere will be *y+ B+* if the *Y*-chromosome breakpoint is proximal to B^S in Y^L, or *y* B^S if the breakpoint is proximal to *y+* in Y^S. Translocations of this type are identified by the property that the $X^D Y^P$ element carries at least one entire arm of the *Y* chromosome, and the reciprocal $Y^D X^P$ element carries no more than one arm of the *Y*. Breakpoints in the *X* chromosome to the right of the centromere or in *Xe* distal to all loci required for viability yield aneuploid $Y^D X^P/Y$ males that carry all known *X*-linked genes and are *y* B^S or *y+ B+* when breaks are proximal to the marker in Y^L or Y^S, respectively. These latter two types of translocation should be rare and may, in most instances, be identified by the presence of less than one arm of the *Y* chromosome on the $Y^D X^P$ element and more than one arm of the *Y* chromosome on the reciprocal $X^D Y^P$ element. Whether the *X*-chromosome breakpoint is at the extreme left or right of the chromosome is distinguishable by either Hoechst 33258 fluorescence cytology or genetic mapping of the Y^D marker on the $Y^D X^P$ element. No translocations with breakpoints far enough distal in the *X* euchromatin to yield viable $Y^D X^P/Y$ males were recovered.

Determination of the relative positions of the translocation breakpoints with respect to *bb+* within Y^S and the *X* heterochromatin was possible for many of the translocations. For translocations broken in Y^L or distal to *ks-1* and therefore distal to *bb+* in Y^S, all $X^D Y^P$/O males carry the *bb+* allele from Y^S and are *bb+* in phenotype. If the *C(1)DX*, *y w f bb*$^-$/$Y^D X^P$ females are invia-

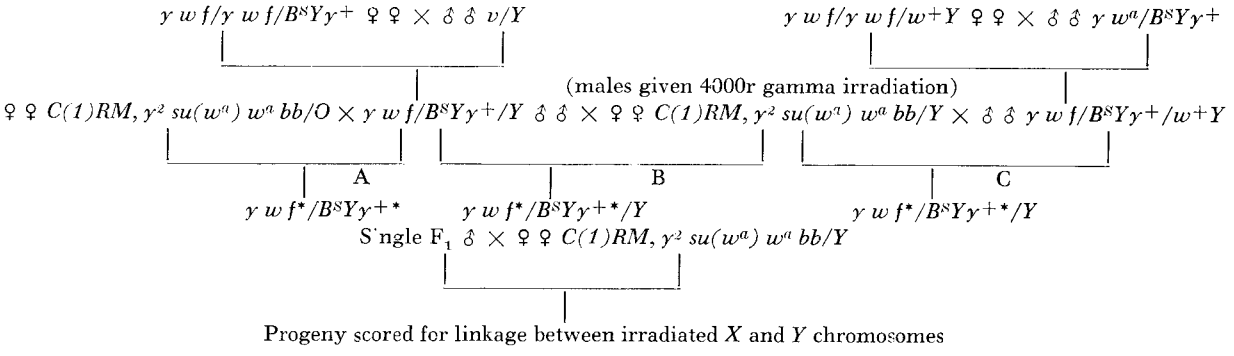

FIGURE 1.—The crossing scheme used in the production and recovery of translocations between the *X* and *Y* chromosomes. Irradiated *X* and *Y* chromosomes are noted with an asterisk. A, B and C refer to Series A, Series B and Series C described in the text.

ble, the *Xh* breakpoint is deduced to be proximal to most or all of *bb*+. If they are viable, but *bb* in phenotype, the breakpoint is deduced to be within *bb*+, the relative position being estimated by the severity of the *bb* phenotype (RITOSSA 1976). Breakpoints distal to most or all of *bb*+ yield *C(1)DX/Y^{D}X^{P}* females that are *bb*+ in phenotype. Translocations broken proximal to *ks-1* in *Y^{S}* may be broken either proximal or distal to *bb*+ and are therefore more complicated. If the breakpoint in *Y^{S}* is proximal to *bb*+ and if the *X* breakpoint is distal to *bb*+, then the *X^{D}Y^{P}* element is *bb*$^{-}$ and *X^{D}Y^{P}/O* males are inviable; *C(1)DX/Y^{D}X^{P}* females are *bb*+. If the *Y^{S}* breakpoint is distal to *bb*+ and the *Xh* breakpoint is proximal to *bb*+, then the *X^{D}Y^{P}/O* males are *bb*+; whereas, the *C(1)DX/Y^{D}X^{P}* females are inviable. When both the *Xh* and *Y^{S}* are broken on the same side of *bb*+ with respect to the centromere, breakpoint positions are ambiguous, since both translocated elements carry *bb*+ and therefore, both *X^{D}Y^{P}/O* males and *C(1)DX/Y^{D}X^{P}* females are viable.

The translocations in Series A were recovered in the absence of a free *Y* chromosome, and, therefore, only aberrations that do not sterilize males were recovered. The translocations in Series B and C were recovered in the presence of an extra unbroken *Y* chromosome, and males were subsequently tested for fertility in the absence of an extra *Y* chromosome. For simplicity, translocations that do not sterilize the males that carry them will be referred to as fertile translocations. Translocations that do sterilize the males that carry them will be referred to as sterile translocations.

