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# Recombination between the X and Y chromosomes and the Sxr region of the mouse

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## Summary

The Sxr (sex-reversed) region that carries a copy of the mouse Y chromosomal testis-determining gene can be attached to the distal end of either the Y or the X chromosome. During male meiosis, Sxr recombined freely between the X and Y chromosomes, with an estimated recombination frequency not significantly different from 50% in either direction. During female meiosis, Sxr recombined freely between the X chromosome to which it was attached and an X-autosome translocation. A male mouse carrying the original $Sxr^a$ region on its Y chromosome, and the shorter $Sxr^b$ variant on the X, also showed 50% recombination between the sex chromosomes. Evidence of unequal crossing-over between the two Sxr regions was obtained: using five markers deleted from $Sxr^b$, 3 variant Sxr regions were detected in 159 progeny (1·9%). Four other variants (one from the original cross and three from later generations) were presumed to have been derived from illegitimate pairing and crossing-over between $Sxr^b$ and the homologous region on the short arm of the Y chromosome. The generation of new variants throws light on the arrangement of gene loci and other markers within the short arm of the mouse Y chromosome.

## 1. Introduction

The X and Y chromosomes of the mouse, as of other mammals, share a region of homology over which pairing takes place during meiotic prophase in male germ cells. Because a single crossover between one chromatid of the X and one of the Y chromosome takes place in every male meiosis (Burgoyne, 1982), the homologous region does not display complete sex linkage and has been termed the pseudoautosomal region.

A rearranged Y chromosome exists in the mouse (Fig. 1*a*), in which a portion of the short arm of the Y chromosome (Yp) has apparently been duplicated and one copy transposed to the tip of the long arm of the Y (McLaren *et al.* 1988; Roberts *et al.* 1988). Because it is now distal to the pseudoautosomal region, this copy has a 50% chance of being transferred to the X chromosome during male meiosis. It is termed the Sxr region, because an X/X embryo to which an X Sxr-carrying sperm has contributed is sex-reversed, and develops as a male. Evidently the Sxr region carries the genetic information responsible for testis determination. X/X Sxr mice are sterile, since X/X spermatogonia degenerate soon after birth, but X/Y Sxr males are fertile (Cattanach *et al.* 1971).

When Sxr is attached to an inactive X chromosome in any cell of an X/X embryo, the inactivation process may spread to include the testis-determining gene. If this occurs in a sufficiently high proportion of the cells contributing to the embryonic gonad, as can be arranged by pairing the X Sxr chromosome with the preferentially active T(X;16)16H X-autosome translocation (T16H), the embryos may develop as females or hermaphrodites rather than as males (McLaren & Monk, 1982; Cattanach *et al.* 1982). T16H/X Sxr females (Fig. 1*b*) are fertile and can be used to produce X Sxr/Y males (Fig. 1*c*).

Given that legitimate X/Y pairing takes place and is always associated with a single crossover event, X/Y Sxr and X Sxr/Y males should both give equal numbers of progeny in which Sxr is carried by the X and by the Y chromosome. However, Cattanach *et al.* (1990) reported that X Sxr/Y males give fewer than

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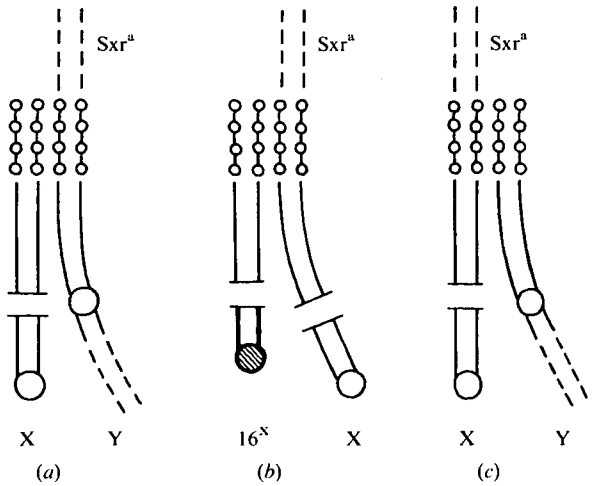


