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# Cytological and genetic analysis of the Y chromosome of *Drosophila melanogaster*

## I. Organization of the fertility factors

Maurizio Gatti and Sergio Pimpinelli
Dipartimento di Genetica e Biologia Molecolare, Città Universitaria, 00185 Roma, Italy

**Abstract.** By applying quinacrine-, Hoechst- and N-banding techniques to neuroblast prometaphase chromosomes the *Y* chromosome of *Drosophila melanogaster* can be differentiated into 25 regions defined by the degree of fluorescence, the stainability after N-banding and the presence of constrictions. Thus these banding techniques provide an array of cytological landmarks along the *Y* chromosome that makes it comparable to a polytene chromosome for cytogenetic analysis. – 206 *Y*-autosome translocations (half of them carrying *Y*-linked sterile mutations) and 24 sterile $y^+ Y$ chromosomes were carefully characterized by these banding techniques and used in extensive complementation analyses. The results of these experiments showed that: (1) there are four linearly ordered fertility factors in $Y^L$ and two fertility factors in $Y^S$. (2) These fertility factors map to characteristic regions of the *Y* chromosome, specifically stained with the N-banding procedure. (3) The most extensively analyzed fertility factors are defined by a series of cytologically nonoverlapping and genetically noncomplementing breaks and deficiencies distributed over large chromosome regions. For example, the breakpoints which inactivate the *kl-5* and *ks-1* loci are scattered along regions that contain about 3,000 kilobases (kb) DNA. Since these enormous regions formally define single genetic functions, the fertility genes of the *Y* chromosome have an as yet unappreciated physical dimension, being larger than euchromatic genes by two orders of magnitude.

### Introduction

The *Y* chromosome of *Drosophila melanogaster* provides an excellent experimental model for studies on the structure and function of heterochromatin. It is completely heterochromatic and has cytological and molecular characteristics that are common to the heterochromatic material of almost all higher eukaryotes. In addition, a series of studies carried out since the early days of *Drosophila* genetics associate this chromosome with a limited but well-defined set of genetic information. This, in conjunction with the fact that the *Y* chromosome is amenable to genetic manipulation, makes it an especially favorable system for asking basic questions about the organization and expression of heterochromatic loci.

The *Y* chromosome of *D. melanogaster* is a submetacentric element which accounts for about 12% of the male genome (Pimpinelli et al. 1978). It is entirely heterochromatic showing positive heteropycnosis at prometaphase and close chromatid apposition at metaphase (Heitz 1933). It appears darkly stained after the C-banding technique which specifically identifies constitutive heterochromatin (Hsu 1971; Pimpinelli et al. 1976), and it is late-replicating as compared to euchromatin (Barigozzi et al. 1966). Four highly repeated, simple-sequence satellite DNAs have been localized in the *Y* chromosome which together account for about 70% of its length (Peacock et al. 1978; Appels and Peacock 1978; Steffensen et al. 1981; Hilliker and Appels 1982). Moreover, it has been suggested that these satellite DNAs are organized in heterochromatic blocks containing long and homogeneous arrays of short sequences, tandemly repeated (Brutlag et al. 1977; Appels and Peacock 1978).

Genetic analysis of the *Y* chromosome has revealed that it carries four sets of functions. It contains the bobbed (*bb*) locus in the short arm which is allelic to the *bb* locus located within the *X* heterochromatin (reviewed by Ritossa 1976). These loci, at which both viable and lethal alleles are known, are the repetitive structural genes for the 18S and 28S ribosomal RNA molecules (Ritossa 1976).

The second set of genetic functions associated with the *Y* chromosome are the fertility factors. These loci are located in both arms of the *Y* chromosome and are essential for male fertility (Bridges 1916; Stern 1929). By complementation analysis between X-ray-induced sterile *Y* chromosomes Brosseau (1960) established a linear order of the fertility factors with five loci in the long arm ($Y^L$) and two in the short arm ($Y^S$). Extensive evidence is available that these loci function in primary spermatocytes and are necessary for spermatid differentiation (reviewed by Williamson 1976; Lindsley and Tokuyasu 1980; see also Marsh and Wieschaus 1978). Recent studies using both the light and electron microscope showed that deletions of different fertility factors produce specific lesions in early spermatogenesis that eventually lead to the complete abortion of the spermatogenic process (Hardy et al. 1981).

The third function carried by the *Y* chromosome is meiotic pairing which is attributable to pairing sites or collochores (Cooper 1964). These are discrete regions localized in the heterochromatin of both the *X* and *Y* chromosomes that are required for the regular pairing and co-orientation of sex chromosomes in spermatocytes, and therefore for their correct meiotic segregation. Cooper (1964) was able to determine that the distal half of $Y^L$ and the distal third of $Y^S$ do not contain collochores.

The fourth function associated with the *Y* chromosome has been inferred from the cytological analysis of meiotic cells in *XO* males. The primary spermatocytes of these males exhibit needle-shaped crystals of a proteinaceous nature (Meyer et al. 1961) and abnormal organelle and chromosome distribution (Lifschytz and Hareven 1977; Lifschytz and Meyer 1977). Recently Hardy and Kennison (1980), Hardy et al. (1981) and Hardy (personal communication) showed that both these phenotypes can be evoked by the same male fertile proximal deficiency of $Y^L$. Although it is not known whether these apparently unrelated defects are pleiotropic effect of the deficiency of a single genetic function, it is clear that the *Y* chromosome carries one or more loci necessary for normal male meiosis.

In addition to its own functions, the *Y* chromosome of *D. melanogaster* also has the interesting property of modifying the expression of position effect variegation (reviewed by Spofford 1976). In most instances, the presence of an extra *Y* chromosome suppresses variegation, and different regions of the *Y* chromosome appear to have different abilities to suppress variegation (Baker and Spofford 1959).

The genetic and cytogenetic analyses of the functions associated with the *Y* chromosome have proven to be extremely difficult. Since the *Y* chromosome does not undergo meiotic recombination even when placed in females, classical genetic mapping of *Y* chromosome loci as well as analysis of their fine structure were precluded. Moreover, the fact that the *Y* chromosome is included in the chromocentre of polytene chromosomes prevented its cytogenetic dissection by salivary banding analysis.

The cytological examination of aceto-orcein stained neuroblast prophases showed that the *Y* chromosome is segmented into a number of heteropycnotic blocks separated by secondary constrictions (Cooper 1959). More recently by a series of banding techniques such as quinacrine and Hoechst 33258 staining or the N-banding procedure a more refined and consistent pattern of longitudinal differentiation was obtained (Holmquist 1975; Gatti et al. 1976; Pimpinelli et al. 1976). By applying these three banding techniques to neuroblast prometaphase chromosomes we can now differentiate the *Y* chromosome into 25 regions, defined by the degree of fluorescence after Hoechst or quinacrine staining, by the stainability after the N-banding procedure, and by the presence of constrictions. Thus, these banding techniques provide a series of cytological landmarks along the *Y* chromosome that make it comparable to a polytene chromosome and amenable to cytogenetic analysis. Moreover, the fact that the heterochromatic blocks visualized by chromosome banding have different cytochemical features permits correlation of the cytological characteristic of a given heterochromatic region with its molecular and functional properties. Our work is aimed at associating the genetic functions carried by the *Y* chromosome with the cytological entities defined by the banding techniques. We believe that these studies can provide insight not only into the genetic organization of the *Y* chromosome heterochromatic loci, but also into the biological function of the satellite DNAs contained in this chromosome.

The present paper is focused on the cytological and genetic analysis of the fertility factors. In this respect we have asked four basic questions: (1) How many fertility factors are contained in the *Y* chromosome? (2) Where do they map? (3) Do they correspond to specific cytological entities? (4) What are their physical dimensions?

## Material and methods

*Stocks.* The isolation and characterization of the *Y*-autosome translocations used here is described in detail by Lindsley et al. (1972). These translocations were generated using a $B^S Yy^+$ and are maintained as *attached-X/T(Y;A)/Balancer* ♀♀ × *attached-XY/T(Y;A)/Balancer* ♂♂. The stocks are *C(1)RM*, $y/Y^S X \cdot Y^L$, *In(1)EN*, $y/T(Y;2)/In(2L+2R)Cy$, *Cy* $cn^2$ (or *In(2LR)SM1*, $al^2$ *Cy* $cn^2$ $sp^2$) for the *T(Y;2)*s and *C(1)M3*, $y^2$ *bb*/$Y^S X \cdot Y^L$, *In(1)EN*, $y/T(Y;3)/In(3LR)TM6$, $ss^- bx^{34e}$ $Ubx^{67b}$ *e* for the *T(Y;3)*s.

All the translocations used in this work were reexamined for male fertility in the absence of a normal *Y* chromosome. $\overline{XY}$, $y/T(Y;A)/Bal$ males were crossed to *y w f* females and their *y w f*/*T(Y;A)*/+ sons were tested for fertility. In some cases the results of these fertility tests differed from those originally reported by Lindsley et al. (1972). Other inconsistencies between our data and those of Lindsley et al. (1972) concern the localization in $Y^L$ or $Y^S$ of some translocation breakpoints. A more detailed account of these discrepancies will be given elsewhere.

The sterile $y^+ Ys$ ($= Y^{st}$) (Brosseau 1960; Schwartz, unpublished) with mutations in $Y^L$ are maintained in stocks as *attached-X*/$Y^{st}$/*attached-*$XY^L$; those with mutations in $Y^S$, as *attached-X*/$Y^{st}$/*attached-*$XY^S$; and those with mutations on both arms, as *attached-X*/$Y^{st}$/*attached-XY*. The type of *attached-X* used to balance Brosseau's *Ys* is unknown, whereas in all the other stocks it is *C(1)A, y*. In all cases the *attached-*$XY^L$ is $X \cdot Y^L$, *y v* and the *attached-*$XY^S$ is $X \cdot Y^S$, *y w*, the *attached-XY* is $Y^S X \cdot Y^L$, *In(1)EN, y B*.

With the exception of the *T(Y;A)*s and the sterile $y^+ Ys$ reported above, some *X-Y* translocations and $w^+ Y2$ (a *Y* chromosome carrying the normal allele for white) both described by Kennison (1981), and the *FM7* balancer chromosome (Merriam 1968), all the mutations, chromosome rearrangements and special chromosomes used in this work are described in detail by Lindsley and Grell (1968). To designate the fertility factors we used the terminology introduced by Brosseau (1960). The fertility genes of $Y^L$ are collectively symbolized by *KL* and those of $Y^S$ by *KS*. Individual fertility loci are designated by lower case letters with a numeral added (e.g., *kl-1, kl-2*..., *ks-1, ks-2*...).

Stocks were grown at 25° ± 1° C, and both cytological and genetic experiments were performed at this temperature.

*Cytology.* Neural ganglia of third-instar larvae were dissected in saline (0.7% NaCl) and immediately transferred to a hypotonic solution (0.5% sodium citrate) for 10 min at room temperature. The ganglia were then fixed for 10–20 s in a fresh mixture of acetic acid, methanol and distilled water (11:11:2) and squashed in 45% acetic acid under a siliconized coverslip. After freezing on dry ice the coverslip was removed with a razor blade and the slides were air dried at room temperature.

Hoechst 33258 and quinacrine staining, N-banding, sequential staining with quinacrine and Hoechst 33258, and sequential staining with Hoechst 33258 and Giemsa were performed as described previously (Gatti et al. 1976; Pimpinelli et al. 1976; Bonaccorsi et al. 1981). Both Hoechst and quinacrine banding were examined under a Zeiss fluorescence microscope equipped with a 200 W mercury light source for incident illumination. Two different combinations of dichroic mirrors and Zeiss filters were used for

observation. Hoechst fluorescence was observed using UG5+BG3 excitation filters, FT460 dichroic mirror and LP475 barrier filter (ultraviolet-violet excitation range). For quinacrine fluorescence 2 X=by BG12 excitation filters FT510 dichroic mirror and LP515 barrier filter (violet-blue excitation range) were used. All microphotographs were taken with Kodak Pan X film.

To compare different kinds of staining sequentially performed on the same chromosomes, microphotographs taken after each step were printed both on normal paper and on the transparent film Kodalith ortho film, type 3. Transparent prints were then overlaid on the normal ones and the different stainings exactly compared.

*Nomenclature for heterochromatic regions.* By applying the above banding techniques the *Y* chromosome of *D. melanogaster* was subdivided into 25 regions. These banding procedures also showed that the *X* and autosomal heterochromatin can be differentiated into several cytological entities. We can now distinguish more than 50 heterochromatic regions in the *D. melanogaster* genome (unpublished). We propose that these regions be numbered sequentially starting from $Y^L$ and continuing with $Y^S$, *XL*, *XR*, *2L*, *2R*, *3L*, *3R* and *4*. To avoid confusion with polytene bands we also propose that the numbers that designate heterochromatic regions be preceded by a lower case h. Any further subdivision of these heterochromatic areas will be indicated by capital letters following the region number.

*Complementation analysis.* Several types of complementation tests were performed. Males carrying different pairwise combinations of sterile rearrangements involving the *Y* chromosome were constructed and tested for fertility.

a) Complementation tests between $T(Y;A)$s: $\overline{XX}$, $y/T(Y;A)_i/Bal$ ♀♀ were crossed to $y\ w\ f/w^+\ Y2/T(Y;A)_j/+$ ♂♂ and their $y\ w\ f/T(Y;A)_i/T(Y;A)_j$ sons were tested for fertility. These males were recognizable because two doses of $B^S$ produce a smaller eye than one dose, and $y^+y^+$ produces a more extreme Hairy wing (*Hw*) effect than $y^+$ (cf. Lindsley et al. 1972).

b) Inter se complementation tests between $y^+Y$s: $y\ w\ f/y\ w\ f/y^+\ Y_i^{st}$ ♀♀ were crossed to $\overline{XY}^L$, $y\ v/y^+\ Y_j^{st}$ (or $\overline{XY}^S$, $y\ w/y^+\ Y_j^{st}$) ♂♂ and their $y\ w\ f/y^+\ Y_i^{st}/y^+\ Y_j^{st}$ sons were identified on the basis of their *Hw* phenotype and tested for fertility.

c) Complementation of the $T(Y;A)$s with $\overline{XY}^L$ and $\overline{XY}^S$: males carrying each sterile $T(Y;A)$ were crossed to both $\overline{XY}^L$, *y v* or $\overline{XY}^S$, *y w* homozygous females and their $\overline{XY}^L$, $y\ v/T(Y;A)/+$ or $\overline{XY}^S$, $y\ w/T(Y;A)/+$ sons were tested for fertility. Fertility of the translocation in combination with $\overline{XY}^L$ or $\overline{XY}^S$ indicates that the *Y* sterile mutation is in the arm of the *Y* carried by the attachment.

d) Complementation tests between $T(Y;A)$s and $y^+Y$s: $y\ w\ f/y\ w\ f/y^+\ Y^{st}$ ♀♀ were crossed with $\overline{XY}$, $y/T(Y;A)/Bal$ ♂♂ and their $y\ w\ f/y^+\ Y^{st}/T(Y;A)/+$ sons showing the *Hw* phenotype were tested for fertility.

e) Complementation tests between $T(X;Y)$s and either $T(Y;A)$s or $y^+Y$s. The two male fertile $T(X;Y)$s used in our complementation analysis, *V24* and *V8* both involve a *y w f X* chromosome and $B^S Yy^+$ (Kennison 1981). *V24* has its translocation breakpoint in $Y^L$ and *V8* in $Y^S$, and both translocations involve the *X* heterochromatin (Kennison 1981). Thus the *X* distal *Y* proximal element ($X^DY^P$) of *V24* is $y\ w\ f\cdot y^+$, while its *Y* distal *X* proximal element ($Y^DX^P$) is marked with $B^S$. In contrast $X^DY^P$ of *V8* is $y\ w\ f\cdot B^S$ and its reciprocal element carries $y^+$. Each element of these translocations was tested separately for complementation. $X^DY^PV24$, $y\ w\ f\cdot y^+/FM7$ ♀♀ were crossed to $\overline{XY}^L$, $y\ v/T(Y;3)S20$ (or $T(Y;3)S21)/+$ ♂♂ (both these $T(Y;3)$s are marked with $y^+$ and are described in detail in Results) and their $X^DY^PV24$, $y\ w\ f\cdot y^+/T(Y;3)S20$ (or $T(Y;3)S21)/+$ sons tested for fertility. $\overline{XX}$, $y/T(Y;3)$ *S20* (or $T(Y;3)S21)/+$ ♀♀ were crossed to $Y^DX^PV24$, $B^S/y\ w\ f/w^+\ Y2$ ♂♂ and their $Y^DX^PV24$, $B^S/y\ w\ f/T(Y;3)S20$ (or $T(Y;3)S21)/+$ sons tested for fertility. $X^DY^PV8$, $y\ w\ f\cdot B^S/FM7$ ♀♀ were crossed either to $\overline{XY}^S$, $y\ w/y^+\ Y^{st}$ or *XY*, $y/T(Y;A)/Bal$ ♂♂ and the $X^DY^PV8$, $y\ w\ f\cdot B^S/y^+\ Y^{st}$ and $X^DY^PV8$, $y\ w\ f\cdot B^S/T(Y;A)/+$ sons tested for fertility. Either $\overline{XX}$, $y/y^+\ Y^{st}$ or $\overline{XX}$, $y/T(Y;A)/Bal$ ♀♀ were crossed to $Y^DX^PV8$, $y^+/y\ w\ f/w^+\ Y2$ ♂♂ and the $Y^DX^PV8$, $y^+/y\ w\ f/y^+\ Y^{st}$ and $Y^DX^PV8$, $y^+/y\ w\ f/T(Y;A)/+$ sons tested for fertility.

*Fertility tests.* At least 16 males (3 to 8-days old) of each genotype were tested for fertility; 2–3 males per vial were mated to 4 *y w f* virgin females and kept at 25° C. After 8 days the parents were discarded and the vials were checked for the presence of larvae. Ten days later the same vials were checked again, and if necessary, the adult progeny were examined. The results of these fertility tests were usually unambiguous; all vials were either fertile or completely sterile. Occasionally 1–2 progeny were observed in the test vials. In these cases males of the genotype under test were constructed again and retested for fertility. These experiments showed that the few genotypes yielding occasional progeny reproducibly give 1–3 progeny per 20 males tested. The chromosomes and genetic combinations yielding such a low number of progeny were considered sterile and were always defective for *kl-3*, *kl-5*, or both. Similar observations were made by Brosseau (1960) and by Kennison (1981, 1983).