The method for analysis of segmental aneuploids of *Y* chromosome regions is shown in Figure 2. Two fertile translocations with breakpoints in *Xh* and at different points in *Y^{L}* are illustrated. Males with the distal part of *Y^{L}* from the first translocation and the proximal part of

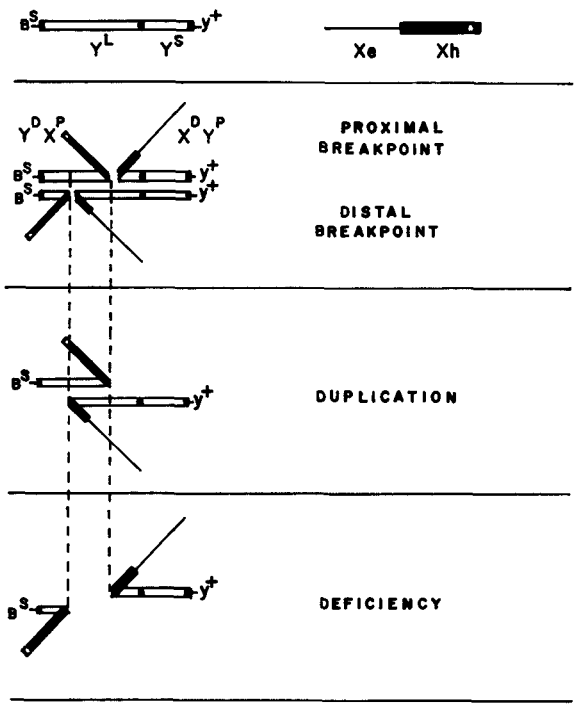

FIGURE 2.—The production of interstitial aneuploids for *Y*-chromosome regions by combining elements of *X-Y* translocations having their *X*-chromosome breakpoint in the centric heterochromatin. Two such *X-Y* translocations with different *Y* chromosome breakpoints are illustrated. The vertical broken lines indicate the homologous positions of both *Y*-chromosome breakpoints.

Y^L from the second translocation carry a duplication for the Y chromosome region between the two breakpoints. Males of the reciprocal class are deficient for the same Y chromosome region. If the segment of the Y chromosome between the two breakpoints is necessary for male fertility, then the deficient males are sterile; if not, they are fertile. In either case, males with the duplication are fertile. In practice, since it is not known which is the deficiency-bearing class and which is the duplication-bearing class, both classes of males are generated for each pair of translocations. If both classes are fertile, there is no Y chromosome material between the two breakpoints that is necessary for male fertility and the breaks are defined to be in the same genetic region of the Y. If one class of males is fertile and the reciprocal class sterile, the sterile class is assumed to be deficient for a Y chromosome region necessary for male fertility. The translocation supplying the proximal part of the broken Y arm to the sterile males has a more proximal Y-chromosome breakpoint than the translocation supplying the distal part of the broken Y arm. The two breakpoints define a region containing at least one factor that is necessary for male fertility, referred to here as a Y-chromosome fertility region. In no case were both reciprocal classes of males sterile.

There are two major assumptions in the technique of segmental aneuploidy employed. The first is that the concommitant duplications and deficiencies for the centric X heterochromatin do not sterilize males. Males with duplications for all of the X heterochromatin are fertile (GERSHENSON 1940; LINDSLEY and SANDLER 1958), as are males deficient for at least 80% of the X centric heterochromatin ($In(1)sc^{4L}sc^{8R}/Y$); thus, sterility resulting from X-chromosome aneuploidy is expected to be rare. The second assumption is that all duplications for Y-chromosome material are male fertile. Since males carrying two doses of the B^SYy^+ chromosome, which was used to generate the translocations, are fertile, all segmental duplications are expected to be fertile.

The real power of the technique of segmental aneuploidy used here lies in its ability to generate male-sterile deficiencies using fertile translocations that themselves lack nothing required for male fertility. The translocations are kept in the absence of any other Y-chromosome material and, thus, are under constant selection against loss or mutation of a Y-chromosome fertility region. This is not true of mutant Y chromosomes or Y-chromosome fragments that are balanced with an entire extra Y chromosome or arm, where loss or mutation at a second site on the Y chromosome could occur and persist undetected.

Complementation tests were performed as follows: X^DY^P/w^+Y2 males from one translocation were crossed to $C(1)A$, y/Y^DX^P females from a second translocation. The parents were removed after six days and the newly emerged progeny transferred to fresh food at 12 and 15 days. Each test vial, containing at least 10 to 20 males and an approximately equal number of their sisters, was scored for the presence or absence of larvae for up to two weeks. Males from a sample of vials were also tested individually with similar results. Males were scored as fertile only if both test vials contained several larvae. Except for several quasi-sterile translocations discussed later, sterile tests produced no larvae over a period of two weeks from a total of 20 or more males in both test vials. Occasionally, one of two test vials would be fertile. In each case, the fertile vial contained one w^+ male resulting from nondisjunction in the parental male. When the remaining w males were retested, they were always sterile.

In Series B, the Y chromosome utilized had been maintained in $X/X/Y$ females for several generations prior to irradiation. Upon analysis it was found that a deletion in Y^S, which had arisen spontaneously in the stock, was present in many translocations. Those translocations broken in Y^L that also carried a spontaneous or independently induced sterile in Y^S were analyzed genetically in the presence of an extra copy of Y^S, either from *Fragment-1* (Y^SX) appended terminally to the X^DY^P element by recombination or from the Y^DX^P element of translocation W-19, which is broken proximal to *ks-1* in Y^S. As a control, several of the fertile translocations broken in Y^L were also retested by complementation crosses, in both the presence and absence of an extra copy of Y^S, with no detectable difference. The sterile translocations broken in Y^S from Series B were not analyzed further. In Series C, the treated Y chromosome was kept under selection for fertility until the last generation before irradiation to avoid the propagation of spontaneous Y steriles.

Mitotic prophases of a selected sample of translocations were examined, using either Hoechst 33258 or quinacrine fluorescence staining by the procedures of GATTI, PIMPINELLI and SANTINI (1976) but omitting the incubation with colchicine to avoid excessive contraction.

RESULTS

The results of the screen for *X-Y* translocations are given in Table 1. Only *X-Y* translocations that are male fertile in the absence of an extra *Y* chromosome were selected in Series A. The translocations selected in Series B and C were male fertile in the presence of an extra *Y* chromosome, but could be either fertile or sterile in its absence.