Fig. 1. X and Y chromosomes pair along the homologous pseudoautosomal region (○—○—○—○). (*a*) In a rearranged mouse Y chromosome, the testis-determining region (----) on the short arm has been duplicated, and the second copy inserted distal to the pairing region (Sxr). Progeny of such X/Y Sxr males include X/X Sxr (sex-reversed) male mice, in which the Sxr region is attached to an X chromosome. (*b*) The Sxr region attached to an X chromosome, and paired with the preferentially active T16H X-autosome translocation. T16H/X Sxr mice may be male, female or hermaphrodite. ◐, Proximal region of chromosome 16. (*c*) Progeny of T16H/X Sxr females include X Sxr/Y males.

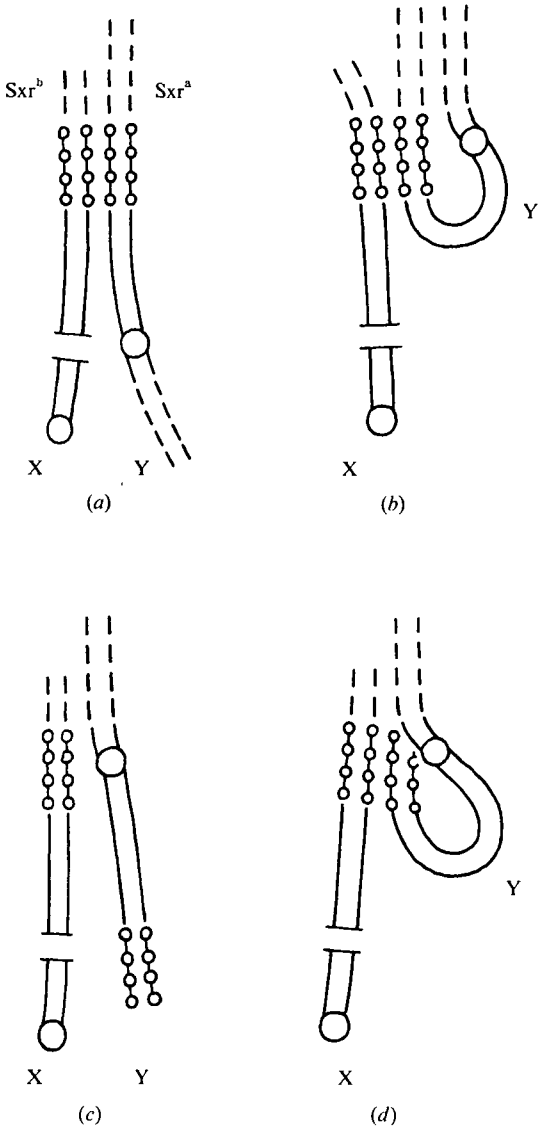


Fig. 2. Possible ways in which pairing can take place between the X and Y chromosomes, when the Y carries an Sxr$^a$ region (----) and the X carries the shortened Sxr$^b$ region (--). (*a*) Legitimate pairing between the X and Y pairing regions (○—○—○—○), and between the two Sxr regions. (*b*) Legitimate pairing between the X and Y pairing regions, with illegitimate 'hairpin pairing' between the Sxr$^a$ region and its testis-determining homologue on the short arm of the Y. (*c*) Illegitimate pairing between the Sxr$^b$ region on the X, and the testis-determining region on the short arm of the Y. (*d*) Illegitimate pairing between the Sxr$^b$ region on the X and the testis-determining region on the short arm of the Y, together with legitimate pairing between the X and Y pairing regions. (For simplicity, it has been assumed that Sxr$^b$ is homologous to the proximal portion of Sxr$^a$, with the distal portion deleted.)