## Results

### *Cytological dissection of the Y chromosome*

*Cytological characterization of the normal Y chromosome and its marked derivatives $y^+Y$ and $B^SYy^+$.* A fine cytological characterization and the construction of an accurate cytological map of the *Y* chromosome is a fundamental step in its cytogenetic dissection by banding analysis. Since the *Y* chromosome rearrangements used to map the male fertility factors were generated employing $y^+Y$ ($=sc^8Y$) and $B^SYy^+$ chromosomes, such characterization must include not only the normal *Y* chromosome but also these marked derivatives.

The $y^+Y$ chromosome, which carries on the long arm the tip of the *X* chromosome with the normal allele of yellow ($y^+$), arose as a product of an exchange in males carrying $In(1)sc^8$ and an unmarked *Y* chromosome (Muller 1948). The $B^SYy^+$ chromosome is a further derivative of $y^+Y$, it carries the Bar Stone ($B^S$) dominant mutation on the long arm and the normal allele of yellow on the short arm (Brosseau et al. 1961). The detailed pedigrees of these marked *Y* chromosomes are reported by Lindsley and Grell (1968) and by Williamson (1976).

To characterize the Oregon-R *Y*, the $y^+Y$, and the $B^SYy^+$ chromosomes we analyzed very long midprophase

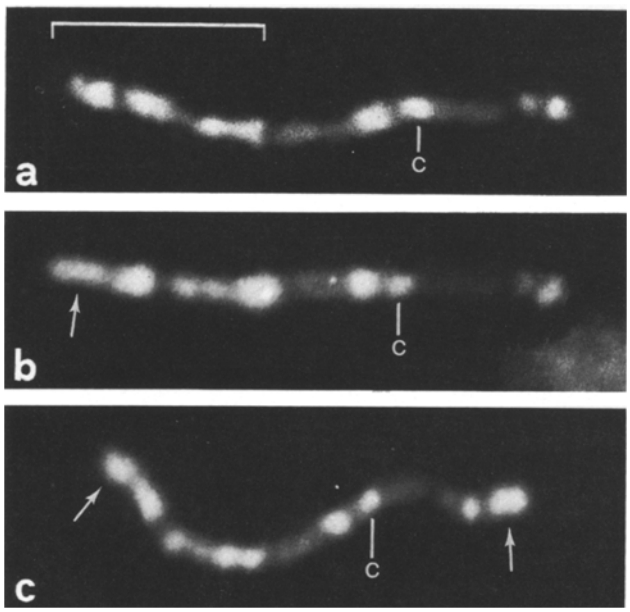


**Fig. 1.** Promethaphase *Y* chromosomes of *D. melanogaster* stained with Hoechst 33258. **a** Oregon-R *Y*, **b** $y^+Y$, **c** $B^SYy^+$. *Arrows* indicate the extra blocks of *X* heterochromatin carried by the $y^+Y$ and $B^SYy^+$ chromosomes; *c*, centromere. Bar represents 5 μm

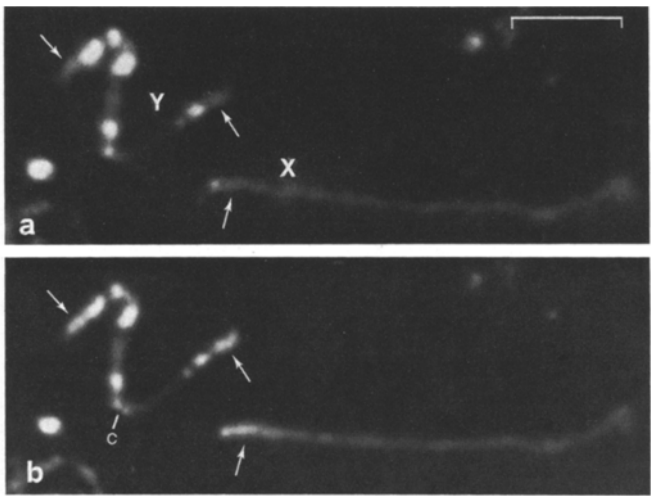


**Fig. 2.** Prometaphase *X* and $B^SYy^+$ chromosomes of *D. melanogaster* sequentially stained with quinacrine (**a**) and Hoechst 33258 (**b**). Note that the terminal blocks of the $B^SYy^+$ and the proximal heterochromatin of the *X* chromosome (*arrows*) are quinacrine-dull and Hoechst-bright; *c*, centromere. Bar represents 5 μm

or early prometaphase *Y* chromosomes processed with three banding techniques: Hoechst 33258 staining, quinacrine staining and N-banding. Each of these techniques provides excellent longitudinal differentiation of the *Y* chromosome (Figs. 1, 2 and 3). Moreover, when these techniques are employed sequentially (quinacrine, Hoechst, N-banding) their power of resolution is greatly increased. For example, some regions that are poorly defined by quinacrine or Hoechst staining are sharply delimited by the N-banding; thus by comparing microphotographs of the same chromosome stained with these two complementary banding methods a very fine longitudinal differentiation is achieved (see Fig. 3).

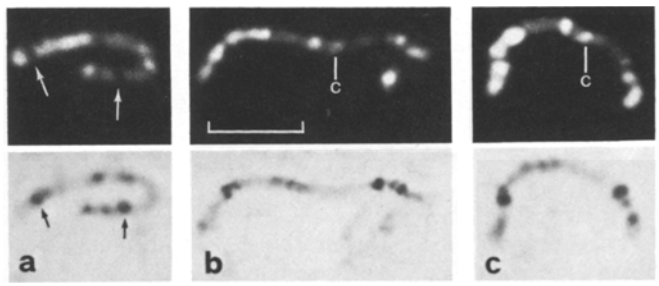


**Fig. 3.** *Y* chromosomes of *D. melanogaster* sequentially stained with Hoechst 33258 and N-banding. **a** Oregon-R *Y*, **b** and **c** $B^SYy^+$ chromosomes. Note that the N-bands correspond to the nonfluorescent areas of the Hoechst-stained chromosomes. *c*, centromere. Bar represents 5 μm

After Hoechst staining the $y^+Y$ exhibits one block of fluorescent material at the end of $Y^L$ that is not found in the Oregon-R *Y* chromosome, while $B^SYy^+$ shows additional bright blocks at both its ends (Fig. 1). Except for these additional fluorescent blocks, the $y^+Y$ and the $B^SYy^+$ chromosomes appear absolutely identical to the Oregon-R *Y* chromosome. To investigate the nature of these extra blocks, these marked *Y* chromosomes were stained sequentially with quinacrine and Hoechst 33258. As can be seen in Figure 2, these heterochromatic blocks are poorly fluorescent after quinacrine staining, but are very bright after staining with Hoechst. Except for these additional quinacrine-dull blocks, the $y^+Y$ and the $B^SYy^+$ are indistinguishable from an Oregon-R *Y* chromosome after quinacrine banding. Since in *D. melanogaster* only the proximal third of the *X* chromosome heterochromatin has the property of being quinacrine-dull and Hoechst-bright (Gatti et al. 1976 and Fig. 2), these data demonstrate that the additional blocks carried by the $y^+Y$ and the $B^SYy^+$ chromosomes are made up of *X* heterochromatin. Moreover, the cytological characterization of $B^SYy^+$-autosome translocations (see Fig. 7) proved that translocations that remove the most distal part of either of the two extra blocks also remove the $B^S$ and $y^+$ markers, indicating that these loci are located at the distal extremes of the blocks. These findings agree well with predictions based on how these marked *Y* chromosomes were generated (Lindsley and Grell 1968; Williamson 1976) (see Fig. 4). On the basis of its origin, the additional block on the $y^+Y$ and the short region of Hoechst-dull material which connects it to $Y^L$ were designated as regions $y^+Xh$ and $y^+Xhj$ respectively. Similarly in the $B^SYy^+$ chromosome the distal block of *X* heterochromatin and the Hoechst-dull region that joins it to $Y^L$ were designated as regions $B^SXh$ and $B^SXhj$ while the block at the end of $Y^S$ was designated as region $Xhy^+$ (Fig. 5).

The third method we used to characterize the Oregon-R *Y*, the $y^+Y$ and the $B^SYy^+$ chromosomes was the N-banding technique (Fig. 3). Except for the extra blocks of *X* heterochromatin, these chromosomes appear substantially identical with only a minor but interesting difference: the most distal N-band of the short arm of the $B^SYy^+$ chromosome appears larger than that seen in either the Oregon-R *Y* or the $y^+Y$ chromosomes (Figs. 3, 8 and 12). We can deduce the origin of this extra N-banded material from the origin of the $B^SYy^+$ chromosome (Fig. 4). The $B^SYy^+$ chromosome arose as a consequence of a spontaneous exchange in a $X/B^SY/bw^+Yy^+$ male (Fig. 4c–d, Brosseau et al. 1961). The $bw^+Yy^+$ chromosome was recovered by

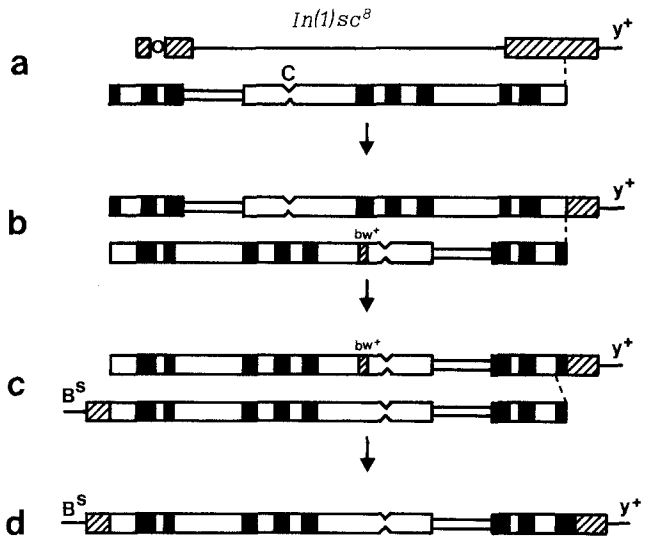


**Fig. 4a–d.** Origin of the $B^S Yy^+$ chromosome. The dark areas along the *Y* chromosome represent the N-bands. See text for explanation

Cooper (1952) as a product of spontaneous recombination in males carrying the $bw^+Y$ and $y^+Y$ chromosomes (Fig. 4b). Since the $y^+Y$ chromosome of Muller (1948) is a product of recombination between an unmarked *Y* chromosome and $In(1)sc^8$ (Fig. 4a), it cannot carry the N-band of the *X* chromosome which is located proximally with respect to the $sc^8$ breakpoint (indeed no N-bands are seen in either the $y^+Xh$ or the $y^+Xhj$ regions of the $y^+Y$). Thus, its derivative $bw^+Yy^+$ chromosome cannot carry *X* chromosome N-banded material. The most likely source of the additional N-banded material of the $B^S Yy^+$ chromosome is a duplication of the region h25 (as seen in both the Oregon-R *Y* and the $y^+Y$ chromosomes) from an unequal exchange between the $B^S Y$ and the $bw^+Yy^+$ chromosomes (Fig. 4c). For this reason we designate the most distal N-band on the short arm of the $B^S Yy^+$ chromosome as region h25D (Fig. 5).

Taken as a whole, these banding studies demonstrate that the $y^+Y$ and the $B^S Yy^+$ chromosomes differ from the Oregon-R *Y* only by the presence of some extra material at their ends. These findings provide the cytological basis for an easy and reliable interpretation of the complementation tests between aberrant chromosomes derived from $y^+Y$ and those derived from $B^S Yy^+$ (see next sections). Moreover, they permit us to relate our genetic data with those of Brosseau (1960), who used a $y^+Y$, or those of Williamson (1970, 1972) and Kennison (1981), who employed a $B^S Yy^+$. Finally, they provide evidence that by cytogenetically analyzing the $y^+Y$ and $B^S Yy^+$ chromosomes, we are in fact studying the cytogenetic organization of a normal *Y* chromosome.

*Detailed description of Hoechst- and N-banding patterns.* By the application of quinacrine-, Hoechst- and N-banding techniques the *Y* chromosome can be subdivided into 25 regions (Fig. 5). The diagrams depicted in Figure 5 represent ideal chromosomes in that the complete array of bands is not usually seen in a single chromosome. These diagrams have been constructed by examining several particularly long midprophase or prometaphase chromosomes that exhibited a very fine differentiation of most but not all the regions. In the more condensed *Y* chromosomes, several regions are not clearly differentiated because some adjacent bands tend to fuse and are no longer distinguishable as separate entities. Thus for a correct interpretation of our diagrams and to facilitate future work on the *Y* chromosome we feel it important to present a detailed description of its cytological organization. Our description of the *Y*

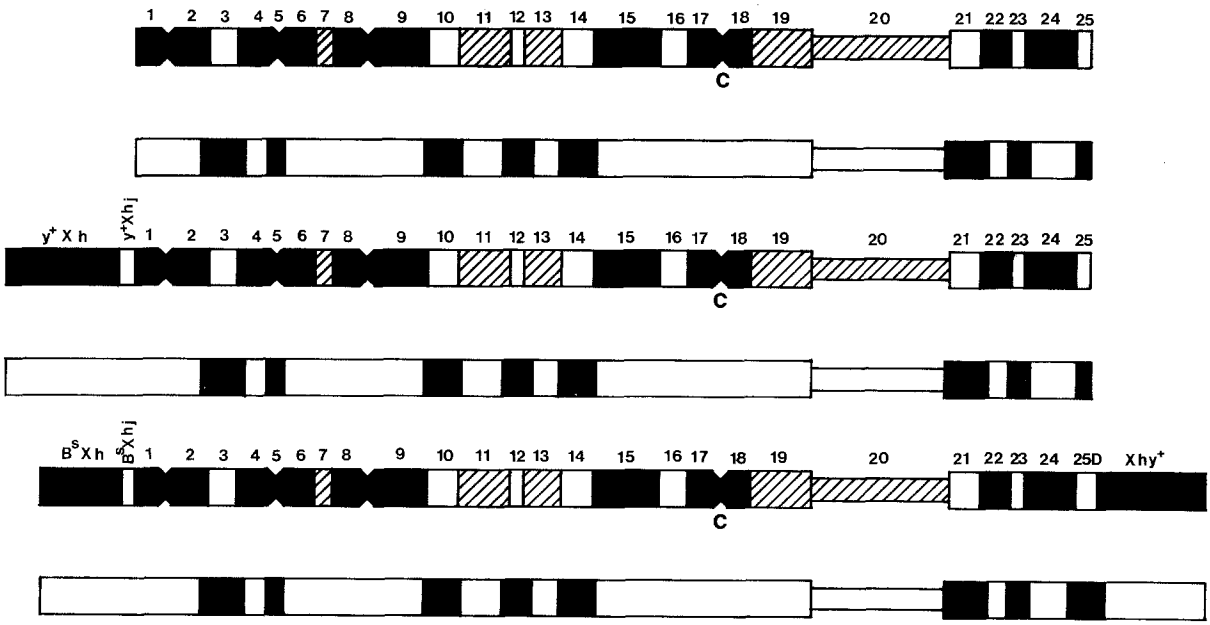


**Fig. 5.** Banding pattern of the *Y* chromosome of *D. melanogaster* and its marked derivatives $y^+Y$ and $B^S Yy^+$. From top to bottom: Hoechst-stained Oregon-R *Y*, N-banded Oregon-R *Y*, Hoechst-stained $y^+Y$, N-banded $y^+Y$, Hoechst-stained $B^S Yy^+$, N-banded $B^S Yy^+$. In the Hoechst-stained chromosomes the dark areas correspond to bright regions; the hatched areas, to dull regions; and the open areas, to nonfluorescent regions. Region h20, which is depicted thinner than the others, corresponds to the nucleolus organizer (Pimpinelli et al., in preparation). *c* centromere

chromosome will refer to the Hoechst-banding pattern after staining with 0.5 μg/ml (Gatti et al. 1976) and to the N-banding pattern. The quinacrine-banding pattern is almost identical to that seen after Hoechst staining; the only difference is region h18, which is quinacrine-dull but Hoechst-bright.

Regions h1 and h2 are usually seen as a single Hoechst brightly fluorescent block and only under very favorable conditions are they separated by an indentation, in which case region h1 appears less fluorescent than region h2. Region h3 is clearly seen in all Hoechst-stained *Y* chromosomes as a rather large non-fluorescent band and, together with region h21, is the most heavily stained area after N-banding. Regions h4, h5 and h6 appear as a single fluorescent block in condensed Hoechst-stained *Y* chromosomes, however, in most prometaphase chromosomes regions h4 and h6 are two distinct bright blocks separated by a non-fluorescing gap (region h5), which corresponds to an N-band. Region h7 is a poorly fluorescent area of variable size that can be seen in all Hoechst-stained *Y* chromosomes. Regions h8 and h9 usually appear as a single Hoechst-bright block and are rarely separated by an indentation. Regions h10–h14 jointly appear as a large Hoechst-dull block. A careful examination of this block reveals that it is subdivided into three less fluorescent regions (regions h10, h12, and h14) and two brighter regions (regions h11 and h13). Such a differentiation is difficult to see even in long prometaphase chromosomes, but by sequential staining with N-banding, regions h10, h12 and h14 appear darkly stained compared to regions h11 and h13 (see Fig. 3). The N-bands h12 and h14 tend to coalesce, and in condensed *Y* chromosomes the three N-banded regions h10, h12 and h14 sometimes appear as a single large N-band. In addition, in most N-band preparations region h10 is less stained than regions h12 or h14. Region h15 is a large Hoechst-bright block clearly visible in all *Y* chromosomes. Region h16 is a rather large nonfluorescent gap in Hoechst-stained chromosomes and is the only nonfluorescent area of the *Y* chromosome which does not correspond to an N-band. Regions h17 and h18 are usually seen as a single Hoechst-bright block and only rarely are separated by a constriction.

Observation of anaphase chromosomes indicates that the centromere is included in an area that comprises regions h17–h18. This conclusion is supported by our studies on *Y*-autosome translocations (see next section) that place the centromere between a left breakpoint involving region h16 and a right one involving region h18. We believe that the centromere is located in the constriction between regions h17 and h18.

Regions h19 and h20 together appear as a long Hoechst-dull segment. However, after sequential staining with aceto-orcein, region h19 always appears as a compact block while region h20 is a large constriction of variable extension. There is evidence that region h20 contains the nucleolus organizer region and hence the ribosomal cistrons. This conclusion is based on a careful cytological characterization by Hoechst and N-banding of six different bobbed *Y* chromosomes. All these chromosomes exhibit deletions of region h20 whose length is proportional to the strength of the bobbed phenotype (Pimpinelli et al., in preparation).