The ratio of translocations broken in the *X* euchromatin to those broken in the *X* heterochromatin as deduced from survival of the $X^{D}Y^{P}/Y$ aneuploid males is 112:154. This is in agreement with the results of NICOLETTI and LINDSLEY (1960) on *X-Y* translocations recovered from daughters of irradiated males. They found that the frequencies of breakpoints in *X* euchromatin and heterochromatin were approximately equal for translocations involving Y^{S} (since Y^{S} was unmarked in their experiments, they were unable to detect translocations involving Y^{L} and the *X* heterochromatin in their screen), but that one quarter of all the euchromatic *X*-chromosome breakpoints were male lethal. The translocations presented here were recovered in males and thus exclude all male-lethal translocations. The ratio of Y^{L} to Y^{S} breakpoints is 97:57 (1.7:1). The metaphase length of the long arm has been estimated to be 1.5 to 2 times that of the short arm (COOPER 1959); therefore, translocation involvement of the two arms of the *Y* chromosome appears to be proportional to metaphase length. The fertility of the *X-Y* translocations in the absence of an extra *Y* chromosome is shown in Table 2. Approximately 67% of the *T(Xe;Y)*'s and 63% of the *T(Y;Ae)*'s are male fertile, while 54% of the *T(Xh;Y)*'s are male fertile; these values are not significantly different by a chi-square test for homogeneity. However, previous observations also indicated that *Y* translocations involving the euchromatic regions of both the *X* chromosome (NICOLETTI, unpublished) and chromosomes *2* and *3* (LINDSLEY *et al.* 1972) are more often fertile than those involving the heterochromatic regions.

TABLE 1

Recovery of T(X;Y)*'s by the methods outlined in Figure 1*

Series Genotype of F_1 male	A X^{*}/Y^{*}	B $X^{*}/Y^{*}/Y$	C $X^{*}/Y^{*}/Y$	Total
F_1 males tested	4948	5072	3245	13,265
F_1 males fertile	3829	3765	2391	9,985
T(Xe;Y)	18	48	46	112
T(Xh;Y)	19	83	52	154
$T(Xh;Y^{S})$	4	31	22	57
$T(Xh;Y^{L})$	15	52	30	97

X-chromosome breakpoints are designated *Xh* if both aneuploid segregants survive and *Xe* if at least one does not. Asterisks (*) designate the treated chromosomes.

TABLE 2

Male fertility of various classes of translocations involving the Y *chromosome*

Aberration	# Recovered	# Tested	# Fertile	% Fertile
T(Xe;Y)	46	39	26	67
T(Xh;Y)	104	95	51	54
T(Xh;Y^S)	22	22	11	50
T(Xh;Y^L)	82	73	40	55
*T(Y;Ae)**	27	27	17	63

* Aneuploid segregants with no *X*-chromosome linkage.
For *T(Xh;Y^L)*, translocations from both Series B and C are included. For all other classes, only translocations from Series C are included.

Table 3 shows the results of fertility tests of males produced by combining complementary elements of 22 of the 23 fertile *T(Xh;Y^S)*'s in all possible pairwise combinations. The remaining fertile translocation was broken in *XR* and had to be analyzed separately. It was broken in *Y^S* distal to all of the genetic material necessary for male fertility. That the breakpoints are indeed in *XR* and distal in *Y^S* was confirmed by Hoechst 33258 fluorescence cytology. Deficiencies generated by crosses between different pairs of fertile translocations broken in *Y^S* reveal two regions that are necessary for male fertility. The more proximal is termed *ks-1* and the more distal *ks-2* (after BROSSEAU 1960). Only one translocation breakpoint (V-8) separates the two regions of *Y^S* necessary for male fertility. Fifty-seven percent (13/23) of all fertile translocations in *Y^S* were broken distal to all of the *Y* chromosome necessary for male fertility.

The sterility of all 11 sterile translocations broken in *Y^S* was mapped by crossing each to several diagnostic fertile translocations. Results of these diagnostic crosses and complete complementation tests between all possible pairs of reciprocal elements from 10 of these sterile translocations are shown in Table 4. One sterile translocation in *ks-1* was lost subsequent to initial testing. The sterile translocations fall into two groups: one group of nine translocations appears to lack *ks-1*; the other group of two translocations appears to lack *ks-2*. Complete complementation was seen among sterile translocations in different groups, while complementation was never found among sterile translocations within the same group.

Table 5 gives the results of complementation tests of 44/57 translocations with male-fertile breakpoints in *Y^L*. These translocations define four regions in *Y^L* necessary for male fertility that correspond to *kl-1*, *kl-2*, *kl-3* and *kl-5* as defined by BROSSEAU (1960) and are so designated; the existence of BROSSEAU's *kl-4* is uncertain (see DISCUSSION). The remaining 13 fertile translocations were broken distal to all of *Y^L* necessary for male fertility; three were broken to the right of the *X*-chromosome centromere and 10 to the left. Fifty-one percent (29/57) of all fertile translocations in *Y^L* were broken distal to all of the *Y* chromosome necessary for male fertility.

TABLE 3

Complementation analysis between fertile T(Xh;Y^S)

$X^D\|Y^P$	$Y^D\|X^P$	y^+ ks-2 ks-1 $bb^+\|bb^+\cdot$		y^+ ks-2 ks-1 $bb^+\|\cdot$ (or) y^+ ks-2 ks-1 $\|bb^+\cdot$						y^+ ks-2 ks-1$\|\cdot$	y^+ ks-2$\|bb^+\cdot$	$y^+\|bb^+\cdot$			$y^+\|\cdot$									
		N-13	N-14	G-1	G-29	L-1	N-1	V-32	W-29	W-19	V-8	N-12	R-12	T-20	G-7	N-10	N-30	P-1	V-4	V-23	V-25	V-36	Z-14	O
	y w f	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
$X\|\cdot KL$	N-13	F	F	F	F	F	F	F	F	-	S	S	S	S	-	-	-	-	-	-	-	-	-	-
	V-14	F	F	F	F	F	F	F	F	-	S	S	S	S	-	-	-	-	-	-	-	-	-	-
$X\ bb^+\|\cdot KL$ or $X\|bb^+\cdot KL$	G-1	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	G-29	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	L-1	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	N-1	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	V-32	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	W-29	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S
$X\ bb^+\|bb^+\cdot KL$	W-19	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S
$X\|$ks-1 $bb^+\cdot KL$	V-8	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S
$X\|$ks-2 ks-1 $bb^+\cdot KL$	N-12	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	R-12	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	T-20	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
$X\ bb^+\|$ks-2 ks-1 $bb^+\cdot KL$	G-7	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	N-10	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	N-30	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	P-1	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	V-4	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	V-23	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	V-25	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	V-36	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	Z-14	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
C(1)DX, y w f bb⁻		+	+	+	+	bb	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-

F and S refer to male fertility and sterility, respectively. + indicates that the tested genotype survives (and therefore carries *bb*+), — that the genotype does not survive (and is *bb*-deficient), and *bb* that the tested genotype is *bb* in phenotype. *KL* refers to the entire fertility complex of Y^L. The left-most column and the top row indicate the genetic contents of the X^DY^P and Y^DX^P translocated elements, respectively, as inferred from the complementation data. A vertical line indicates the translocation breakpoint.

Thirty sterile translocations broken in Y^L were crossed to several diagnostic fertile translocations in order to map the sterility. Twenty-two were sterile at only one fertility region, while eight behaved as deficiencies for two or more adjacent fertility regions. These results are shown in Figure 3. Results of the diagnostic crosses and complementation tests among 21 of the steriles that do not

TABLE 4

Complementation analysis between male-sterile T(Xh;Y^S) *and diagnostic crosses with selected male-fertile* T(Xh;Y^S)

$X^D\|Y^P$	$Y^D\|X^P$	*V-14*	*W-19*	T-10	W-9	Z-3	T-21	V-33	V-54	W-1	Z-13	*V-8*	T-17	T-6	*O*
		y+ ks-2 ks-1 bb+|bb+·	y+ ks-2 ks-1|·	y+ *ks-2*|*bb*+·			y+ *ks-2*|·					y + ks-2|bb+·	y+|*bb*+·	y+|·	
X|·KL	*V-14*	*F*	—	*S*	*S*	*S*	—	—	—	—	—	*S*	*S*	—	—
X bb+|bb+·KL	*W-19*	*F*	*F*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
	T-10	*F*	*F*	S	S	S	S	S	S	S	S	*S*	S	S	S
X|bb+·KL	W-9	*F*	*F*	S	S	S	S	S	S	S	S	*S*	S	S	S
	Z-3	*F*	*F*	S	S	S	S	S	S	S	S	*S*	S	S	S
	T-21	*F*	*F*	S	S	S	S	S	S	S	S	*S*	S	S	S
	V-33	*F*	*F*	S	S	S	S	S	S	S	S	*S*	S	S	S
X bb+|bb+·KL	V-54	*F*	*F*	S	S	S	S	S	S	S	S	*S*	S	S	S
	W-1	*F*	*F*	S	S	S	S	S	S	S	S	*S*	S	S	S
	Z-13	*F*	*F*	S	S	S	S	S	S	S	S	*S*	S	S	S
X|ks-1 bb+·KL	*V-8*	*F*	*F*	*F*	*F*	*F*	*F*	*F*	*F*	*F*	*F*	*F*	*S*	*S*	*S*
X|ks-1 bb+·KL	T-17	*F*	*F*	F	F	F	F	F	F	F	F	*F*	S	S	S
X bb+|ks-1 bb+·KL	T-6	*F*	*F*	F	F	F	F	F	F	F	F	*F*	S	S	S
C(1)DX, y w f bb$^-$		+	—	*bb*	+	*bb*	—	—	—	—	—	+	+	—	—

Crosses involving the selected male-fertile translocations are indicated in *italics*. The left hand column and the top row list only the nonmutant fertility loci. All other symbols are explained in the Table 3 footnote.

behave as deficiencies are shown in Table 6. All possible combinations of sterile translocations within the same region, as defined by the diagnostic crosses, were tested, but combinations of sterile translocations in different regions were only sampled. Complementation was seen between all pairs of sterile translocations with breakpoints in different regions, but not between pairs with breakpoints in the same region, implying that all sterile translocations within a region are defective for the same genetic locus.

In several complementation tests involving sterile translocations with breakpoints in *kl-3* and *kl-5*, a few progeny were obtained from matings expected to be completely sterile. This slight fertility was observed only in crosses involving sterile translocations and was never observed among the fertility tests of over 1800 males with segmental deficiencies for *kl-3* or *kl-5* generated from fertile translocations. Retests of males with the original quasisterile translocations gave the same low level of fertility. This fertility was on the order of one adult progeny