Region h21 is a rather large Hoechst-nonfluorescent band which is clearly visible even in condensed chromosomes and is heavily stained in N-banding. Region h22 is a small Hoechst-bright block, its degree of fluorescence is lower than the other bright bands of the *Y* chromosome and in some preparations it appears as an almost Hoechst-dull element. Region h23 is a short, but usually clearly visible, nonfluorescent gap which corresponds to an N-band. Region h24 is a fluorescent block which in Hoechst-stained Oregon-R *Y* chromosomes appears as the most distal region of $Y^S$. However, if after Hoechst staining chromosomes are N-banded, a short heavily stained band distal to region h24 is observed. Thus distal to region h24 there is a Hoechst-nonfluorescent region, region h25, which corresponds to an N-band.

In some N-band preparations regions h7, h16 and h19 are palely stained (Fig. 12e). However, the occurrence of these slightly N-banded regions is rather rare, and they are always much less stained than the other N-banded material. Thus they were not classified as N-bands.

*Localization of secondary constrictions.* In his classic study on aceto-orcein stained chromosomes Cooper (1959) reported that in early prophase the *Y* chromosome is often subdivided into a number of heterochromatic blocks separated by constrictions. Judging from Cooper's camera lucida drawings the pattern of constrictions along the *Y* chromosome is rather variable; moreover, most constrictions do not exhibit morphological characteristics that permit them to be distinguished from one another. Despite these difficulties Cooper (1959) was able to construct a cytological map of the *Y* chromosome on the basis of the appearance and location of the secondary constrictions.

To relate the banding pattern of the *Y* chromosome to the array of secondary constrictions, several *Y* chromosomes with different degrees of condensation were sequentially stained with Hoechst and either aceto-orcein or Giemsa, and the microphotographs taken after each step were compared. As shown in Figure 6, this analysis clearly established that: (1) condensed metaphase *Y* chromosomes exhibit only the nucleolar constriction in $Y^S$ (region h20) and in some cases they also show an indentation at the level of the major secondary constriction of $Y^L$ (region h8–h9); (2) in long midprophase or prometaphase *Y* chromosomes the Hoechst-nonfluorescent gaps that are selectively stained by N-banding do not correspond to secondary constrictions, being on the contrary the most prominent regions of the *Y* chromosome; (3) also region h16 which is the only Hoechst nonfluorescent gap without N-banding properties does not correspond to a constriction; (4) the secondary constrictions correspond to some Hoechst-bright blocks that after orcein staining appear as stretched areas of variable extension (Fig. 6).

By combining these observations with banding studies it can be concluded that the *Y* chromosome is a continuous array of heterochromatic blocks having very different cytological characteristics. During the process of mitotic contraction some of these blocks exhibit a delayed condensation appearing as secondary constrictions.

### *Cytological characterization of the Y-autosome translocations*

To localize the *Y* chromosome fertility factors we used a series of translocations involving the $B^S Yy^+$ chromosome and either the second or the third chromosome. These *Y*-autosome translocations were generated by Lindsley et al.

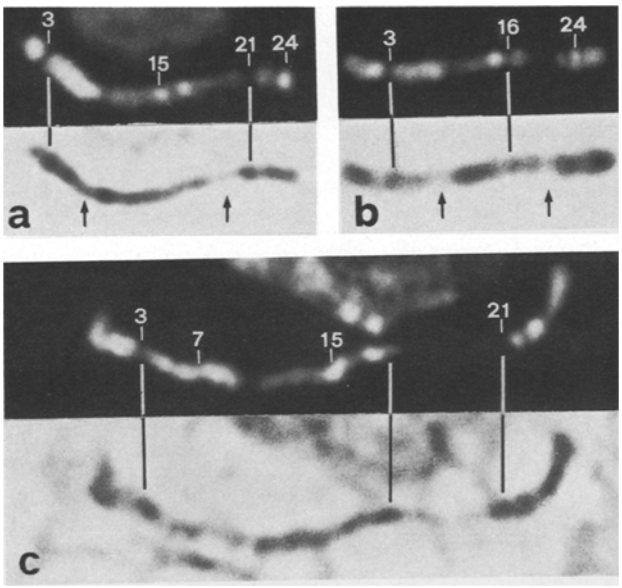


**Fig. 6.** *Y* chromosomes of *D. melanogaster* sequentially stained with Hoechst (upper) and Giemsa (lower). **a** Oregon-R *Y*, **b** and **c** $B^S Yy^+$ chromosomes. The numbers along the *Y* chromosome indicate some of the cytological landmarks diagrammed in Figure 5. Note that the Hoechst-bright blocks correspond to the thin regions of Giemsa-stained chromosomes. In contrast the Hoechst-negative areas correspond to the most prominent regions seen after Giemsa staining. Arrows on the right point to the nucleolar constriction (region h20) and on the left to the main constriction of $Y^L$ (regions h8–h9). The same pattern of constrictions shown in **b** and **c** is also observed in the absence of previous staining with Hoechst and using aceto-orcein

(1972) and were originally employed to construct genomes with segmental aneuploidy for the autosomal complement. About half of these translocations are sterile in the absence of a normal *Y* chromosome and must therefore bear recessive mutations involving at least one of the *Y* chromosome fertility factors. On the assumption that those fertility factors located at or near *Y* chromosome breakpoints are inactivated, we characterized 96 fertile and 110 sterile translocations by Hoechst banding in order to determine whether they had different *Y* chromosome breakpoints.

To obtain a precise definition of the *Y* chromosome breakpoints only translocations involving autosomal euchromatin were analyzed. The points of junction between *Y* heterochromatin and autosomal euchromatin can be easily determined since (1) euchromatin is homogeneously Hoechst-dull whereas *Y* heterochromatin is often Hoechst-bright or Hoechst-negative and (2) in contrast to autosomal euchromatin *Y* heterochromatin exhibits close apposition of sister chromatids. By using these two criteria translocation breakpoints were assigned to either the Hoechst highly fluorescent or to the Hoechst-negative regions (Fig. 7). For the translocations involving the Hoechst-bright regions the position of the breakpoints within the region was determined exactly by measuring the relative dimensions of its two subdivisions. This was not usually possible for the translocations involving the Hoechst-nonfluorescent areas because these regions do not stand out from euchromatin with the same contrast as the Hoechst-bright regions. Thus most of these translocations, after Hoechst staining, were processed with N-banding which specifically stains Hoechst-negative areas, and the translocation breakpoints within these regions were determined precisely (Fig. 8).

The exact localization of breakpoints involving regions h10–h14 turned out to be rather difficult. In fact, in most Hoechst-stained preparations these regions appear as a unique block having the same fluorescence as autosomal euchromatin, and the three nonheavily stained N-banded regions h10, h12, and h14 often tend to fuse obscuring the presence of regions h11 and h13. In addition, in this area (h10–h14) sister chromatid apposition is not as tight as in the rest of the *Y* chromosome so that euchromatic-heterochromatic junctions of translocations are not always sharply defined. Thus, while the translocation breakpoints could be assigned to the different regions of this area, their localization within a specific region is only tentative. However, the localizations of the breakpoints at the interfaces of regions h9–h10 and h14–h15 have been unambiguously determined.

The results of this extensive cytological analysis are summarized in Figures 9 and 10 which show the distribution of the translocation breakpoints in Hoechst-stained chromosomes. Of the 99 *T*(*Y*; *2*)s examined, 79 are simple reciprocal translocations (Fig. 9), 13 exhibit a *Y* chromosome deletion in addition to the translocation (Fig. 10), 3 have an additional inversion, 1 has an additional inversion plus a deletion, 1 is an insertional translocation and 2 carry additional rearrangements in $Y^L$ difficult to resolve cytologically (see Table 3). Similarly, of the 107 *T*(*Y*, *3*)s, 90 are simple translocations (Fig. 9), 12 have an additional deletion (Fig. 10 and Table 4), 3 have an additional inversion and 2 are insertional translocations (see Table 4).

An examination of Figure 9 reveals that the *Y* chromosome breakpoints of these translocations exhibit a striking nonrandomness in their distribution along the $B^S Yy^+$ chromosome. Most breakpoints are clustered either in the Hoechst-bright blocks of *X* heterochromatin located at both ends of the $B^S Yy^+$ chromosome (72/169) or at the level of the nonfluorescent heavily N-banded regions h3, h21, h23 and h25D (74/169). In contrast the *Y* chromosome highly fluorescent blocks exhibit an extremely low incidence of breaks (4/169), the only exception is region h1–h2 which is involved in 4 translocation events. However, these 4 breakpoints are in fact localized at the level of the junction between regions h1 and h2. The Hoechst-dull regions h7, h11, h13, h19 and h20 also show a relatively low frequency of breakpoints (9/169) and the nonheavily stained N-banded regions h10, and h14 exhibit far fewer breakpoints (5/169) than the heavily stained N-banded blocks.

This nonrandom distribution of the *Y* chromosome breakpoints may reflect nonrandom recovery of the translocations. Kennison (1983) isolated a sample of 88 male sterile *Y*-autosome translocations from daughters of $\gamma$-irradiated fathers and found that over one half of these translocations sterilize males in combination with an $\overline{XY}$ chromosome and thus behave as dominant male sterile mutations. Since the translocations we characterized cytologically were selected by testing for pseudolinkage in $\overline{XY}$-bearing sons of irradiated fathers (Lindsley and Sandler et al. 1972), it is possible that our sample of *Y*-autosome translocations is biased by the loss of a large class of translocations that behave as dominant male steriles. Unfortunately we cannot assess the extent of such bias because Kennison (1983) did not characterize his sample of *Y*-autosome translocations cytologically. However, he did characterize by Hoechst banding

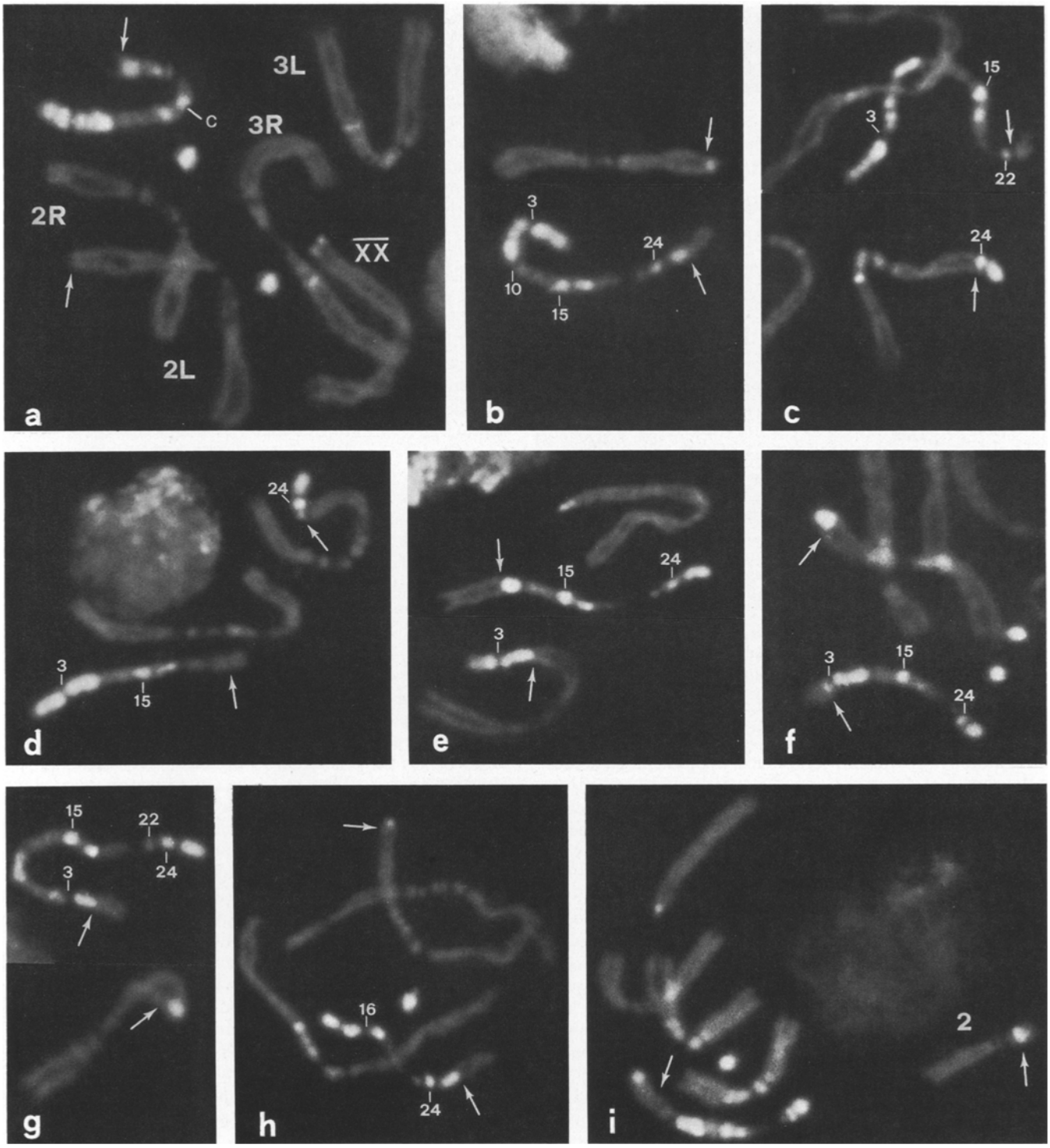


**Fig. 7.** *Y*-autosome translocations stained with Hoechst 33258. The numbers along the *Y* chromosome indicate cytological landmarks (cf. Fig. 5) and the *arrows* point to translocation breakpoints. **a** *R104*, breakpoints in $Xhy^+$ and 58A ($Xhy^+$; 58A). Note that no fluorescent material is translocated on the tip of *2R*. This suggests that the $y^+$ marker is located at the distal terminus of the $Xhy^+$ region and that the translocation occurred between the Hoechst-bright *X* heterochromatin and the $y^+$ locus. A similar situation was recorded for some translocations broken in $Y^L$ that remove the $B^S$ marker without transferring fluorescent material on the autosomes. **b** *B66* ($Xhy^+$; 28C). **c** *R78* (h23; 98E). **d** *P8* (h21; 24D). **e** *G74* (h7; 34B). **f** *J94* (h1/h2; 66B). **g** *R147* ($B^SXhj$; 27E). **h** *H52*, translocation breakpoints in $Xhy^+$ and 27E, and carrying an interstitial deficiency of the *Y* chromosome spanning region h3 to region h16. **i** *H64*, complex insertional translocation: autosomal euchromatin inserted into region h3 and $B^SXh$ fluorescent material translocated on chromosome *2*

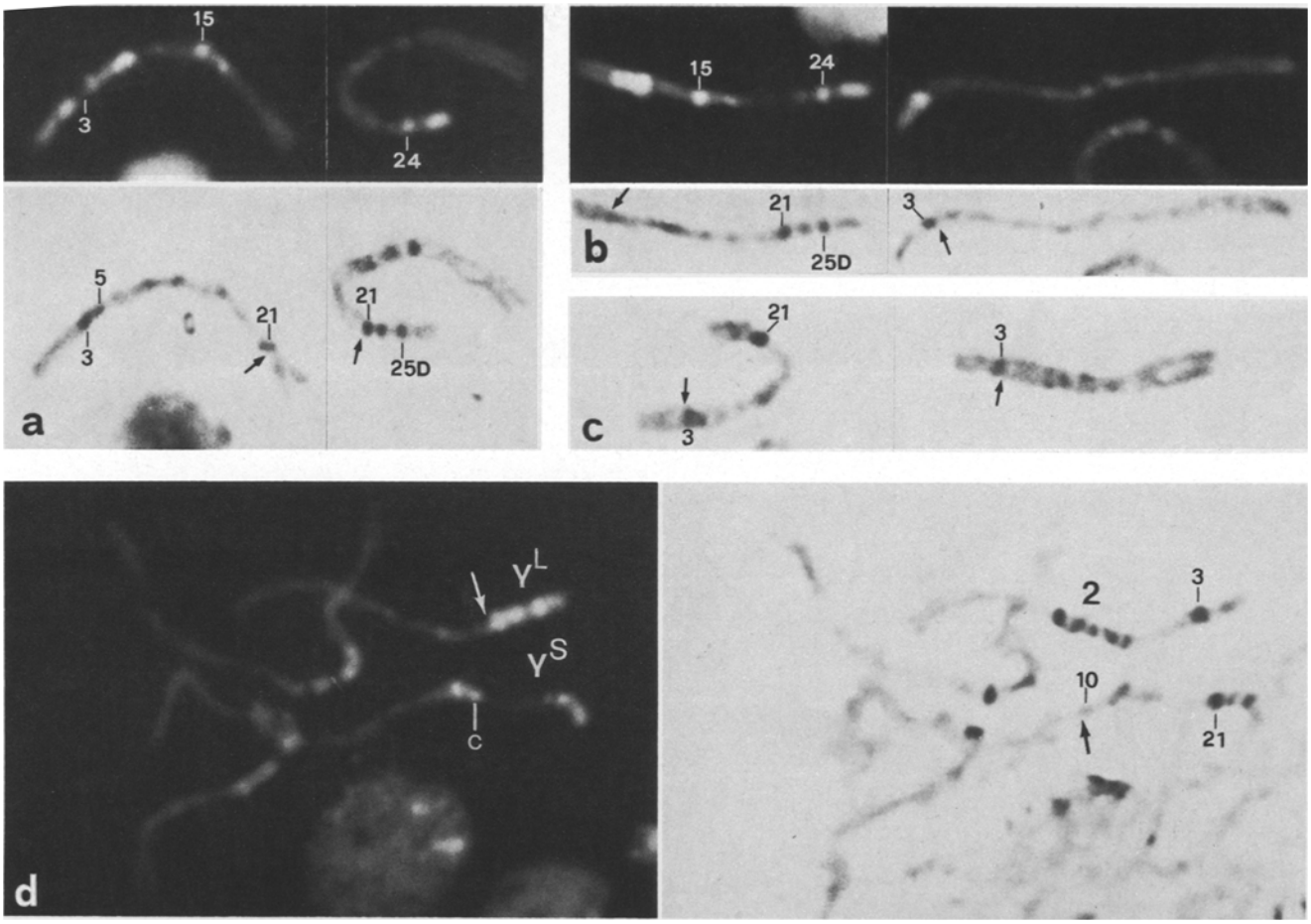


**Fig. 8.** *Y*-autosome translocations processed with the N-banding technique. **a**, **b** and **d** show sequential staining with Hoechst and N-banding. The numbers along the *Y* chromosome indicate cytological landmarks (cf. Fig. 5) and the *arrows* point to translocation breakpoints. **a** *A162*, breakpoints in the central third of region h21 and in 32F. **b** *G122*, breakpoints in the proximal third of region h3 and in 67C. **c** *A24*, breakpoints in the central third of region h3 and in 46A. **d** *P57*, breakpoints at the junction between regions h9 and h10 and in 38B, note the five N-bands of chromosome *2*

a group of translocations between the *X* heterochromatin and the $B^S Yy^+$ chromosome and found a distribution of *Y* chromosome breakpoints rather similar to that reported here (Kennison 1981). The *Y* breakpoints of Kennison's translocations are clustered in the same regions as the *T*(*Y*;*A*)s, but he found a somewhat higher frequency of breakpoints involving the central part of the *Y* chromosome (region h10–region h20). Since *X*–*Y* translocations are very seldom dominant male steriles (Nicoletti and Lindsley 1960), the distribution of their *Y* chromosome breakpoints should reflect the real frequency with which the different regions of the *Y* chromosome are involved in translocation events. Thus, the distribution of the *Y* chromosome breakpoints of our sample of *T*(*Y*;*A*)s which is comparable to that of Kennison's *T*(*X*;*Y*)s (Kennison 1981) should result, at least in part, from a non-random induction of translocations. Moreover, since the $B^S Yy^+$ chromosome participates in translocations with autosomes with a frequency proportional to its metaphase length (Lindsley and Sandler et al. 1972), these observations indicate that some *Y* chromosome regions are involved in rearrangements much more frequently than the rest of the genome while others are refractory to rearrangements (Fig. 9). Whether this differential sensitivity to rearrangements is due to the different breakability of the various *Y* chromosome regions or to their different rejoining capability is unknown.