TABLE 5

Complementation analysis between fertile T(Xh;Y^L)'s

X^DY^P	Y^DX^P	B^S *kl-5 kl-3 kl-2 kl-1*|·							B^S *kl-5 kl-3 kl-2*|·															B^S *kl-5 kl-3*|·	B^S *kl-5*|·					B^S|·																
		F-15	F-14	F-12	W-3	W-28	W-33	R-43	G-25	V-63	E-15	G-8	S-13	Z-2	E-1	V-43	P-7	J-2	N-29	S-7	V-17	S-28	V-31	W-27	V-47	S-38	R-17	S-32	V-24	G-24	V-27	W-2	W-30	G-15	G-22	E-5	G-30	K-1	N-16	P-9	E-12	E-17	F-6	W-8	V-13	0
X	*y w f*	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	F-15	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	F-14	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	F-12	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
X|·*KS*	W-3	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	W-28	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	W-33	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	R-43	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	G-25	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	V-63	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	E-15	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	G-8	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	S-13	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	Z-2	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	E-1	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
X|*kl-1*·*KS*	V-43	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	P-7	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	J-2	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	N-29	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	S-7	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	V-17	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	S-28	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	V-31	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
X|*kl-2 kl-1*·*KS*	W-27	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	V-47	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	S-38	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
X|*kl-3 kl-2 kl-1*·*KS*	R-17	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	S-32	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	V-24	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	G-24	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	V-27	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	W-2	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	W-30	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	G-15	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	G-22	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	E-5	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	G-30	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
X|*KL*·*KS*	K-1	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	N-16	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	P-9	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	E-12	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	E-17	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	F-6	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	W-8	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
	V-13	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
C(1)DX, y w f bb$^-$		bb	+	bb	-	-	-	-	+	+	+	+	+	+	bb	bb	-	-	-	-	-	-	-	-	+	bb	-	-	-	+	+	+	+	+	bb	bb	bb	bb	bb	bb	bb	-	-	-	-	-

KS refers to the entire fertility complex of Y^S. All other symbols are explained in the Table 3 footnote.

per forty males tested and was observed only if newly emerged males were allowed to mate with their sisters as soon as they were sexually mature. It could be eliminated completely by either etherizing or aging the males. Addition of an extra Y^S to the genome enhanced the fertility. Fertility was also greater in flies reared at 25° than in flies reared at 18°. We have interpreted this slight fertility as the result of a variegated position effect on the Y-chromosome fertility regions

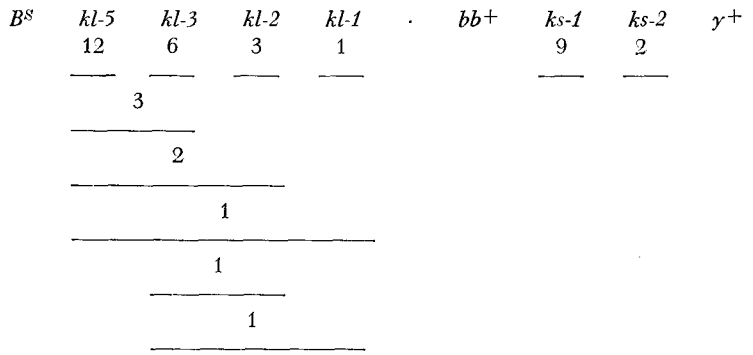

FIGURE 3.—The top line represents the genetic map of B^SYy+. Below it, the horizontal lines indicate the Y-chromosome functions absent in the various sterile X-Y translocations. The figure above each line is the number of sterile translocations of that type.

from a translocation breakpoint near, but not within, the region. There does not appear to be any correlation with the position of the breakpoint in the X heterochromatin. This slight fertility was observed only in the two most distal of four regions in Y^L (4/6 translocations involving *kl-3* and 8/11 translocations involving *kl-5*). BROSSEAU (1960) also encountered slight fertility only in males bearing X-ray-induced Y-chromosome mutations in the three most distal of five loci in Y^L (*i.e.*, *kl-3*, *kl-4*, and *kl-5*). Of recently isolated gamma-ray-induced and EMS-induced mutant Y chromosomes (unpublished data), only males bearing those defective in *kl-3* or *kl-5* exhibit slight fertility.

In addition to the 57 fertile and 30 sterile translocations broken in Y^L already discussed, there were 10 translocations for which one element of the translocation was either not recovered or was lost before complete analysis. The genetic content of each remaining element was determined by complementation tests with known fertile translocations and the distribution of their breakpoints was consistent with that of complete translocations.

Figure 4 shows a genetic map of the Y chromosome constructed from X-Y translocation breakpoints. The length of each genetic region is proportional to the number of analyzed translocation breakpoints within that region. Only translocations from Series B and C (where both elements of the translocation were recovered) were used to construct the map of Y^L. Sterile translocations that inactivated more than one fertility region were assumed to be three-break rearrangements with a breakpoint in each of the two outermost fertility regions affected. Only translocations from Series C were used to construct the genetic map of Y^S, but the length was corrected for the unanalyzed Y^S translocations recovered in Series B.

In order to determine whether the most proximal breakpoints recovered in both Y^S and Y^L were proximal to all of the Y chromosome necessary for male fertility, males deficient for the Y centromere were generated. These males, $X/Y^{LD}X^P/Y^{SD}X^P$, had one normal X chromosome, one X centromere with the distal part of Y^L (Y^DX^P from a translocation broken in Y^L) and another X cen-

TABLE 6

Complementation analysis between male-sterile T(Xh;Y^L)*'s and diagnostic crosses with selected male-fertile* T(Xh;Y^L)*'s*

X^DY^P	Y^DX^P	B^S k1-5 k1-3 k1-2 k1-1|·	B^S *kl-5 kl-3 kl-2*|·	B^S k1-5 k1-3 k1-2|·	B^S *kl-5 kl-3*|·	B^S k1-5 k1-3|·	B^S *kl-5*|·	B^S k1-5|·	B^S|·
		F-12	Z-6	*E-15*	R-5 R-19 V-30	*W-27*	S-2 S-1 R-39 S-24 T-13 V-57	*V-24*	S-25 N-18 T-4 R-36 N-20 L-2 T-2 S-20 T-11 R-29 R-21
X|·KS	*F-12*	F	S	S	S S S	S	S S S S S S	S	S S S S S S S S S S
X|·KS	Z-6	F	S	S		S		S	
X|kl-1·KS	*E-15*	F	F	F	S S S	S	S S S S S S	S	S S S S S S S S S S
X|kl-1·KS	R-5	F		F	S S S	S		S	
	R-19	F		F	S S S	S		S	
	V-30	F		F	S S S	S	S	S	S
X|kl-2 kl-1·KS	*W-27*	F	F	F	F F F	F	S S S S S S	S	S S S S S S S S S S
X|kl-2 kl-1·KS	S-2	F		F		F	S S S S S S	S	
	S-1	F		F		F	S S S S S S	S	
	R-39	F		F		F	S S S S S S	S	
	S-24	F		F		F	S S S S S S	S	
	T-13	F		F	F	F	S S S S S S	S	S S S S
	V-57	F		F	F F F	F	S S S S S S	S	S S S S S S S S
X|kl-3 kl-2 kl-1·KS	*V-24*	F	F	F	F F F	F	F F F F F F	F	S S S S S S S S S S
X|kl-3 kl-2 kl-1·KS	S-25	F		F	F	F	F F	F	S S S S S S S S S S
	N-18	F		F		F	F F	F	S S S S S S S S S S
	T-4	F		F		F		F	S S S S S S S S S S
	R-36	F		F		F		F	S S S S S S S S S S
	N-20	F		F		F		F	S S S S S S S S S S
	L-2	F		F		F		F	S S S S S S S S S S
	T-2	F		F	F	F	F F	F	S S S S S S S S S S
	S-20	F		F	F	F	F F	F	S S S S S S S S S S
	T-11	F		F		F	F F	F	S S S S S S S S S S
	R-29	F		F		F		F	S S S S S S S S S S
	R-21	F		F		F		F	S S S S S S S S S S
C(1)DX, y w f bb-		bb	-	+	- - -	-	+ - - - - - -	-	+ +bb bb bb - - - - - -

Crosses involving the selected male fertile translocations are indicated in *italics*. The left hand column and the top row list only the nonmutant fertility loci. *KS* refers to the entire fertility complex of Y^S. All other symbols are explained in Table 3.

tromere with the distal part of Y^S (Y^DX^P from a translocation broken in Y^S). The results from several combinations of translocations are presented in Table 7. In all cases, if both translocation elements were from translocations broken proximal to all previously identified fertility regions, the males were fertile; thus, there are no additional regions necessary for male fertility in the pericentric region.

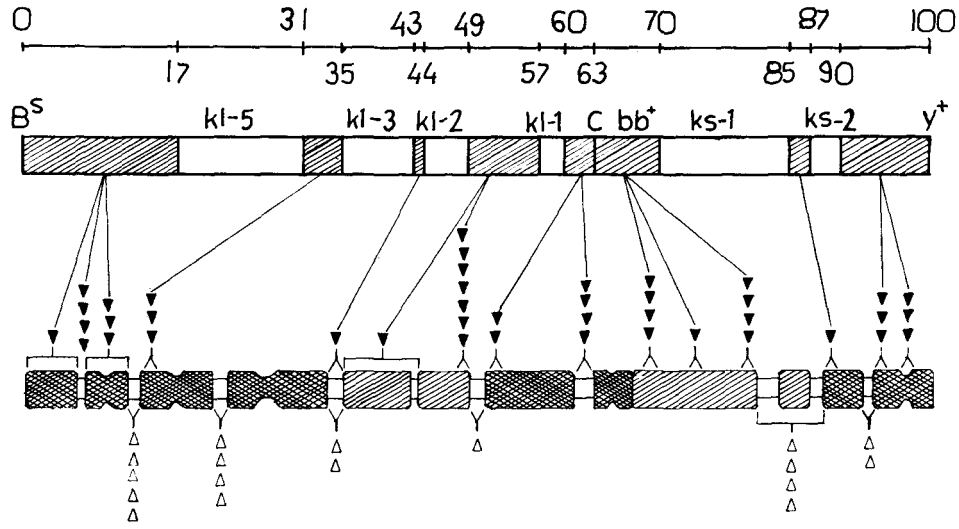

FIGURE 4.—Comparison of the genetic and cytological maps of the *Y* chromosome. The upper map is a breakage map in which the relative lengths of the different segments are proportional to the numbers of translocations with *Y*-chromosome breakpoints within them. Hatched segments are male-fertile *T(Xh;Y)*'s, and open segments are male-sterile ones. The numbers across the top estimate the percentage of breakpoints falling into the different segments. The ratio of Y^L to Y^S and the relative lengths of the segments within Y^L were estimated from the results of experiments B and C. The relative lengths of Y^S segments were estimated from experiment C alone. The positions of the genetically defined factors on the *Y*-chromosome are also indicated on the breakage map. The lower map represents the cytological appearance of the *Y*-chromosome when stained with Hoechst 33258 and observed in the fluorescence microscope (GATTI and PIMPINELLI, personal communication). Cross-hatched regions indicate highly fluorescent blocks, hatched regions indicate dully fluorescent blocks, and white regions indicate nonfluorescent blocks. The open triangles below the cytological map represent the cytological breakpoints of selected male-sterile translocations. All male-sterile *X-Y* translocations examined appear to have breakpoints either within or near the edges of nonfluorescent blocks; fertile translocations, represented by filled triangles above the cytological map, can also be shown to have breakpoints near the boundary between differentially staining blocks. The genetic and cytological maps, based on 64 analyzed cases, are completely co-linear.

Combinations with at least one of the translocations broken more distally did, as expected, sterilize males. The *Y* centromere itself does not appear to be necessary for male fertility.

Males with three *Y* chromosomes are sterile (COOPER 1956) and this has been demonstrated to be due to three copies of Y^L (WILLIAMSON and MEIDINGER 1979). The region responsible for male sterility in three doses was mapped by producing males with segmental duplications for various *Y*-chromosome regions and an extra w^+Y2 chromosome. The only region of the entire *Y* chromosome that was male sterile in three doses was the region including *kl-3* (X^DY^P, V-24/Y^DX^P, W-27/w^+Y2).