Another level at which the distribution of the *Y* chromosome breakpoints (bkp) is clearly nonrandom concerns the localization of the fertile breakpoints with respect to the sterile ones (Fig. 9). Most fertile breakpoints involve either the $B^S Xh$ and the $B^S Xhj$ regions (27 fertile bkp/27 bkp) or the $Xhy^+$ region (44 fertile bkp/45 bkp) which indeed are not expected to contain any fertility factors since they are made up of *X* heterochromatin. The fertile breakpoints involving *Y* chromosome material are rather few and are located in the Hoechst-bright blocks h15 (1 fertile bkp/1 bkp), h18 (1 fertile bkp/1 bkp) and h24 (2 fertile bkp/2 bkp), in the Hoechst-dull regions h11 (2 fertile bkp/2 bkp), h13 (3 fertile bkp/3 bkp), h19 (1 fertile bkp/1 bkp) and h20 (1 fertile bkp/1 bkp), in the Hoechst-negative region h16 (1 fertile bkp/1 bkp), and especially in the N-banded region h25D (12 fertile bkp/13 bkp). In contrast, sterile breakpoints are specifically located at the level of the N-banded regions h3 (30 sterile bkp/30 bkp), h10 (2 sterile bkp/2 bkp), h14 (3 sterile bkp/3 bkp), h21 (21 sterile bkp/21 bkp) and h23 (9 sterile bkp/10 bkp), in the junction between regions h1 and h2 (4 sterile bkp/4 bkp) and in region h7 (2 sterile bkp/2 bkp). Thus with the exception of region h25D all

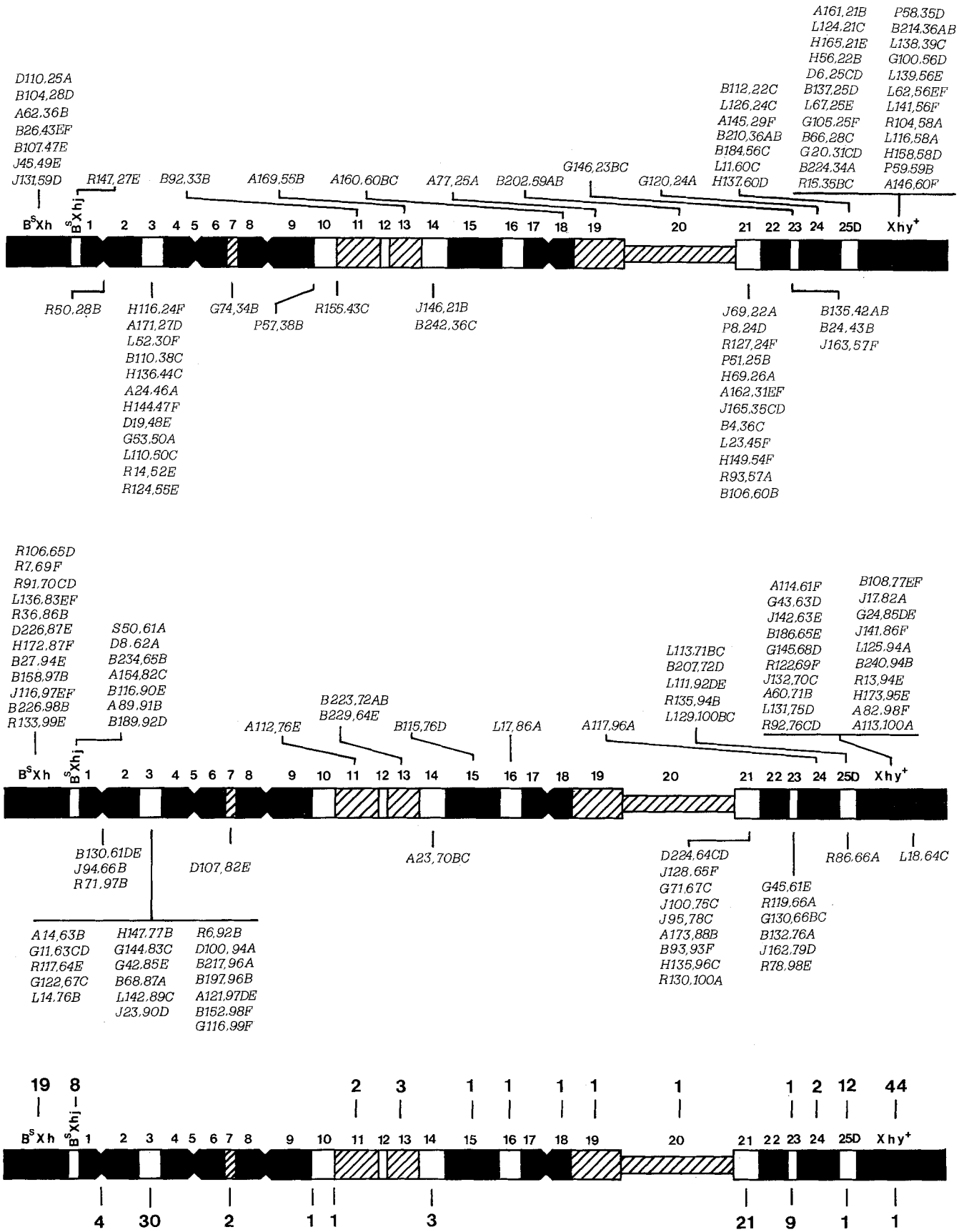


**Fig. 9.** Distribution of the *Y* chromosome breakpoints in *Y*-autosome translocations. Top: *T(Y;2)*s; middle: *T(Y;3)*s; bottom: summary of *T(Y;2)*s and *T(Y;3)*s. The diagram of the *Y* chromosome is representative of a $B^S Yy^+$ chromosome stained with Hoechst 33258. The translocations listed above each diagram are male fertile in the absence of an extra *Y* chromosome; those listed below are male sterile

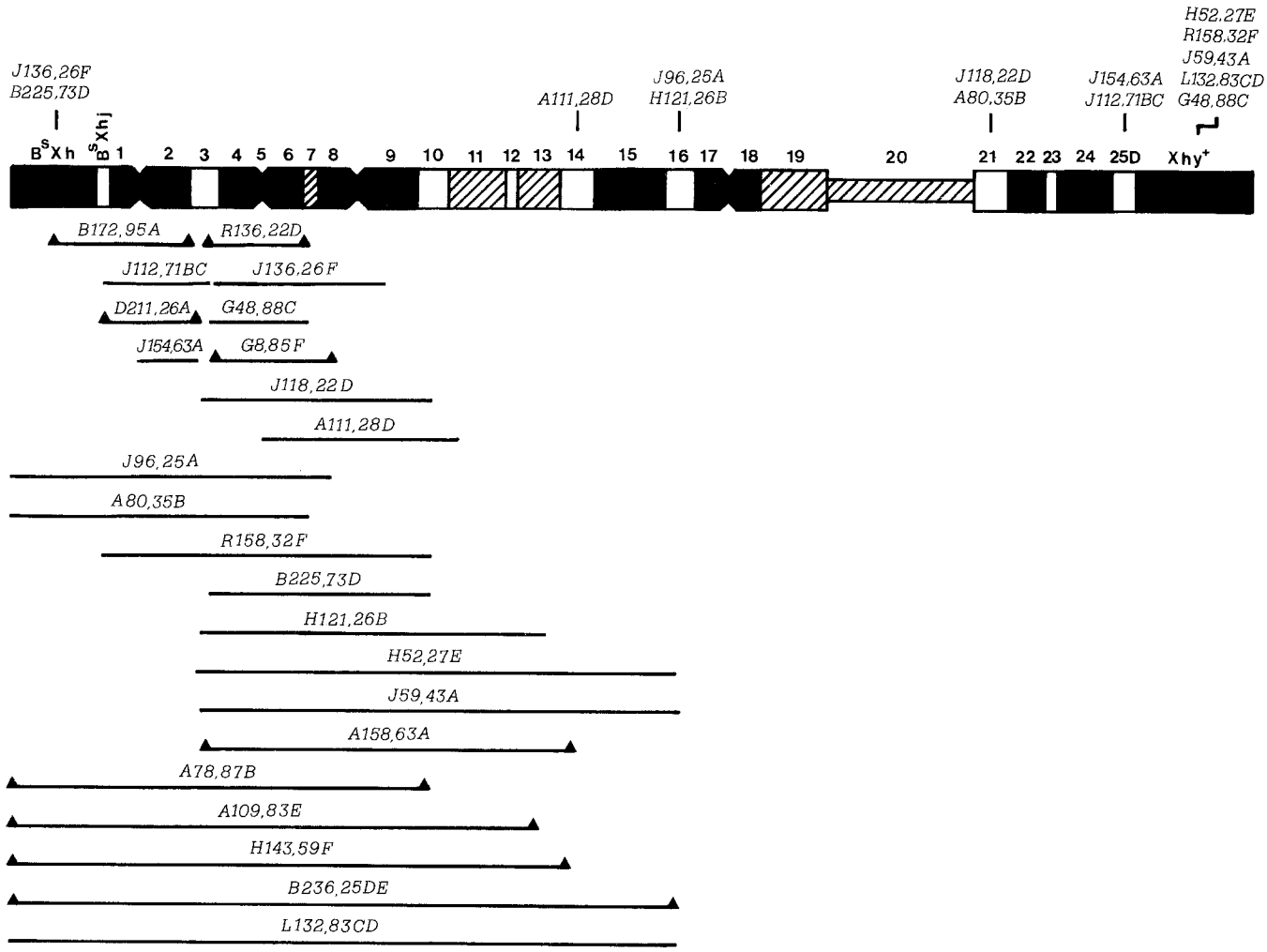


**Fig. 10.** Diagram of *Y*-autosome translocations carrying *Y* chromosome deficiencies. The deficiencies delimited by triangles are included between the translocation breakpoints; i.e., the *Y* material proximal to the deficiency is joined to autosomal euchromatin whereas the *Y* material distal to the deficiency is appended to the centric autosomal piece. The deficiencies depicted as simple bars represent *Y* chromosome deletions which occurred independently of the translocation event

the breakpoints involving N-bands appear to confer sterility. However since region h25D is larger than the corresponding region h25 observed in a normal *Y* chromosome, it might contain a duplicated fertility factor that is not inactivated by a single breakpoint.

In conclusion, our data suggest the existence of five regions in $Y^L$ and two regions in $Y^S$ whose integrity is essential for male fertility. All except two (regions h1/h2 and h7) are specifically stained by the N-banding technique.

### *Genetic analysis*

*Complementation tests between Y-autosome translocations.* The most straightforward way of asking whether the regions identified by the sterile *Y* chromosome breakpoints correspond to separate genetic loci is testing for complementation between two sterile $T(Y;A)$s, broken in different regions of the *Y* chromosome. However, $T(Y;A)_i/T(Y;A)_j$ males turned out to be sterile even when one or both translocations were fertile (Table 1). Table 1 reveals that the sterility of males carrying two $T(B^SYy^+;A)$s is not related to either the autosomal or the *Y* chromosome breakpoint of the translocations. Such synthetic sterility was quite unexpected because $X/B^SYy^+/B^SYy^+$ males are fertile (Williamson 1970, 1972). Clearly the sterility of $T(B^SYy^+;A)_i/T(B^SYy^+;A)_j$ males is chromosomal and not genic; it is caused by a disruption of normal chromosome organization in the genome and not by gene mutation. Other cases of chromosomal sterility are reviewed in detail by Lindsley and Tokuyasu (1980). One example of disruption of fertility caused by the combination of two fertile chromosome rearrangements is the synthetic sterility shown by males carrying a *Y*-autosome translocation and an *X* chromosome deficient for *bb* in the proximal heterochromatin (see Lindsley and Tokuyasu 1980). These rearrangements interact in a dominant manner because the sterility of $Df(1)bb^-/T(Y;A)$ males cannot be overcome either by the presence of an extra *Y* chromosome or by the addition of a duplication for the entire *X* heterochromatin (Lindsley and Tokuyasu 1980). Furthermore, it has been shown that the element of the $T(Y;A)$ which interacts with the *bb* deficiency to produce sterility is the capped autosome, whereas the capped *Y* chromosome is fertile in the presence of $Df(1)bb^-$ (Lindsley, personal communication). These findings together with our observation (Table 6) that males carrying two

**Table 1.** Complementation analysis between *T(Y;A)*s. Number of progeny/number of males tested reported in each box. The *Y* chromosome breakpoints are in parentheses and an asterisk indicates fertile *T(Y;A)*s

| | *D110* ($B^S Xh$)* | *R91* ($B^S Xh$)* | *R50(h1/h2)* | *G53(h3)* | *G74(h7)* | *P57(h9/h10)* | *R155(h10/h11)* | *B92*(h11)* | *B242(h14)* | *P8(h21)* | *G45(h23)* | *P58* ($Xhy^+$)* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *D110*, 25A* | – | 11/28 | 0/24 | 0/24 | 0/23 | 0/18 | 2/17 | 0/15 | 0/16 | 0/20 | 0/18 | 0/16 |
| *R91*, 70CD* | | – | 0/19 | 0/20 | 9/20 | 0/19 | 0/16 | 0/13 | 0/15 | 0/14 | 0/12 | 0/14 |
| *R50, 28B* | | | – | 0/26 | 0/21 | 0/16 | 0/19 | 0/15 | 0/22 | 0/15 | 0/8 | 0/15 |
| *G53, 50A* | | | | – | 0/10 | 0/27 | 0/20 | 0/21 | 0/15 | 8/16 | 5/9 | 0/7 |
| *G74, 34B* | | | | | – | 0/14 | 0/22 | 0/13 | 0/21 | 0/16 | 23/10 | 1/14 |
| *P57, 38B* | | | | | | – | 0/14 | 0/11 | 0/8 | 0/7 | 13/9 | 0/10 |
| *R155, 43C* | | | | | | | – | 0/11 | 0/16 | 0/15 | 0/7 | 0/19 |
| *B92*, 33B* | | | | | | | | – | 4/15 | 0/16 | 0/13 | 0/12 |
| *B242, 36C* | | | | | | | | | – | 0/15 | 0/7 | 0/17 |
| *P8, 24D* | | | | | | | | | | – | 0/17 | 29/23 |
| *G45, 61E* | | | | | | | | | | | – | 7/14 |
| *P58*, 35D* | | | | | | | | | | | | – |

*Y*-autosome translocations, one involving a $B^S Yy^+$ (with two extra pieces of *X* heterochromatin appended at the ends) and the other a $y^+ Y$ chromosome (with only one extra block of *X* heterochromatin) are fertile, suggest that in $T(Y;A)_i/T(Y;A)_j$ males the amount and arrangement of *X* heterochromatin in the genome is also critical for male fertility. It should be noted that males carrying a $B^S Yy^+$-autosome translocation in combination with a $B^S Yy^+$, although less fertile than $X/y^+ Y/T(B^S Yy^+;A)$ males, consistently produce some progeny (unpublished observations). The causes of these phenomena of synthetic sterility are unknown. One interpretation is that in certain rearranged genomes an abnormal amount and location of *X* heterochromatin disrupts the control of chromosome inactivation during spermatogenesis (see Lindsley and Tokuyasu 1980).

*Complementation tests between sterile T(Y;A)s and Brosseau's sterile Y chromosomes.* Since complementation tests between sterile $B^S Yy^+$-autosome translocations with different *Y* chromosome breakpoints did not indicate whether these breakpoints corresponded to separate fertility factors, we performed a series of complementation tests between sterile *T(Y;A)*s and sterile $y^+ Y$ chromosomes. A sample of eight $y^+ Y$ sterile chromosomes, originally isolated by Brosseau (1960), was kindly provided by Dr. Kiefer. Before use in complementation tests with *T(Y;A)*s, these *Y* chromosomes were characterized genetically and cytologically (Table 2 and Figs. 11 and 13). Complementation tests in all the possible pairwise combinations revealed that these eight $y^+ Y$ chromosomes belong to only three complementation groups (Table 2). The first group includes *Y* chromosomes designated *kl-1*$^-$, *N* and *kl-3*$^-$*-4*$^-$, *N*, the second group *kl-5*$^-$, *Br*, *kl-4*$^-$*-5*$^-$, *N* and *kl-2*$^-$, *N*, and the third group *ks-2*$^-$, *Br*, *ks-2*$^-$, *N* and *kl-2*$^-$, *Br* (*Br* indicates direct acquisition by Kiefer from Brosseau and *N* from Brosseau by way of Novitski). The cytological analysis by Höchst and N-banding (Fig. 11) showed that the *kl-1*$^-$, *N* and *kl-3*$^-$*-4*$^-$, *N* chromosomes (Fig. 11b) are cytologically identical, both carry a pericentric inversion with one breakpoint at the junction between regions h14 and h15 and the other in region h19. The *kl-5*$^-$, *Br* chromosome (Fig. 11d–f) carries a short paracentric inversion with breakpoints in region h1/h2 (junction between regions h1 and h2) and in the middle of region h3, whereas *kl-4*$^-$*-5*$^-$, *N* and *kl-2*$^-$, *N* both exhibit the same complex rearrangement involving an inversion plus a deletion (Fig. 11c). The *ks-2*$^-$, *Br*, ks-2$^-$, *N* and *kl-2*$^-$, *Br* chromosomes are also cytologically identical and show a deletion of most of the short arm with the breakpoint located very distally in region h20 (Fig. 11a).