For all translocations except those broken proximal to *ks-1* in Y^S or proximal to *kl-1* in Y^L, the *X*-heterochromatin breakpoint can be inferred from the data. Of

TABLE 7

Data on fertility of males with deficiencies for the centric region of the Y *chromosome*

$Y^{LD}X^{P}$	$Y^{SD}X^{P}$	N-13	V-14	G-1	G-29	y^{+} *ks-2 ks-1*| L-1	N-1	V-32	W-29	W-19	y^{+} *ks-2*| V-8	*O*
B^{S} *kl-5 kl-3 kl-2 kl-1*|	F-12	F	F	F	F	F	F	F	F	F	S	S
	F-14	F	F	F	F	F	F	F	F	F	S	S
	F-15	F	F	F	F	F	F	F	F	F	S	S
	W-3	F	F	F	F	F	F	F	F	F	S	S
	W-28	F	F	F	F	F	F	F	F	F	S	S
	W-33	F	F	F	F	F	F	F	F	F	S	S
B^{S} *kl-5 kl-3 kl-2*|	E-1	S	S	S	S	S	S	S	S	S	S	S
	E-15	S	S	S	S	S	S	S	S	S	S	S
	O	S	S	S	S	S	S	S	S	S	S	S

F and S refer to male fertility and sterility, respectively. The left hand column and the top row indicate the genetic contents of the $Y^{LD}X^{P}$ and $Y^{SD}X^{P}$ translocated elements, respectively, as inferred from the complementation data of Tables 3 and 5. A vertical line indicates the translocation breakpoint. All males carried, in addition to the translocated elements listed, a normal sequence *X* chromosome with the markers *y w f*.

the 105 translocations with breakpoints in the centric heterochromatin of the *X* chromosome, 4 have breakpoints in *XR* and 101 have breakpoints in *XL*. Of these latter, all but one were tested for the presence of bb^{+} or *bb* in the $Y^{D}X^{P}$ element, using *C(1)DX*. Of the $Y^{D}X^{P}$ elements from these translocations, 28 are bb^{+}, 17 are *bb*, and 55 are bb^{-}.

Fluorescent staining of mitotic prophase chromosomes was used to determine the breakpoints of a sample of these *X-Y* translocations. After Hoechst 33258 or quinacrine staining, the centric *X* heterochromatin and the *Y* chromosome have characteristic appearances (HOLMQUIST 1975; GATTI, PIMPINELLI and SANTINI 1976). The cytological map of the *Y* chromosome in Figure 4 is as drawn by GATTI and PIMPINELLI (personal communication). After Hoechst 33258 staining, the centric heterochromatin of the *X* chromosome consists of a brightly fluorescent proximal region and a less brightly fluorescent distal region; consequently proximal *X*-chromosome breakpoints interfere with precise localization of *Y*-chromosome breakpoints within or at the edge of a fluorescent block. In a similar manner, distal *X*-heterochromatin breakpoints interfere with precise localization of *Y*-chromosome breakpoints near or within less brightly fluorescent regions. Thus, only a fraction of the translocations examined by Hoechst 33258 fluorescence gave precise breakpoint information. After quinacrine staining, the proximal brightly fluorescent region of the centric *X* heterochromatin is much smaller. Several translocations that were not clearly resolvable with Hoechst

33258 staining were therefore examined using quinacrine staining to localize those breakpoints more accurately.

Of the male-fertile translocations for which a precise breakpoint could be determined, all appeared to have a *Y*-chromosome breakpoint near the junction of a fluorescent and a nonfluorescent block; the fluorescent block was clearly visible on one of the translocation elements, while the nonfluorescent block was visible on the reciprocal element. In no case was a highly fluorescent block clearly divided by a translocation breakpoint. All fertile translocations with genetic breakpoints between the same two fertility regions were broken between the same two nonfluorescent blocks. In every case, fertile translocations that were broken proximal to one fertility region genetically had breakpoints proximal to a particular nonfluorescent block, while fertile translocations breakpoints distal to the region genetically were always broken distal to that nonfluorescent block.

The correspondence between the nonfluorescent blocks and the fertility regions extends to the male sterile translocations as well. All of the sterile translocations examined appeared to have *Y*-chromosome breakpoints within or near the edges of nonfluorescent blocks. All sterile translocations that failed to complement genetically had breakpoints at the same nonfluorescent block. The order of the fertility regions with respect to the centromere is the same for both the genetic map and the cytological map; the two maps are completely co-linear. A similar cytogenetic map has been constructed by GATTI and PIMPINELLI (personal communication) by examining *Y*-autosome translocations with euchromatic autosomal breakpoints.

DISCUSSION

One hundred and twenty-one reciprocal translocations between the *X* and *Y* chromosomes, 87 with breakpoints in Y^L and 34 with breakpoints in Y^S, were analyzed genetically and a selected sample examined cytologically. Fertility tests of 31,000 males with more than 2700 different segmental aneuploid genotypes are consistent with the existence of only six regions of the *Y* chromosome necessary for male fertility, two regions in Y^S and four in Y^L. These regions appear to be associated with the nonfluorescent blocks of the *Y* chromosome that appear after Hoechst 33258 fluorescence staining.

This small number of fertility regions is consistent with earlier findings. NEUHAUS (1938, 1939) used complementation tests between sterile *T(Y;4)*'s to define five complementation groups in Y^S and four groups in Y^L All of his translocations appeared to be broken distally in the *Y* chromosome when examined in oogonial metaphases. BROSSEAU (1960) used complementation between different X-ray-induced sterile *Y* chromosomes to define five fertility factors in Y^L(*kl-1* being most proximal and *kl-5* most distal) and two in Y^S (*ks-1* being proximal to *ks-2*, but probably distal to bb^+). One of the distal factors in Y^L, *kl-4*, was defined only by the failure of one sterile chromosome in the *kl-3* deficient group to complement one sterile chromosome in the *kl-5* deficient group. As a consequence, these *Y* chromosomes were designated $kl\text{-}3^-\text{-}4^-$ and $kl\text{-}4^-\text{-}5^-$, respectively.

That these two *kl-4*-deficient chromosomes of BROSSEAU failed to complement for some reason other than an overlapping deficiency is suggested by data from detachments of compound-*X* chromosomes involving the *Y* chromosome that were induced by irradiating oocytes (BROSSEAU 1964; LUCCHESI 1965; PARKER 1967). Induced interchanges between *kl-3* and *kl-5* should yield one pair of reciprocal products from breaks distal to *kl-4* and another pair of reciprocal products from breaks proximal to *kl-4*. Of the four types of fragments expected, only two non-reciprocal types were recovered. All 11 distal fragments that complemented *kl-5*$^-$, but not *kl-3*$^-$, also complemented *kl-4*$^-$*-5*$^-$ (*i.e.*, if they contained *kl-5*$^+$ they also contained *kl-4*$^+$); therefore, all arose from breaks between *kl-3*$^-$ and *kl-4*. The 19 proximal fragments that complemented *kl-3*$^-$, but not *kl-5*$^-$, also complemented *kl-3*$^-$*-4*$^-$ (*i.e.*, if they contained *kl-3*$^+$, they contained *kl-4*$^+$); thus, they arose from breaks between *kl-4* and *kl-5*. These results are most easily explained by the nonexistence of *kl-4*. In contrast, ANDREWS and WILLIAMSON (1975) presented evidence from complementation tests between BROSSEAU's sterile chromosomes and *Y*-chromosome fragments recovered from irradiated oocytes that *kl-4* does exist. Complementation tests between newly isolated X-ray-induced male-sterilizing *Y* chromosomes failed to resolve more than four complementation groups in Y^L (KAUFMAN, HAZELRIGG and FORNILI, personal communictaion).

When the translocations are examined in mitotic prophase with Hoechst 33258 fluorescence staining, the distribution of the *Y*-chromosome breakpoints of the cytologically tractable translocations appears to be nonrandom at two levels. The first and most striking departure from a nonrandom distribution is found among the precise breakpoints localized. Most, if not all, of these breakpoints appear to be within nonfluorescent blocks or near junctions between differentially staining regions. When the genetic and cytological data are combined, it appears that there are also more breakpoints in the distal and proximal portions of each arm than expected from random recovery. More than half of all the male-fertile translocations analyzed were broken distal to all of the *Y* material necessary for male fertility. Half-translocations recovered from irradiated oocytes show similar concentrations of breakpoints distal and proximal in each arm (BROSSEAU 1964; LUCCHESI 1965; PARKER 1967; ANDREWS and WILLIAMSON 1975). One possible cause for the nonrandom distribution of *Y* chromosome breakpoints is nonrandom induction of translocations, either from differential sensitivity to breakage of different regions or from differential rejoining. Another possibility is biased recovery of translocations from loss of a large class of dominant lethal or sterile aberrations. It could also reflect preferential involvement of regions of homology between the *X* heterochromatin and the *Y* chromosome; however, a nonrandom distribution of *Y* chromosome breakpoints was also observed for translocations between the *Y* chromosome and the euchromatic regions of chromosomes *2* and *3* (GATTI and PIMPINELLI, personal communication). Whatever the cause of the nonrandom distribution of the *Y*-chromosome breakpoints, the regions that are involved in translocations must be more sensitive to rearrangement than the average chromosome region in the genome, since the total frequency of translocations involving the *Y*, second, third and fourth chromosomes is roughly propor-

tional to their metaphase lengths (KIRSANOV 1946; LINDSLEY *et al.* 1972). This type of differential sensitivity for rearrangement is reminiscent of the block hypothesis first suggested for the proximal heterochromatin of the *X* chromosome (MULLER and GERSHENSON 1935; MULLER *et al.* 1937).

Little is known about the genetic content or function of individual fertility regions. Whereas we have found no evidence of complementation among sterile translocation breakpoints within the same region, WILLIAMSON (1972) reported extensive complementation among EMS-induced sterile mutations within single regions (as defined by BROSSEAU's sterile chromosomes), which he has interpreted as interallelic complementation. Because of the high frequency of breaks induced in heterochromatin by EMS (WILLIAMSON 1970, 1972; OLSON and LIM 1976), the nature of the EMS-induced mutations is uncertain; however, WILLIAMSON's results suggest that the genetic organization within single fertility regions may be complex. Similar complex patterns of complementation were also found for EMS-induced lethal mutations in the centric heterochromatin of chromosome *2* (HILLIKER 1976).

In conclusion, whereas the *Y* chromosome of *Drosophila melanogaster* contains one-tenth of the chromatin of a normal diploid male, it contains very few of the many genes required for male fertility. These few genes appear to be distributed along the *Y* chromosome in highly breakable regions that are interspersed between large blocks of Hoechst 33258 staining material that do not participate in *X-Y* translocations. The function of this unusual genetic organization and its relation to regulation during spermatogenesis is unknown.

I am grateful to D. L. LINDSLEY for his support and guidance during the course of this work; to M. GATTI and S. PIMPINELLI for their invaluable instruction and advice on the fluorescence cytology and for sharing their unpublished data; and to them, R. HARDY and T. KAUFMAN for discussions. I wish also to thank D. L. LINDSLEY and A. T. C. CARPENTER for their many helpful comments on the manuscript and S. ROKOP for technical assistance.

This work was supported by a predoctoral traineeship from the Public Health Service 5T32 GM07199 and a contract from the Dept. of Energy (DE-AS03-76SF00034, Project Agreement no. DE AT03-76EVT0150).

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