**Table 2.** Complementation tests between Brosseau's sterile *Y*s. F = male fertile combination; S = male sterile combination

| | *kl-1*$^-$, *N* | *kl-3*$^-$*-4*$^-$, *N* | *kl-4*$^-$*-5*$^-$, *N* | *kl-2*$^-$, *N* | *kl-5*$^-$, *Br* | *kl-2*$^-$, *Br* | *ks-2*$^-$, *N* | *ks-2*$^-$, *Br* |
|---|---|---|---|---|---|---|---|---|
| *kl-1*$^-$, *N* | S | S | F | F | F | F | F | F |
| *kl-3*$^-$*-4*$^-$, *N* | | S | F | F | F | F | F | F |
| *kl-4*$^-$*-5*$^-$, *N* | | | S | S | S | F | F | F |
| *kl-2*$^-$, *N* | | | | S | S | F | F | F |
| *kl-5*$^-$, *Br* | | | | | S | F | F | F |
| *kl-2*$^-$, *Br* | | | | | | S | S | S |
| *ks-2*$^-$, *N* | | | | | | | S | S |
| *ks-2*$^-$, *Br* | | | | | | | | S |

These data clearly establish that several of these *Y* chromosomes no longer correspond to what their label reports them to be. It appears therefore that they were mislabeled or they accumulated additional mutations since being generated. Based on the rearrangements they carry out, we have renamed *kl-1*$^-$, *N* and *kl-3*$^-$*-4*$^-$, *N* as *In(Y)B1*, *kl-4*$^-$*-5*$^-$, *N* and *kl-2*$^-$, *N* as *In(Y)B2*, *kl-5*$^-$, *Br* as *In(Y)B3*, and *ks-2*$^-$, *Br*, *ks-2*$^-$, *N* and *kl-2*$^-$, *Br* as *Df(Y)B4*.

Tables 3 and 4 show the results of complementation tests between these $y^+ Y$ chromosomes and the 110 sterile *T(Y;A)*s previously characterized with banding techniques. These sterile translocations were also tested for fertility in combination with both an attached $XY^L$ and an attached $XY^S$. An inspection of Tables 3 and 4 reveals that most translocations with the breakpoint in one *Y* chromosome

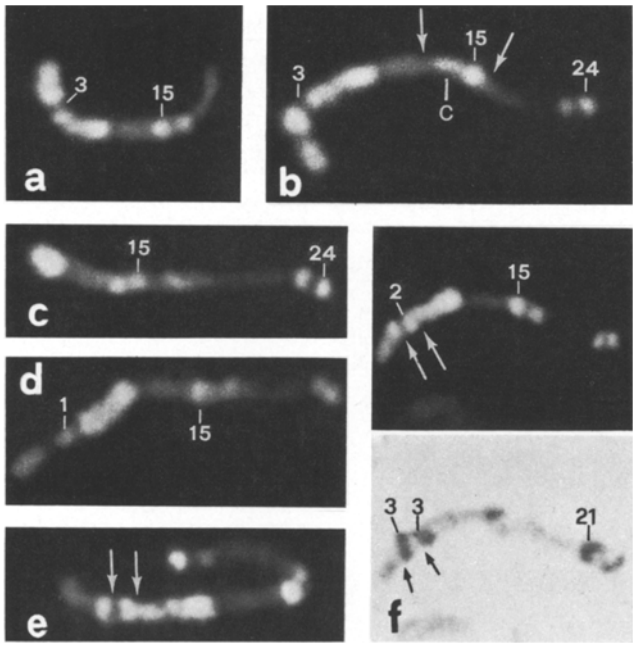


**Fig. 11.** Brosseau's sterile $y^+Y$ chromosomes. **a** *ks-2*$^-$, *Br*=*ks-2*$^-$, *N*=*kl-2*$^-$, *Br* and renamed *Df(Y)B4*, carries a deletion of $Y^S$ material distal to region h20. **b** *kl-1*$^-$, *N*=*kl-3*$^-$*-4*$^-$, *N* and renamed *In(Y)B1* (h14; h19). **c** *kl-4*$^-$*-5*$^-$, *N*=*kl-2*$^-$, *N* and renamed *In(Y)B2*, carries an inversion plus a deletion, see Figure 13 for a diagram of this complex rearrangement. **d**, **e** and **f** *kl-5*$^-$, *Br* renamed *In(Y)B3* (h1/h2; h3) stained with Hoechst 33258 (**d**), quinacrine (**e**) and sequentially stained with Hoechst 33258 and N-banding (**f**). *Arrows* indicate inversion breakpoints

arm have a sterile mutation in the same arm. Of the 9 translocations carrying sterile mutations on both arms of the *Y* chromosome, 6 exhibit aberrations involving both $Y^S$ and $Y^L$, and 3 have an apparently normal non-translocated arm. It should be noted that *R86*, which is sterile even though it is broken in region h25D, has a cytologically undetectable sterile mutation in $Y^L$. Moreover, *L18*, which is broken in region $Xhy^+$ and exhibits a cytologically normal $Y^S$, is sterile in combination with $\overline{XY^L}$ and thus carries a sterile on $Y^S$ which, however, is not associated with the translocation breakpoint.

An examination of the results of complementation tests between the *T(Y;A)*s and the Brosseau's testers reveals that all the translocations broken in the same region of the *Y* chromosome behave in the same way. The 3 translocations broken in region h14 all fail to complement *In(Y)B1*, whereas they do complement *In(Y)B2*, *In(Y)B3* and *Df(Y)B4*. *R155* (Table 3), which is broken at the junction between regions h10 and h11, complements all the four Brosseau's testers, whereas *P57* (Table 3), which is broken at the junction between regions h9 and h10, fails to complement only *In(Y)B2*. The 2 translocations broken in region h7, the 30 translocations broken in region h3, and the 4 translocations broken in region h1/h2 are all sterile in combination with either *In(Y)B2* or *In(Y)B3* and fertile with *In(Y)B1* and *Df(Y)B4*. The 21 translocations broken in region h21 and the 9 broken in region h23 are sterile with *Df(Y)B4*, but fertile with the other 3 *Y* chromosomes.

Several conclusions can be drawn from these complementation experiments. First, they clearly show that a $B^SYy^+$-autosome translocation in combination with a $y^+Y$

**Table 3.** Complementation tests between sterile *T(Y;2)*s, $\overline{XY^L}$, $\overline{XY^S}$ and Brosseau's *Y*s

| Translocation(s) | *Y* breakpoint(s)[a] | $\overline{XY^S}$ | *In(Y)B1* | *In(Y)B2* | *In(Y)B3* | *Df(Y)B4* | $\overline{XY^L}$ |
|---|---|---|---|---|---|---|---|
| *R50* | h1/h2 | S | F | S | S | – | F |
| *G53* | h3 | S | F | S | S | F | F |
| *A24, A171, B110, D19, H116, H136, H144, L52, L110, R14* | h3 | S | F | S | S | – | F |
| *R124*[b] | h3 | S | F | S | S | S | S |
| *G74* | h7 | S | F | S | S | F | F |
| *P57* | h9/h10 | S | F | S | F | F | F |
| *R155* | h10/h11 | S | F | F | F | F | F |
| *B242, J146* | h14 | S | S | F | F | F | F |
| *H69* | h21 | F | F | F | F | S | S |
| *A162, B4, B106, H149, J69, J165, L23, P8, P51, R93, R127* | h21 | F | – | – | – | S | S |
| *B24, B135, J163* | h23 | F | – | – | – | S | S |
| *A80* | h21+Df($B^S$Xh; h7) | S | F | S | S | S | S |
| *J96* | h16+Df($B^S$Xh; h8) | S | F | S | S | – | F |
| *R158* | $Xhy^+$+Df($B^S$Xhj; h10) | S | F | S | S | F | F |
| *J118* | h21+Df(h3; h10) | S | F | S | S | S | S |
| *H121* | h16+Df(h3; h13) | S | F | S | S | – | F |
| *J59* | $Xhy^+$+Df(h3;h16) | S | S | S | S | F | F |
| *H52* | $Xhy^+$+Df(h3;h16) | S | S | S | S | – | F |
| *J136* | $B^S$Xh+Df(h3; h9) | S | F | S | S | – | F |
| *A111* | h14+Df(h5; h11) | S | S | S | F | F | F |
| *H143* | T=Df($B^S$Xh; h14) | S | S | S | S | – | F |
| *B236* | T=Df($B^S$Xh; h16) | S | S | S | S | – | F |
| *D211* | T=Df($B^S$Xhj; h3) | S | F | S | S | – | F |
| *R136* | T=Df(h3; h7) | S | F | S | S | – | F |
| *J70*[c] | h11+In(h3; h21) | S | F | S | S | S | S |
| *A120*[d] | h21+In(h16; h21) | F | – | – | – | S | S |
| *B80*[e] | $Xhy^+$+In(h21; $Xhy^+$) | F | – | – | – | S | S |
| *H64*[f] | Ins(h3) | S | F | S | S | – | F |
| *J122*[g] | $Y^L$ | S | F | S | S | – | F |
| *J166*[h] | h25D+$Y^L$ | S | F | S | S | F | F |
| *D106*[i] | In($Y^L$)+Df($Y^L$)+Df($Y^S$) | S | F | S | S | S | S |

[a] Df: deficiency; In: inversion; T: translocation; Ins: insertional translocation. T=Df: translocation and deficiency with common breakpoints. A diagrammatic representation of *T(Y; A)*s carrying *Y* deficiencies is given in Figure 10

[b] No cytological defects in $Y^S$

[c] Inversion: *Y* proximal element: $B^SXh$-h3/h21–h11/autosomal euchromatin. *Y* distal element: autosomal centric piece/h11-h3/h21-$Xhy^+$

[d] Inversion: *Y* proximal element: $B^SXh$-h16/h21-h16/autosomal euchromatin. *Y* distal element: autosomal centric piece/h21-$Xhy^+$

[e] Inversion: *Y* proximal element: $B^SXh$-h21/$Xhy^+$-h21/autosomal euchromatin. *Y* distal element: autosomal centric piece/$Xhy^+$

[f] Autosomal euchromatin inserted into region h3

[g] Complex rearrangement involving the distal part of $Y^L$; difficult to resolve cytologically

[h] Carries a rearrangement in $Y^L$; difficult to resolve cytologically

[i] Complex: inversion plus deletions of regions h3–h11 and h21-$Xhy^+$

**Table 4.** Complementation tests between sterile *T(Y;3)*s, $\overline{XY^L}$, $\overline{XY^S}$ and Brosseau's *Y*s

| Translocation(s) | *Y* Breakpoint(s)[a] | $\overline{XY^S}$ | *In(Y)B1* | *In(Y)B2* | *In(Y)B3* | *Df(Y)B4* | $\overline{XY^L}$ |
|---|---|---|---|---|---|---|---|
| *J94* | h1/h2 | S | F | S | S | F | F |
| *B130, R71* | h1/h2 | S | F | S | S | – | F |
| *A14, A121, B68, B152, B197, B217, D100, G11, G42, G116, G122, G144, H147, J23, L14, L142, R6, R117* | h3 | S | F | S | S | – | F |
| *D107* | h7 | S | F | S | S | – | F |
| *A23* | h14 | S | S | F | F | – | F |
| *A173, B93, D224, G71, H135, J95, J100, J128, R130* | h21 | F | – | – | – | S | S |
| *J162* | h23 | F | F | F | F | S | S |
| *B132, G45, G130, R78, R119* | h23 | F | – | – | – | S | S |
| *R86*[b] | h25D | S | F | S | S | F | F |
| *L18*[c] | $Xhy^+$ | F | F | F | F | S | S |
| *L132* | $Xhy^+ + Df(B^SXh;$ h16) | S | S | S | S | F | F |
| *J112* | $h25D + Df(B^SXhj;$ h3) | S | F | S | S | F | F |
| *J154* | h25D + Df(h1/h2; h3) | S | F | S | S | F | F |
| *G48* | $Xhy^+ + Df(h3; h7)$ | S | F | S | S | F | F |
| *B225* | $B^SXh + Df(h3; h10)$ | S | F | S | S | – | F |
| *A78* | $T = Df(B^SXh; h10)$ | S | F | S | S | – | F |
| *A109* | $T = Df(B^SXh; h13)$ | S | F | S | S | F | F |
| *B172* | $T = Df(B^SXh; h3)$ | S | F | S | S | – | F |
| *G8*[d] | T = Df(h3; h8) | S | F | S | S | S | S |
| *A158* | T = Df(h3; h14) | S | S | S | S | – | F |
| *H138*[e] | $h21 + Df(Y^L)$ | S | F | S | S | S | S |
| *B155*[f] | $h3 + Df(Y^L)$ | S | F | S | S | S | S |
| *R98*[g] | h11 + In(h3; h16) | S | F | S | S | F | F |
| *D221*[h] | $h23 + In(Y^L)$ | S | F | S | S | S | S |
| *B21*[i] | $Y^S$ | F | F | F | F | S | S |
| *A81*[j] | Ins(h3; h10) | S | F | S | S | – | F |
| *G110*[k] | $Ins(B^SXh;$ h3) | S | F | S | S | – | F |

[a] See footnote of Table 3
[b] No cytological defects visible in $Y^L$
[c] No other cytological defects visible in $Y^S$
[d] No cytological defects in $Y^S$
[e] Exhibits a small deficiency of fluorescent material in $Y^L$ not resolvable cytologically
[f] Carries a small deficiency of fluorescent material in $Y^L$ not resolvable cytologically. No cytological defects in $Y^S$
[g] Inversion: *Y* distal element: $B^SXh$-h3/h16-h11/autosomal centric piece. *Y* proximal element: autosomal euchromatin/h11-h3/h16-$Xhy^+$
[h] Carries a short inversion on $Y^L$ probably involving regions h3 and h6
[i] Complex: inversion plus translocation in $Y^S$ not resolvable cytologically
[j] Region h3–h10 inserted into the autosomal euchromatin
[k] Region $B^SXh$-h3 inserted into the autosomal euchromatin and autosomal euchromatin appended to region h3

complements regularly: when the *T(Y;A)*s and the $y^+Y$ testers have breakpoints or deletions involving different regions of the *Y* chromosome, the males are fertile; but when they share common cytological defects, the males are sterile.

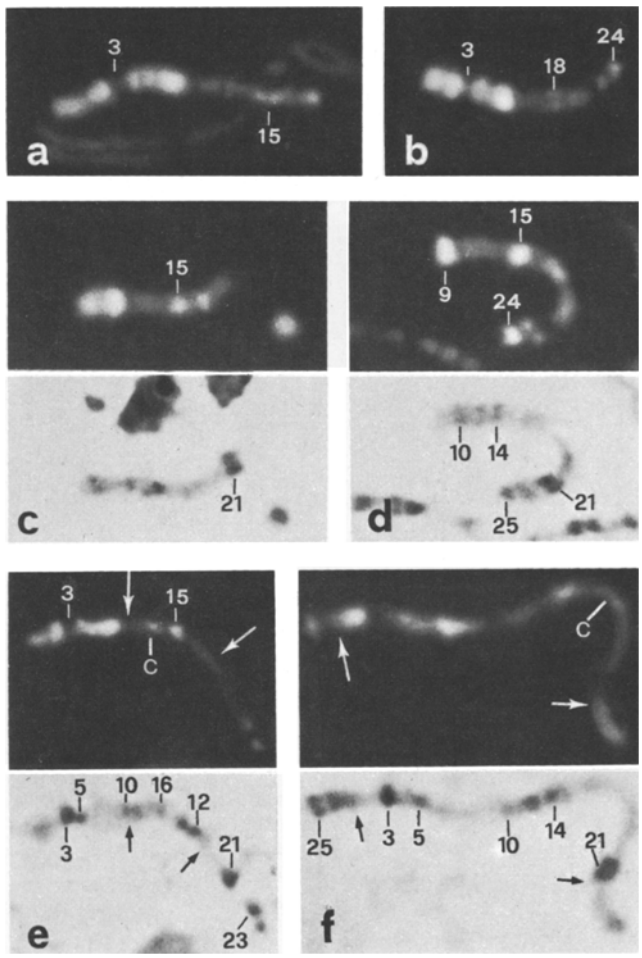


**Fig. 12.** Cytological characterization of γ-ray-induced sterile $y^+Y$ chromosomes. A diagram of these rearrangements is given in Figure 13. **a** *Df(Y)S12* (h19; h25). **b** *Df(Y)S6* (h13; h17). **c** *Df(Y)S13* [*Df(Y)S13L* (h3; h8) + *Df(Y)*S13R (h22–h25)]. **d** *Df(Y)S8* ($y^+Xh$; h8). **e** *In(Y)S18* (h10/h11; h20). Note that in the N-banded *Y* chromosome, region h10 is less heavily stained than both regions h12 and h14. Moreover in this particular preparation regions h16 and h19, which normally are N-band negative, are palely stained. **f** *In(Y)S19* ($y^+Xhj$; h21). *Arrows* point to the inversion breakpoints; numbers indicate cytological landmarks

(The translocations broken in region h7 do not behave in this way and will be discussed later.) Moreover, the fact that most translocations broken in a given region of the *Y* chromosome fail to complement with rearrangements involving the same region strongly suggests that their sterility is associated with their translocation breakpoint.

These complementation data identify four genetic units in $Y^L$ and one unit in $Y^S$: the first unit in $Y^L$ is defined by the translocations broken in region h14, the second by *R155*, the third by *P57*, and the fourth by the translocations broken in region h1/h2, h3 and h7, the only complementation group in $Y^S$ is defined by breakpoints in both regions h21 and h23. Thus these complementation experiments were not sufficient to determine whether regions h1/h2, h3 and h7 of $Y^L$, and regions h21 and h23 of $Y^S$ identify different fertility factors.

*Cytological and genetic characterization of a group of newly isolated sterile Y chromosomes.* To integrate and confirm


**Fig. 13.** Diagram of sterile $y^+Ys$. *In(Y)S16* carries two inversions; *In(Y)B2* carries an inversion plus a deficiency; *T(Y;3)S21* is involved in a translocation with *3R* and also carries a deficiency; *T(Y;3)S20* has its translocation breakpoint in region $y^+Xh$ and, although it is male sterile, does not exhibit any other apparent cytological defect. Similarly *In(Y)S15*, although it is *kl-1*$^-$ (see later), does not show defects involving regions h13–h15

the previous data we needed further sterile $y^+Y$ chromosomes to use in complementation tests with sterile *T(Y;A)*s. A sample of 20 putative sterile $y^+Y$ chromosomes was generously provided by D. Lindsley. These chromosomes were isolated 5 years ago by M. Schwartz after $\gamma$-irradiation (unpublished), and since then were kept in Lindsley's laboratory without further characterization. Complementation tests with $\overline{XY^S}$ and $\overline{XY^L}$ had shown that 12 of these chromosomes had mutation(s) in *KL*, 6 had mutation(s) in *KS* and 2 had mutations in both *KL* and *KS*.

The characterization by Hoechst and N-banding revealed that 18 of these 20 $y^+Ys$ carry chromosome rearrangements (Figs. 12 and 13): 7 carry deficiencies in $Y^L$, 1 has a deficiency in $Y^S$ and 2 have deficiencies in both $Y^L$ and $Y^S$ (these chromosomes were designated as *Df(Y)S5* through *Df(Y)S14*), 2 carry paracentric inversions on $Y^L$ (*In(Y)S15*, *In(Y)S16*), 1 has a paracentric inversion in $Y^S$ (*In(Y)S17*) and 2 carry pericentric inversions (*In(Y)S18*, *In(Y)S19*), 1 has a translocation involving $Y^L$ (*T(Y;3)S20*), 1 has a translocation plus a deficiency in $Y^L$ (*T(Y;3)S21*) and 1 has a translocatin involving $Y^S$ (*T(Y;3)S22*). Of these sterile $y^+Y$ chromosomes 2 do not exhibit any cytologically detectable defect and were designated as *ms(Y)S23* and *ms(Y)S24*.

The 14 sterile rearrangements with mutations in $Y^L$ and the 3 Brosseau's *Ys* defective in $Y^L$ were tested for complementation in all possible pairwise combinations. The results of this analysis show that these 17 *Y* chromosomes define five complementation groups (Table 5). If these complementation groups are designated as *kl-1* through *kl-5* with *kl-1* identified by the most proximal rearrangements (*In(Y)B1*, *Df(Y)S5* and *Df(Y)S6*) and *kl-5* by the most distal one (*In(Y)B3*), the *kl-4* complementation group is defined only by the two noncomplementing translocations *T(Y,3)S20* and *T(Y;3)S21*. Both these translocations are broken in *3R* euchromatin and each of them has an additional sterile in a complementation group other than *kl-4*: *T(Y;3)S20* is also *kl-3*$^-$ and *T(Y;3)S21* is also *kl-5*$^-$. The findings that (1) *T(Y;3)S20* and *T(Y;3)S21* do not have common *Y* chromosome breakpoints (Fig. 13) and (2) flies carrying two heterozygous rearrangements both involving *3R* sometimes are male sterile (Kennison, personal communication) suggested that these translocations may fail to complement for reasons other than being defective in the same *Y* fertility factor. To test this possibility males carrying either *T(Y;3)S20* or *T(Y;3)S21* in combination with either reciprocal element of *T(X;Y)V24* were tested for fertility. *T(X;Y)V24* is male fertile and according to Kennison (1981) breaks the *Y* chromosome between the two most distal fertility factors of $Y^L$ (these two factors were designated by Kennison as *kl-3* and *kl-5* and are genetically the same as our *kl-3* and *kl-5*). If our putative *kl-4* really existed, one of the two reciprocal elements of *V24* would be *kl-4*$^-$ and thus fail to complement both *T(Y;3)S20* and *T(Y;3)S21*. Alternatively, if *kl-4* did not exist, the $Y^DX^P$ element of *V24* would be *kl-5*$^+$ *kl-3*$^-$ and complement *T(Y;3)S21* but not *T(Y;3)S20*, whereas the $X^DY^P$ element would be *kl-5*$^-$ *kl-3*$^+$ and complement only *T(Y;3)S20*. The results of these complementation tests clearly showed the second alternative to be correct and indicated that the 17 aberrant *Y* chromosomes tested for complementation define only four genetic units in $Y^L$.

A complementation map which summarizes the above genetic experiments is shown in Figure 14. In this map we designate the three more proximal fertility factors as *kl-1*, *kl-2* and *kl-3* respectively and the most distal one as *kl-5*. We are using this nomenclature because the 4 fertility factors in $Y^L$ defined by our genetic tests do probably correspond to *kl-1*, *kl-2*, *kl-3* and *kl-5* of Brosseau, while the existence of Brosseau's *kl-4* is uncertain (see Discussion).

**Table 5.** Complementation tests between sterile $y^+$ *Ys* with mutations in *KL*

| | *In(Y)B1, Df(Y)S5, Df(Y)S6* | *In(Y)S15* | *Df(Y)S14* | *In(Y)S18* | *In(Y)S16* | *Df(Y)S9, Df(Y)S10, Df(Y)S11* | *T(Y;3)S20* | *In(Y)B2, Df(Y)S7, Df(Y)S8, Df(Y)S13* | *T(Y;3)S21* | *In(Y)B3* |
|---|---|---|---|---|---|---|---|---|---|---|
| *In(Y)B1, Df(Y)S5, Df(Y)S6* | S | S | S | F | F | F | F | F | F | F |
| *In(Y)S15* | | S | S | F | S | S | S | S | F | F |
| *Df(Y)S14* | | | S | S | S | S | S | S | S | S |
| *In(Y)S18* | | | | S | S | S | F | F | F | F |
| *In(Y)S16* | | | | | S | S | S | S | F | F |
| *Df(Y)S9, Df(Y)S10, Df(Y)S11* | | | | | | S | S | S | S | S |
| *T(Y;3)S20* | | | | | | | S | S | S | F |
| *In(Y)B2, Df(Y)S7, Df(Y)S8, Df(Y)S13* | | | | | | | | S | S | S |
| *T(Y;3)S21* | | | | | | | | | S | S |
| *In(Y)B3* | | | | | | | | | | S |

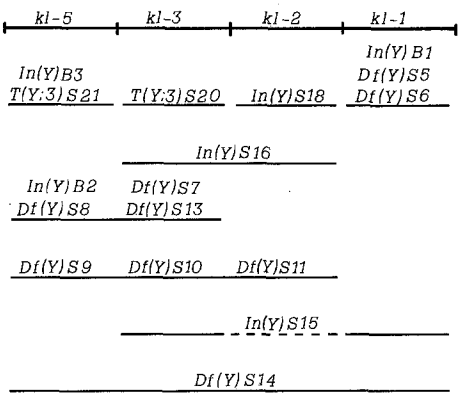


**Fig. 14.** Complementation map for fertility factors on the long arm of the *Y* chromosome. *T(Y;3)S20/T(Y;3)S21* males are sterile, but these translocations fail to complement for reasons other than being defective for the same fertility factor (see text for explanation)

The fact that our putative *kl-4* did not exist is not related in any way to the choice of this nomenclature. The same nomenclature has been used by Kennison (1981) to designate the four fertility factors of $Y^L$ identified by adjacent *Y* chromosome deficiencies generated by combining complementary elements of two *T(X; Y)*s with displaced *Y* chromosome breakpoints. Complementation tests between Kennison's *T(X; Y)*s and our sterile *Ys* have shown that his fertility factors are genetically the same as ours (Kennison, unpublished; Gatti and Pimpinelli, unpublished).

The cytological characterization of the nine *Y* chromosomes with mutations in $Y^S$ is shown in Figures 12 and 13. All these rearranged *Ys* fail to complement each other in all the possible pairwise combinations (complementation matrix not shown). However, when they are tested for complementation with the $X^D Y^P$ element of *T(X; Y)V8*, which carries *KL* and *ks-1*$^+$ but not *ks-2*$^+$ (Kennison 1981), they can be grouped in two categories: (1) those lacking only *ks-1* (*ms(Y)S23*, *ms(Y)S24*, *In(Y)S19* and *T(Y;3)S22*) and (2) those lacking both *ks-1* and *ks*-2 (*Df(Y)B4*, *Df(Y)S12*, *Df(Y)S13*, *Df(Y)S14* and *In(Y)S17*).

*Complementation tests between representative sterile T(Y; A)s and rearranged sterile $y^+Y$ chromosomes.* Since all translocations broken in regions h3 and h21 behaved the same in complementation tests with Brosseau's *Ys*, only some representative translocations with different breakpoints within these regions were tested for complementation with the group of rearranged $y^+$ *Ys* described above.

The results of complementation tests between rearrangements with sterile mutations in *KL* (Table 6) show that the three translocations broken in region h14 are *kl-1*$^-$, *R155* (broken between regions h10 and h11) is *kl-2*$^-$ and *P57* (broken between regions h9 and h10) is *kl-3*$^-$. *G74* and *D107*, both broken in region h7, behave as double mutants in that they are sterile in combination with both *kl-3*$^-$ and *kl-5*$^-$ chromosomes. Finally, the translocations broken in region h3 and those broken in region h1/h2 show identical complementation patterns and are *kl-5*$^-$. Thus, these complementation experiments failed to identify fertility factors other than the four identified by complementation tests between rearranged $y^+$ *Y* chromosomes (Fig. 14).

The finding that translocations broken in region h1/h2 as well as those broken in region h3 identify the same fertility factor is striking. This is not due to a failure of the *Y* deficiencies against which these translocations were tested to separate these breakpoints. In fact, as shown in Figure 15, translocations broken in region h1/h2 and distally in region h3 fail to complement *Df(Y)S9*, *Df(Y)S11* and *Df(Y)S13L* which delete *Y* chromosome material proximal to the breakpoints of these translocations. Moreover *Df(Y)S21* which is deficient only for regions h1 and h2 fails to complement *Df(Y)S9*, *Df(Y)S11* and *Df(Y)S13L* carrying deficiencies proximal to region 2 and *B110*, *D100* and *G122* which are broken in the proximal part of region h3. Thus translocation breakpoints proximal, internal and

**Table 6.** Complementation tests between representative sterile *T(Y;A)*s and $y^+$ *Y* chromosomes with mutations in *KL*. The *Y* breakpoints of the translocations are in parentheses

| | (h14) *A23* *B242* *J146* | (h10/h11) *R155* | (h9/h10) *P57* | (h7) *D107* *G74* | (h3) *A24* *A121* *B110* *D19* *D100* *G42* *G53* *G122* | (h1/h2) *B130* *J94* *R50* *R71* |
|---|---|---|---|---|---|---|
| *In(Y)B1, Df(Y)S5, Df(Y)S6 (kl-1⁻)* | S | F | F | F | F | F |
| *In(Y)S18(kl-2⁻)* | F | S | F | F | F | F |
| *T(Y;3)S20(kl-3⁻)* | F | F | S | S | F | F |
| *In(Y)B3, T(Y;3)S21(kl-5⁻)* | F | F | F | S | S | S |
| *In(Y)S15(kl-1⁻-3⁻)* | S | F | S | S | F | F |
| *Df(Y)S14*[a]*(kl-1⁻-2⁻-3⁻-5⁻)* | S | S | S | S | S | S |
| *In(Y)S16(kl-2⁻-3⁻)* | F | S | S | S | F | F |
| *Df(Y)S9, Df(Y)S10, Df(Y)S11(kl-2⁻-3⁻-5⁻)* | F | S | S | S | S | S |
| *In(Y)B2, Df(Y)S7, Df(Y)S8, Df(Y)S13*[a]*(kl-3⁻-5⁻)* | F | F | S | S | S | S |

[a] Also deficient for *KS*

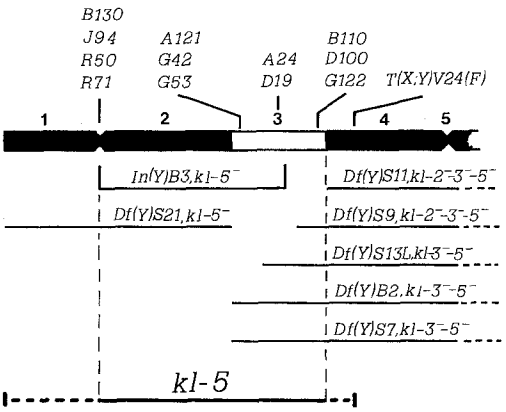


**Fig. 15.** Cytogenetic map of the *kl-5* locus. All the rearrangements listed below the diagram of the distal section of $Y^L$ do not complement each other and do not complement the *T(Y;A)s* listed above. The three groups of translocations represented by *A121*, *A24* and *B110*, are broken in the distal, middle and proximal third of region h3, respectively. *T(X;Y)V24* is male fertile (Kennison 1981). The *solid line* represents the extent of *kl-5* as defined by the breakpoints of the sterile noncomplementing rearrangements. The *broken line* indicates the proximal and distal outside limits of the locus defined by the nearest fertile breakpoints. See text for further explanation

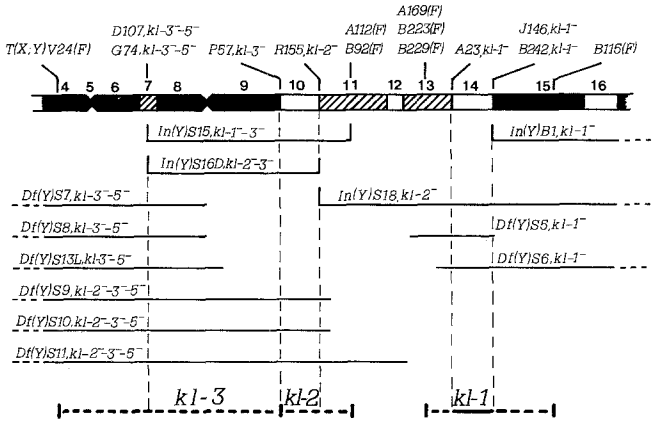


**Fig. 16.** Cytogenetic map of *kl-1*, *kl-2* and *kl-3*. *A23*, *B242* and *J146* do not complement *In(Y)S15*, *kl-1⁻-3⁻* which exhibits an apparently normal h13–h15 region. Moreover *D107*, *G74* and *P57* do not complement *T(Y;3)S20*, *kl-3⁻* which is broken in region $B^S Xh$, but has an apparently normal $Y^L$. All the translocations followed by (F) are male fertile. See text and legend of Figure 15 for further explanation

distal to region h3 as well as those localized in region h1/h2 all identify the same genetic functional unit. In other words, *kl-5* is an enormous genetic locus extending at least from region h1/h2 to the junction between regions h3 and h4. The outside proximal and distal limits of *kl-5* are not precisely determined. However, they should not extend beyond the distal part of region h4, where the male fertile *T(X;Y)V24* is broken (Kennison 1981, and confirmed in the present study), and the junction between regions h1 and $B^S Xhj$ which corresponds to the end of an unmarked *Y*.

Due to the paucity of breakpoints inactivating the three more proximal fertility factors of $Y^L$ the cytogenetic maps of *kl-1*, *kl-2* and *kl-3* are much less well defined than that of *kl-5* (Fig. 16). *kl-1* appears to be a large locus involving at least all of region h14. This is suggested by the failure of complementation between *A23* and *In(Y)B1*, broken distally and proximally to region h14 respectively. The current proximal outside limit of *kl-1* is determined by the breakpoint in region h15 of the male fertile translocation *B115*, and its distal outside limit by the breakpoints in region h13 of the fertile translocations *A169*, *B223* and *B229* (Fig. 16). *kl-2* is defined by three rearrangements (*R155*, *In(Y)S18* and *In(Y)S16D*), all localized at the junction between regions h10 and h11. Its outside proximal limit is defined by the two fertile translocations broken in region h11 (*B92* and *A112*) and its distal limit by *P57* broken at the junction between regions h9 and h10 which is *kl-2⁺ kl-3⁻*. The sterility of *P57* could be due either to its breakpoint or to an independent, cytologically undetectable, lesion in region h7 (which is broken in four *kl-3⁻* rearrange-

**Table 7.** Complementation tests between representative sterile *T(Y;A)*s and $y^+Y$ chromosomes with mutations in *KS*. The *Y* breakpoints of the translocations are in parentheses

| | (h21) | (h23) | (fertile |
|---|---|---|---|
| | *A162*[a] | *B24* | controls) |
| | *H135* | *D221*[a] | *A77* (h19) |
| | *H138* | *G45*[a] | *R135* (h25D) |
| | *H149* | *G130*[a] | |
| | *J165* | *J162* | |
| | *P8*[a] | *J163* | |
| | *P51* | *R78* | |
| | *R93* | *R119* | |
| *ms(Y)S23, ks-1⁻* | S | S | F |
| *ms(Y)S24, ks-1⁻* | S | S | F |
| *In(Y)S19, ks-1⁻* | S | S | F |
| *T(Y;3)S22, ks-1⁻* | S | S | F |
| *Df(Y)B4, ks-1⁻-2⁻* | S | S | F |
| *Df(Y)S12, ks-1⁻-2⁻* | S | S | F |
| *Df(Y)S13, ks-1⁻-2⁻*[b] | S | S | F |
| *Df(Y)S14, ks-1⁻-2⁻*[c] | S | S | F |
| *In(Y)S17, ks-1⁻-2⁻* | S | S | F |

[a] Sterile in combination with $Y^DX^PV8$, $ks\text{-}1^-ks\text{-}2^+$ and fertile with $X^DY^PV8$, $ks\text{-}1^+ks\text{-}2^-$

[b] Also deficient for *kl-3-5*

[c] Also deficient for *KL*

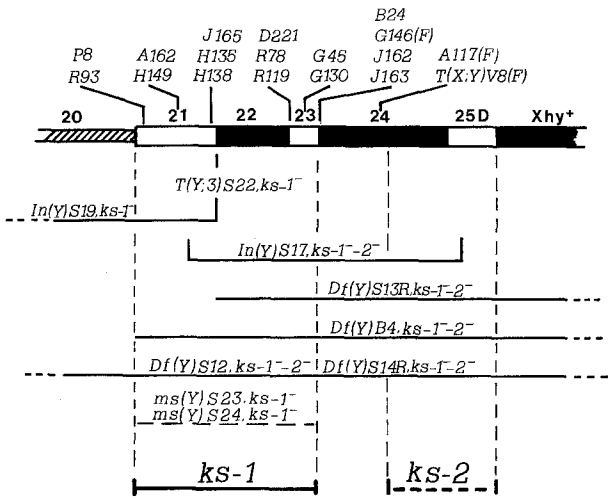


**Fig. 17.** Cytogenetic map of *KS*. All the *T(Y;A)*s drawn above the diagram (except *A117* and *G146*) fail to complement the rearranged $y^+Y$s drawn below and the two cytologically normal *ms(Y)S23* and *ms(Y)S24*. The three groups of translocations represented by *P8*, *A162* and *J165* are broken in the proximal, middle and distal third of region h21, respectively. Similarly, the three groups of translocations represented by *D221*, *G45* and *B24* involve the proximal, middle and distal part of region h23, respectively. A careful cytological analysis of *G146* has shown that it is broken at the junction between regions h23 and h24. The translocations followed by (F) are male fertile. The localization of *ks-2* is defined by: (1) the breakpoint of the male fertile *T(X;Y)V8* that separates *ks-1* from *ks-2* (Kennison 1981) and (2) by the breakpoint of the male fertile *T(Y;3)A117* that has been used to generate a male sterile deficiency extending distally from the middle of region h24 and including region h25D (see next section)

ments). In either case the breakpoint of *P57* should determine the outside distal limit of *kl-2*. However, while in the first case *kl-3* would extend at least from region h7 to the junction between regions h9 and h10, in the second case, it would be defined only by the breakpoints in region h7.

Surprisingly, the two translocations broken in region h7, *G74* and *D107*, although having cytologically normal *kl-5* regions, are both $kl\text{-}3^-\text{-}5^-$. It appears unlikely that this is due to chance because the probability that a translocation has a cytologically undetectable mutation not associated with its breakpoint is less than 5% (see Tables 3 and 4). *kl-5* and *kl-3* are genuinely two separate complementation groups, since *In(Y)S15* and *In(Y)S16*, which are both broken in region h7, are $kl\text{-}3^-$ but not $kl\text{-}5^-$. In addition, Kennison (1981) described four *T(Xh;Y)*s broken in region h7 which are only $kl\text{-}3^-$, and three *T(Xh;Y)*s broken in region h4 which are male fertile. It appears therefore that most breakpoints in region h7 or distal to region h7 do not exhibit a long-range effect leading to the inactivation of the *kl-5* locus in region h3. Thus, although it cannot be excluded that the $kl\text{-}5^+$ function is impaired when region h7 is joined to autosomal euchromatin, the simplest hypothesis is that both *G74* and *D107* are real $kl\text{-}3^-\text{-}5^-$ double mutants.

The results of complementation tests between *T(Y;A)*s and rearranged $y^+Y$s with mutations in *KS* are given in Table 7. Translocations broken in either regions h21 or h23 fail to complement either $ks\text{-}1^-$ or $ks\text{-}1^-\text{-}2^-$ testers. In addition, these translocations do not complement the *Y* distal element of *T(X;Y)V8*, but are fertile in combination with its reciprocal *Y* proximal element. (*V8* is a male fertile *Xh-Y* translocation which separates *ks-1* from *ks-2* (Kennison 1981), and we confirmed by both Hoechst and N-banding that it is broken in region h24). Thus breaks in regions h21 and h23 both affect the same genetic functional unit. The complementation data that lead to the conclusion that *ks-1* is also a physically enormous genetic locus are shown diagrammatically in Figure 17. The proximal outside limit of *ks-1* is most likely the junction between regions h20 and h21. This is suggested by the fact that several bobbed lethal *Y* chromosomes deficient for the entire region h20, but retaining region h21, are fertile in combination with a $bb^+X$ chromosome (Pimpinelli et al., in preparation). The distal outside limit of *ks-1* is the junction between regions h23 and h24 where the fertile translocation *G146* is broken.

The breakpoint in region h24 of the male fertile *T(X;Y)V8* (broken between *ks-1* and *ks-2*) delimits proximally the region which contains *ks-2*. This locus cannot extend distally beyond the junction between regions h25D and $Xhy^+$ because this junction corresponds to the end of an unmarked *Y* chromosome (Fig. 17). Thus, *ks-2* appears to be in region h25D and, in contrast to the other fertility factors, is not inactivated by translocation breakpoints: 12 of 13 translocations broken in region h25D are fertile and the only one which is sterile has a second-site sterile mutation in *KL*. This fact, rather than being a peculiarity of *ks-2*, might be related to the particular organization of this locus in the $B^SYy^+$ chromosome. Since region h25D is about twice as large as the corresponding region h25 of a normal *Y*, it may contain a duplicate *ks-2* locus which is not inactivated by a single breakpoint.

*Generation of deficiencies and duplications of the Y chromosome.* *Y*-autosome translocations not only offer the opportunity of generating segmental aneuploidy for autosomal material (Lindsley et al. 1972) but can also be employed

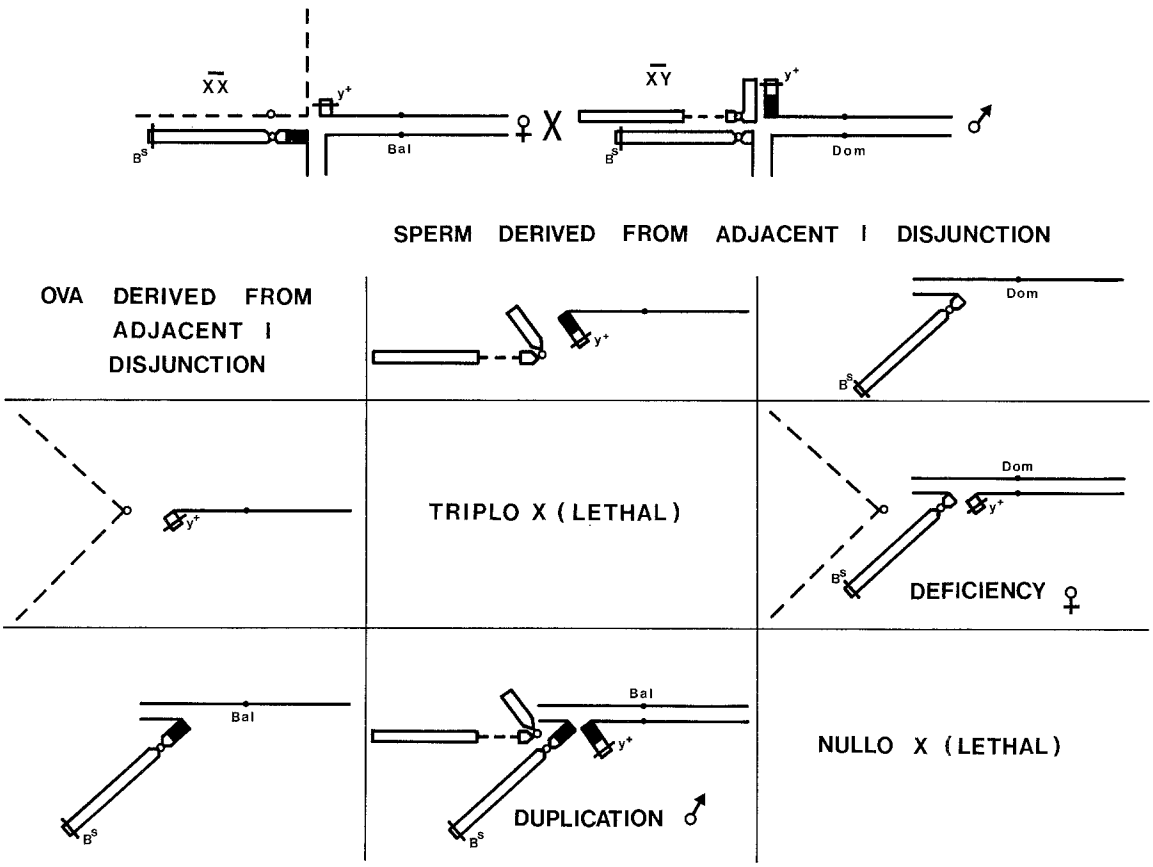


**Fig. 18.** The production of *Y* chromosome duplications and deficiencies by combining products of adjacent I disjunction from parents heterozygous for different *Y*-autosome translocations with displaced *Y* chromosome breakpoints and close autosomal breakpoints. The translocated *Y* chromosomes, represented by bars, are marked with $B^S$ at the end of $Y^L$ and $y^+$ at the end of $Y^S$. Autosomal material and *X* chromosome material are represented by solid lines and broken lines respectively. *Bal* signifies a balancer chromosome that is *In(2LR)SM1*, $al^2$ *Cy* $cn^2$ $sp^2$ in crosses involving *T(Y;2)*s, and *In(3LR)TM6*, $ss^-$ $bx^{34e}$ $Ubx^{67b}$ *e* in crosses involving *T(Y;3)*s. *Dom* signifies a normal sequence dominant-bearing chromosome that is *Sco* in crosses involving *T(Y;2)*s and *Sb* in crosses involving *T(Y;3)*s. In the case shown the *Y* chromosome breakpoint of the paternal *T(Y;A)* is proximal to that of the maternal *T(Y;A)* and the region delimited by these breakpoints is represented as a solid bar. If the autosomal breakpoint of the paternal translocation is proximal to that of the maternal one, an autosomal deficiency is generated in males concomitant with the *Y* chromosome duplication, whereas a corresponding autosomal duplication accompanies the *Y* chromosome deficiency in females. The opposite occurs when the paternal *T(Y;A)* has an autosomal breakpoint distal to that of the maternal *T(Y;A)* (see Lindsley et al. 1972). If the paternal *T(Y;A)* is used as maternal translocation and vice versa, the *Y* chromosome duplication depicted in males will be present in females, whereas the deficiency present in females will occur in males. All the *Y* chromosome deficiencies and duplications generated in our experiments were recovered in males carrying an attached *XY*. These males were then crossed with *y w f* virgins and their sons bearing either a *Y* chromosome deficiency or duplication were tested for fertility. This procedure rules out the possibility that our results are biased by dominant effects on males fertility caused by autosomal segmental aneuploidy

to generate deficiencies and duplications of the *Y* chromosome. Although not pointed out by Lindsley et al. (1972), the same rationale and experimental details used for the construction of autosomal segmental aneuploidy can also be applied to generate duplications and deficiencies for *Y* chromosome material. Figure 18 illustrates the genetic technique for constructing duplications and deficiencies of $Y^L$ using two translocations with displaced *Y* chromosome breakpoints in the long arm and similar autosomal breakpoints. An analogous experimental scheme can be used to generate duplications and deficiencies of $Y^S$. The main limitation of this technique is that autosomal segmental aneuploidy is generated concomitantly with duplications or deficiencies of the *Y*. Thus, only translocations that produce heterozygous viable autosomal duplications or deficiencies can be employed.

The first question we asked using this technique was whether region h25D really contains the *ks-2* locus. We constructed males carrying an attached *XY* and the *Y* distal, autosomal proximal element of the male fertile *T(Y;3)H173* ($Xhy^+$; 95E) both received from their father, plus the *Y* proximal, autosomal distal element of the male fertile *T(Y;3)A117* (h24, 96A) and the *SM1* balancer chromosome received from their mother (the genetic constitution of these males can be symbolized $\overline{XY}/Y^DA^PH173/A^DY^PA117/SM1$). These males were crossed with *y w f* females and their *y w f*/$Y^DA^PH173/A^DY^PA117$/+ sons carrying a deficiency extending from region h24 to the proximal third of region $Xhy^+$ were tested for fertility. As shown in Table 8, 85 males deficient from this region gave no progeny, this confirms our previous inference that region h25D is essential for male fertility.

A second point we wanted to confirm was the physically enormous size of the *kl-5* and *ks-1* loci. We therefore tested

**Table 8.** Fertility of *Y* chromosome deficiencies (Df) and duplications (Dp) generated by the segmental aneuploidy technique

| Genome tested | *Y* chromosome[a] constitution | Autosomal constitution | No. of males tested | No. of progeny |
|---|---|---|---|---|
| $A^DY^PA117$ $Y^DA^PH173$ | Df h24-Xhy$^+$ | Df 95E-96A | 85 | 0 |
| $A^DY^PR71$ $Y^DA^PA121$ | Dp h1/h2-h3d | Dp 97B-97DE | 215 | 0 |
| $A^DY^PJ94$ $Y^DA^PG122$ | Dp h1/h2-h3p | Df 66B-67C | 120 | 0 |
| $A^DY^PB110$ $Y^DA^PP57$ | Dp h3p-h9/h10 | Dp 38B-38C | 33 | fertile |
| $A^DY^PB110$ $Y^DA^PB242$ | Dp h3p-h14p | Dp 36C-38C | 23 | fertile |
| $A^DY^PG130$ $Y^DA^PH138$ | Dp h21d-h23m | Dp 66B-66BC | 232 | 0 |
| $A^DY^PB112$ $Y^DA^PJ69$ | Dp h21-h25D | Dp 22A-22C | 36 | fertile |

[a] The letters p, m and d following the region designation indicate that the translocation breakpoint involves the proximal, the middle or the distal part of the region

for fertility males carrying segmental duplications of either the h1/h2–h3 or h21–h23 region of the *Y* chromosome, constructed by combining the complementary elements of two translocations with displaced breakpoints within these regions (cf. Fig. 18). To test whether the complementary elements of two $B^SYy^+$-autosome translocations complement regularly, we also constructed and tested for fertility males carrying h3–h9/h10, h3–h14 and h21–h25D segmental duplications. The two h1/h2–h3 duplications and the h21–h23 duplication are male sterile in the absence of a normal *Y*, whereas duplications h3–h9/h10, h3–h14 and h21–h25D are male fertile (Table 8). These results indicate that segmental *Y* chromosome duplications are fertile when they are constructed from two $T(B^SYy^+;A)$s carrying sterile mutations in different loci. Thus, the sterility of the h1/h2–h3 and h21–h23 segmental duplications is attributable to a failure of complementation between the translocation elements used to generate them. This is consistent with the conclusion that *kl-5* and *ks-1* extend from region h1/h2 through h3 and h21 through h23 respectively.

The observation that $Y^DA^PP57/A^DY^PB110$, $Y^DA^PB242/A^DY^PB110$ and $Y^DA^PJ69/A^DY^PB112$ males are fertile suggested a way to further investigate the phenomenon of synthetic sterility of males carrying two complete $B^SYy^+$-autosome translocations. We constructed males carrying the four elements of each pair of translocations that had been employed to generate these fertile duplications. Fifteen males of each of the three genotypes ($ywf/T(Y;2)B110/T(Y;2)P57$, $ywf/T(Y;2)B110/T(Y;2)B242$ and $ywf/T(Y;2)B112/T(Y;2)J69$) were tested for fertility and were completely sterile. This provides direct evidence that the sterility of males carrying two $B^SYy^+$-autosome translocations is not related in any way to either the *Y* chromosome or the autosomal breakpoint.

## Discussion

### *The cytological organization of the Y chromosome*

The cytological analysis by banding techniques has shown that the *Y* chromosome of *D. melanogaster* is differentiated into a series of heterochromatic blocks having different cytochemical features. Three basic types of heterochromatin can be distinguished along the *Y* chromosome: 1) Hoechst and quinacrine brightly fluorescent, and N-banding negative regions (h1, h2, h4, h6, h8, h9, h15, h17, h22 and h24), 2) Hoechst and quinacrine dully fluorescent, and N-banding negative regions (h7, h11, h13, h19 and h20), 3) Hoechst and quinacrine nonfluorescent, and N-banding positive regions (h3, h5, h10, h12, h14, h21, h23 and h25). The different behavior of these three types of heterochromatin probably reflect a different DNA base composition. This can be inferred by coupling the studies on the molecular basis of quinacrine, Hoechst and N-banding with those on the biochemical characterization and chromosomal localization of highly repetitive DNAs of *Drosophila*.

Quinacrine binds to DNA by intercalation without an appreciable specificity for DNA base composition. However, experiments in vitro showed that the fluorescence of quinacrine bound to DNA strongly depends on base composition: AT-rich DNA enhances quinacrine fluorescence while GC-rich DNA quenches the fluorescence of quinacrine (for reviews see Schnedl 1978; Comings 1978). In vitro studies with Hoechst 33258 indicated that it is bound to DNA by an external attachment to the major groove of the double helix and not by intercalation. These studies also showed that Hoechst 33258 fluorescence is markedly enhanced by AT-rich DNA while the enhancement is less with GC-rich DNA (Comings 1975; Latt and Wohlleb 1975). Thus the fluorescence properties of both quinacrine and Hoechst 33258 would indicate that they preferentially stain AT-rich chromosome regions.

The present cytological studies have shown that the *Y* chromosome of *D. melanogaster* contains several heterochromatic blocks which are extremely quinacrine- and Hoechst-bright as compared to euchromatin. We suggest that each of these fluorochrome-bright blocks contains one of the three AT-rich satellite DNAs mapped on the *Y* chromosome by in situ hybridization (Peacock et al. 1978; Appels and Peacock 1978; Steffensen et al. 1981; Hilliker and Appels 1982). In fact, the localization of the bright bands along the *Y* chromosome is comparable with that of the 1.672, 1.686 and 1.697 g/cm$^3$ satellite DNAs of *Drosophila* (see Figure 11 of Appels and Peacock 1978 and Figure 10 of Steffensen et al. 1981).

The N-banding technique, as developed by Matsui and Sasaki (1973) and Funaki et al. (1975), essentially consists in heating the slides for 15 min at 96° C in a phosphate solution at acidic pH. By this technique, Funaki et al. (1975) were able to show that in 27 animal and plant species the N-bands specificially identify the nucleolus organizer regions (NOR). Since the technical procedure they used involved the extraction of DNA, RNA and histones, they suggested that certain nonhistone proteins specifically linked to the NOR were selectively stained in N-banding.

In several species of the genus *Drosophila* (Pimpinelli et al. 1976), in some mammals (Faust and Vogel 1974), and in wheat (Gerlach 1977) the N-bands do not specifically stain the NORs or at least not only the NORs. Furthermore, in *D. melanogaster* the N-bands do not correspond at all to the NORs, in fact both the NOR in the middle of the *X* heterochromatin and the nucleolus organizer region of the *Y* chromosome (region 20, Pimpinelli et al., in preparation) are completely negative in N-band preparations. *D. melanogaster* exhibits 17 N-bands that are restricted to heterochromatin and localized as follows. There are 8 N-bands in the *Y* chromosome, 5 in $Y^L$ and 3 in $Y^S$, in condensed chromosomes some of these N bands tend to coalesce so that a contracted *Y* exhibits a rather large subterminal N-banded region at each end and another N-banded area in the proximal part of $Y^L$. The *X* chromosome shows only one small N-band localized very proximally while the second chromosome has five heavily stained N-bands. There are 3 N-bands in the third chromosome: two very tiny and difficult to see in *3L* heterochromatin, and one large and heavily stained in the distal part of *3R* heterochromatin. Chromosome *4* exhibits only a small proximal N-band.

If the chromosomal distribution of N-bands is compared with that of satellite DNAs observed after in situ hybridization (Appels and Peacock 1978; Steffensen et al. 1981), an almost absolute correspondence between their localization and that of 1.705 g/cm$^3$ satellite DNA is found (cf. Figs. 3, 8, 11 and 12 of this paper with Figs. 7 and 11 of Appels and Peacock 1978, and Fig. 10 of Steffensen et al. 1981). We suggest therefore that the nonfluorescing N-banded heterochromatic blocks specifically contain the relatively GC-rich 1.705 g/cm$^3$ satellite DNA of *Drosophila*. Gerlach (1977) has already suggested that N-bands corresponded to 1.705 g/cm$^3$ satellite DNA.

In conclusion, by combining the biochemical and in situ hybridization studies on satellite DNAs of *Drosophila* (reviewed by Brutlag et al. 1978; Peacock et al. 1978; Appels and Peacock 1978) with our cytological observations, we suggest the following model for the organization of the *Y* chromosome. The *Y* chromosome is a continuous and heterogeneous array of heterochromatic blocks having different cytological properties. With the exception of region h20 which contains the ribosomal cistrons (Pimpinelli et al., in preparation) and of regions h7, h11, h13, h16 and h19 whose molecular content is unknown, each of these blocks is probably composed of a specific satellite DNA and perhaps of one of the isomeric forms of a satellite DNA (Brutlag et al. 1978; Appels and Peacock 1978). Consequently, each block would contain a long array of a short DNA sequence tandemly repeated. This model does not exclude the possibility that a few unique sequence DNA molecules are interspersed within or between the heterochromatic blocks of the *Y* chromosome. However, the existence of such molecules has not yet been demonstrated.

### *The number of fertility factors*

The present study has shown that there are four linearly ordered fertility factors in $Y^L$ and two fertility factors in $Y^S$. The same number of fertility factors was recently found by Kennison (1981) who employed a large series of male fertile *X-Y* translocations broken in the *X* heterochromatin to generate adjacent segmental deficiencies along the entire length of the *Y* chromosome. He identified four regions in $Y^L$ and two in $Y^S$ that are necessary for male fertility. In addition he performed a series of complementation tests between male sterile translocations broken in each of these regions and failed to resolve more than a single complementation group per region. Four complementation groups in $Y^L$ and two in $Y^S$ were also found by Hazelrigg, Fornili and Kaufman by complementation analysis with newly isolated X-ray-induced sterile $B^S Y y^+$ chromosomes (T. Kaufman, personal communication).

A total of six *Y*-linked fertility factors is consistent with the earlier findings of Neuhaus (1938, 1939) and Brosseau (1960). By complementation between sterile *Y-4* translocations with different *Y* chromosome breakpoints, Neuhaus defined four genetic units necessary for male fertility in $Y^L$ and five units in $Y^S$. Brosseau performed a complementation analysis on a set of X-ray-induced sterile $y^+ Y$ chromosomes and concluded that there were five linearly ordered fertility factors in $Y^L$ and two in $Y^S$. He argued that the experimental scheme used by Neuhaus could have led to the misclassification of the genotype of some of the males tested for fertility and thus to an overestimation of the number of fertility factors in $Y^S$. Brosseau (1960) designated the genes in $Y^L$ as *kl-5* through *kl-1* (*kl-5* being the most distal one) and those in $Y^S$ as *ks-1* and *ks-2* (*ks-2* being distal to *ks-1*). However, just as was our putative *kl-4*, Brosseau's *kl-4* was defined only by two noncomplementing *Y* chromosomes which were $kl\text{-}3^-\text{-}4^-$ and $kl\text{-}4^-\text{-}5^-$ respectively. We believe it likely that these chromosomes also failed to complement for reasons other than being defective in the same fertility factor. As pointed out by Kennison (1981), this is suggested by the results of complementation tests between Brosseau's testers and a series of detachments of compound *X* chromosomes generated by exchanges with the *Y* chromosome (Brosseau 1960, 1964; Lucchesi 1965). If *kl-4* really exists, then exchanges between *kl-3* and *kl-4* should produce a pair of $kl\text{-}3^+\text{-}4^-$ and $kl\text{-}3^-\text{-}4^+$ reciprocal elements, whereas exchanges between *kl-4* and *kl-5* should give rise to $kl\text{-}4^+\text{-}5^-$ and $kl\text{-}4^-\text{-}5^+$ products. Alternatively if *kl-4* does not exist, only reciprocal $kl\text{-}3^+\text{-}5^-$ and $kl\text{-}3^-\text{-}5^+$ detachments should be generated each of them complementing at least one of the two putative $kl\text{-}3^-\text{-}4^-$ and $kl\text{-}4^-\text{-}5^-$ chromosomes of Brosseau. The finding that 8 of 26 detachments tested were $kl\text{-}4^+\text{-}5^-$ and 18, $kl\text{-}3^-\text{-}4^+$, whereas none were $kl\text{-}4^-\text{-}5^+$ or $kl\text{-}3^+\text{-}4^-$, argues strongly for the nonexistence of Brosseau's *kl-4*. Similar results were obtained by Parker (1967) who tested 6 *Y-4* half translocations with breakpoints between *kl-3* and *kl-5* for complementation with Brosseau's *Y*s and found that none of them was $kl\text{-}4^-$. In contrast, Andrews and Williamson (1975) found that 18 of 116 *Y* chromosome fragments from irradiated oocytes with breakpoints between *kl-3* and *kl-5* were $kl\text{-}4^-$.

Taken together these complementation studies indicate that the existence of Brosseau's *kl-4* is uncertain and strongly suggest that the *Y* chromosome of *D. melanogaster* contains but four fertility factors in $Y^L$ and two in $Y^S$. Since the fertility factors identified in the present study probably do correspond, with the exception of *kl-4*, to those defined by Brosseau, we have felt it useful to retain his nomenclature. Thus we designate the four fertility factors in $Y^L$ as *kl-5*, *kl-3*, *kl-2* and *kl-1* and the two factors in $Y^S$ as *ks-1* and *ks-2*.

*The localization of fertility factors*

By combining cytological analysis by Hoechst and N-banding with complementation experiments we mapped the six male fertility factors in specific regions of the *Y* chromosome (Figs. 15, 16 and 17). A comparable cytogenetic map was constructed by Kennison (1981) who examined by Hoechst banding a series of *X-Y* translocations broken in the *X* heterochromatin.

Our results indicate that there is a strict correspondence between the regions necessary for male fertility and those specifically stained in N-band preparations. Cytologically, the N-bands correspond to the nonfluorescent areas seen after Hoechst and quinacrine staining but do not correspond to constrictions; on the contrary they are the most prominent regions of the *Y* chromosome in orcein- or Giemsa-stained preparations. Moreover, as previously suggested, they probably contain the 1.705 g/cm$^3$ satellite DNA of *Drosophila*.

We have shown that five of the six fertility factors (*kl-5*, *kl-2*, *kl-1*, *ks-1* and *ks-2*) are associated with or contain at least one N-band (Figs. 15, 16 and 17). However, this was not demonstrated for the *kl-3* locus (Fig. 17). The *Y* chromosome area in which *kl-3* maps does contain the N-banded region h5, but no breaks involving the N-band itself were found in any of the inversions and translocations examined in the present work. Consequently it was not possible to determine whether breakpoints in region h5 are male sterile and inactivate a specific fertility factor. However, by analogy with the breakpoints involving the other N-bands those in region h5 are also likely to be male sterilizing. Moreover, on the basis of the extensive complementation data previously discussed, they are not expected to identify a fifth fertility factor in $Y^L$. Thus we believe that region h5 is an integral part of the *kl-3* locus and that interruptions in chromosomal continuity in this region would result in *kl-3*$^-$ rearrangements.

Region h12 is the only N-banded area which does not correspond to a fertility factor. Although this region was never found broken in any of the *Y* chromosome rearrangements analyzed, it is deleted in *Df(Y)S11* (Fig. 13). By combining the *Y* distal *X* proximal element of *P7* (*P7* is a male fertile *X-Y* translocation broken in region h11, Kennison 1981 and confirmed by Pimpinelli et al., in preparation) with *Df(Y)S11* we constructed males carrying a *Y* chromosome deletion spanning region h11 to h13 and thus lacking region h12. These males are fertile, but behave as meiotic mutants showing a characteristic meiotic phenotype. They exhibit needle-shaped crystals in primary spermatocytes and produce an elevated frequency of both sex chromosomal and autosomal nondisjunction (Pimpinelli et al., in preparation). Thus although region h12 does not contain a fertility factor, it does include a *Y*-linked heterochromatic locus necessary for normal male meiosis. The separation of the factor responsible for the repression of crystal formation from *kl-2* was observed earlier by Hardy (personal communication).

These results indicate that each N-band identifies a specific genetic function carried out by the *Y* chromosome of *D. melanogaster*. However there is no absolute one-to-one correspondence between N-bands and heterochromatic functions in that the *ks-1* locus contains two N-banded regions separated by a brightly fluorescent block, and the bobbed locus is devoid of N-bands. The correspondence between N-bands and heterochromatic functions may very well extend to the other chromosomes of the *Drosophila* genome. In this respect it is very suggestive to recall that the heterochromatin of chromosome *2* which contains five distinct N-bands (Fig. 8), also carries five to eight vital genes defined by deletion mapping and EMS-induced lethals (Hilliker and Holm 1975; Hilliker 1976). A chromosome *2* carrying a deletion of a single N-band of *2R* heterochromatin has been recently constructed in our laboratory (Dimitri, unpublished). This chromosome was synthesized by recombination between two brown-variegated inversions with displaced heterochromatic breakpoints within *2R* heterochromatin. Complementation analysis with Hilliker's lethals and deficiencies has shown that the absence of this specific N-band results in the loss of a single vital function, defined by the complementation group III of Hilliker (1976). These preliminary results on chromosome *2* are consistent with our findings on the *Y* chromosome and strongly suggest that N-bands identify a class of heterochromatic loci having different genetic functions but similar cytogenetic organization.

*The organization of the fertility factors*

The most interesting result to emerge from this study is the enormous physical size of the *kl-5*, *kl-1* and *ks-1* loci (Figs. 15, 16 and 17). For example *kl-5* is defined by a series of cytologically nonoverlapping, sterile breaks and deficiencies scattered over a long region of the *Y* chromosome in which no fertile breakpoints are found. Thus these sterile, noncomplementing rearrangements operationally define a single functional unit necessary for male fertility.

Our data show that chromosomal continuity within the h1/h2–h3 and h21–h23 regions is crucial for the functioning of the *kl-5* and *ks-1* loci respectively. The most straightforward interpretation of these results is that these regions are coextensive with the functional domains of the *kl-5* and *ks-1* fertility factors. Alternatively regions h1/h2–h3 and h21–h23 might identify chromosome areas within which particular breakpoints exert long-range position effects leading to gene inactivation. Although the latter possibility cannot be excluded, we believe it is unlikely because we examined several kinds of rearrangements involving either the *kl-5* or *ks-1* region and each of these leads to male sterility. Our complementation analysis was performed between *Y*-autosome translocations involving either the second or the third chromosome euchromatin, and rearranged free *Y* chromosomes in which different types of heterochromatin, that are not normally contiguous, are juxtaposed. Moreover, *Y*-autosome translocations involving the heterochromatin of chromosome *2* and broken in regions h3 and h21 failed to complement the *kl-5*$^-$ and *ks-1*$^-$ testers respectively (unpublished results). Similarly male sterile *X-Y* translocations involving *X* heterochromatin and broken in regions h3 and h21–h23 (Kennison 1981) were sterile in combination with *kl-5*$^-$ and *ks-1*$^-$ *Y* chromosomes, respectively (unpublished). These results suggest that, regardless of the type of genetic material involved in the rearrangement, any interruption of chromosomal continuity in any part of the *kl-5* or *ks-1* region acts in a cis-dominant fashion impairing the function of the entire complex.

It has been estimated that the diploid genome of *D. melanogaster* contains approximately 340,000 kb DNA (Rasch et al. 1971), consequently the *Y* chromosome, which

accounts for 12% of the *Drosophila* genome (Pimpinelli et al. 1978), should contain about 40,000 kb. Assuming a uniform distribution of DNA along the *Y* chromosome, we can calculate that *kl-5*, *kl-1* and *ks-1*, which span 8%, 3% and 8% of the *Y* chromosome length, contain 3,200, 1,200 and 3,200 kb DNA respectively. Thus the physical dimension of these heterochromatic genes is two orders of magnitude greater than that of the euchromatic genes of *D. melanogaster* (cf. Judd et al. 1972; Levis et al. 1982).

Due to the paucity of breakpoints inactivating *kl-3*, *kl-2* and *ks-2*, no firm conclusions can yet be reached about the dimensions of these loci. However, since there is no reason to believe that their genetic organization differs from that of the other fertility factors, we propose that these loci also have an extent comparable to those of *kl-5*, *kl-1* and *ks-1*.

These findings provide insight into the specifically high mutability of the *Y* chromosome fertility factors following treatments with both ionizing radiations and chemical mutagens. Whereas the *Y* chromosome contains only six fertility genes, it has been estimated that the *X* chromosome carries 100–350 genes that can mutate to male sterility (Lindsley and Tokuyasu 1980). Nevertheless a series of experiments have clearly established that the sensitivity of the *Y* chromosome to the induction of male sterile mutations by both ionizing radiations and EMS is 2–3 times higher than that of the *X* chromosome (for review see Lindsley and Tokuyasu 1980). It appears therefore that the *Y* fertility genes are 50–150 times more mutable than those on the *X*. Our data provide an explanation for this paradox by suggesting that the *Y* chromosome fertility factors offer much larger physical targets than *X*-linked euchromatic genes, thereby leading to extreme sensitivity to the induction of mutations.

Williamson (1970, 1972) induced a series of sterile *Y* chromosomes with ethyl methanesulfonate (EMS) that were first tested for fertility in combination with Brosseau's *Ys* and then used in an inter se complementation analysis. He found extensive complementation within each of the complementation units defined by the Brosseau's testers. For example he subdivided the *kl-5* and *ks-1* loci into five and three complementation groups, respectively. Williamson (1972) interpreted these results as evidence for interallelic complementation, however they could also be interpreted as indicating the presence of multiple functional subunits within each fertility factor. In contrast, recent studies of Kennison (1983) failed to find complementation among EMS-induced sterile *Y* chromosomes with mutations in the same fertility factor. Although Kennison's analysis was performed on a relatively small number of EMS-induced sterile *Ys*, the reason for this discrepancy is not clear. Thus, until further studies clarify this matter, the possibility exists that the fertility regions of the *Y* chromosome (as defined by complementation tests between rearranged *Ys*) accommodate several functional subunits which could be independently inactivated by point mutations. However the disruption of chromosomal continuity within these regions would affect the expression of all the functional subunits of the complex.

The results of our cytological and genetic analysis of the *Y* chromosome fertility factors strongly suggest that both the *kl-5* and *ks-1* regions (region h1/h2–h3 and h21–h23, respectively) contain very long arrays of tandemly repeated, simple sequence satellite DNA. Although these findings do not clarify the biological function of satellite DNA, they permit it to be delimited within specific functional regions necessary for normal sperm development.

Recent studies have shown that the regions containing *kl-5*, *kl-3* and *kl-2* are responsible for three different high-molecular-weight polypeptides that are structural components of sperm axoneme (Goldstein et al. 1983). Electron microscope observations and electrophoretic analysis indicate that the *kl-5* and *kl-3* polypeptides are components of the axonemal dynein arms (Hardy et al. 1981; Goldstein et al. 1983). Moreover dose variation experiments and the analysis of a temperature-sensitive *kl-5*$^{-}$ mutation led Goldstein and coworkers to suggest that the *kl-5* and *kl-3* loci contain the coding sequences for the dynein polypeptides. This would imply that, in addition to satellite DNA, the fertility factors also contain unique DNA sequences that are regularly transcribed and translated. If this is the case, the highly repetitive DNA of the fertility factors may play some kind of regulatory role in the expression of the structural sequences located in its vicinity.

Although the most straightforward model for the *Y* chromosome fertility factors is that of an enormous region composed mainly of satellite DNA blocks with a few unique DNA sequences interspersed between or within them, there are alternative ways in which these loci could be organized. The possibility exists that the *Y* chromosome fertility factors do not contain any coding sequences and that they regulate the expression of genes located elsewhere in the genome by interacting with their products in some unknown fashion. An example of an interaction between a *Y* chromosome region and an *X*-linked locus has been recently provided by Lovett (1983). She found that deficiencies encompassing the *kl-2-kl-1* region of the *Y* chromosome lead to a 30–40 fold increase of a testis-specific RNA encoded by the 12F1,2 region of the *X* chromosome. These data may be interpreted as indicating that in the absence of a specific *Y* chromosome region, there is an altered metabolism of this particular RNA.

We have not systematically compared our results with those obtained on the heterochromatic *Y* chromosome of *D. hydei*. This system has been subjected to extensive cytological, genetic and molecular studies that indicate that in many aspects it is different from that of *D. melanogaster* (for recent references see Hennig 1978; Bonaccorsi et al. 1981; Hackstein et al. 1982; Vogt et al. 1982; Lifshytz et al. 1983). However, there is evidence that some fertility loci of *D. hydei* also have large physical dimensions. Complementation experiments using a large array of male sterilizing mutations revealed 16 fertility genes on the *Y* chromosome of *D. hydei*, 5 of them are specifically associated with lampbrush-like loops. Cytogenetic analysis showed that no more than a single complementation group can be detected in each of the lampbrush-loop forming sites (Hackstein et al. 1982). Since the lampbrush loops of *D. hydei* contain on average about 500 kb DNA (Hennig 1978), these findings are consistent with ours and indicate that these heterochromatic fertility genes are also much larger than the euchromatic ones.

In conclusion, by coupling cytological and genetic analysis we demonstrate that the *Y* chromosome fertility factors extend over chromosomal regions containing about 3,000 kb DNA. Heterochromatin is currently believed to be composed of essentially genetically inert material in which a few conventional genes are embedded. Our findings

lead us to doubt this view and to propose that heterochromatin is an almost continuous array of genetic functional units having enormous physical dimensions.

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