50% recombinant progeny. In the present paper we have examined the progeny of X/Y Sxr males, X Sxr/Y males and T16H/X Sxr females, in order to compare the frequency of recombination of Sxr from the Y to the X, from the X to the Y, and between two X chromosomes.

Because the Y chromosome, and the Sxr region in particular, contains a high proportion of repeated DNA sequences, mismatched pairing and unequal crossing-over occurs relatively frequently. Within the Sxr region, new restriction fragment-length polymorphisms have been shown to arise in approximately 3% of all meiotic events in the male (Epplen *et al.* 1988). By mating T16H/X Sxr females with X/Y Sxr males, X Sxr/Y Sxr males can be produced, in which the two Sxr regions can presumably pair and recombine. If one sex chromosome carries the original Sxr$^a$ region, and the other carries the Sxr$^b$ variant (McLaren *et al.* 1984), from which a large portion has been deleted (Bishop *et al.* 1988), mismatched pairing and unequal crossing-over could result in progeny with intermediate amounts of Sxr DNA (Fig. 2*a*). In the present paper this possibility has been examined in the progeny of an X Sxr$^b$/Y Sxr$^a$ male, using as markers for the deleted region the DNA probe Sx1 (Bishop *et al.* 1988) and four genes, *Sby* that encodes a ubiquitin-activating enzyme (Mitchell *et al.* 1991), *Zfy*-2 that codes for a zinc-finger protein (Mardon *et al.* 1989), *Spy* that is required for spermatogenesis (Burgoyne *et al.* 1986) and *Hya*, which controls expression of the histocompatibility antigen H-Y (McLaren *et al.* 1984). All five markers are present in Sxr$^a$ but missing in Sxr$^b$.

Finally, illegitimate pairing may take place between an Sxr region and the homologous region on Yp. When the Sxr region is carried on the Y chromosome, illegitimate pairing of this nature will result in a

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hairpin-like configuration of the Y during male meiosis, with the looped Y chromosome either separate or simultaneously paired with the X over the pseudoautosomal region (Fig. 2*b*). Chandley & Speed (1987) detected both these configurations by electron microscopy of testis preparations from X/Y Sxr mice. When the Sxr region is carried on the X chromosome, illegitimate pairing will result in Yp pairing with Sxr at the distal end of the X (Fig. 2*c*). The adjacent pseudoautosomal region of the X may also pair with the pseudoautosomal region of the Y (Fig. 2*d*). Recombination following illegitimate pairing of Yp and an $Sxr^a$ region will be hard to detect; if an $Sxr^b$ region is involved, however, some or all of the deleted sequences of $Sxr^b$ may be replaced by corresponding sequences from Yp, as in the anomalous crossing-over event reported by McLaren *et al.* (1988). Other examples are reported in the present paper.

## 2. Materials and Methods

### (i) *Mice*

The source of the stocks of mice used in the present study is given in McLaren & Monk (1982), which also gives details of the procedures used to classify the progeny of some of the crosses. The T16H/X females mated to X/Y $Sxr^a$ and X $Sxr^a$/Y males were derived by crossing T16H/X females from a T16H maintenance stock with males from the C3H/HeHa strain into which the *Pgk-1*$^a$ allele had been introduced, so the translocated X carried Pgk-$1^b$ and the normal X Pgk-$1^a$. The X $Sxr^a$/Y males were derived by crossing T16H/X $Sxr^a$ females with $X^{Ta}$/Y males from the original stock in which tabby (*Ta*) had arisen and been maintained (Falconer, 1953). The Y chromosome on to which $Sxr^a$ recombined in this study came from this stock, and so did the Y chromosome on to which the $Sxr^b$ variant recombined after it was first detected in a T16H/X Sxr female (McLaren *et al.* 1984), but it is uncertain whether it represented the original 1953 Y chromosome or whether there had been one or more outcrosses with males of a different stock. In the present study, T16H/X $Sxr^a$ females were mated with either *Ta* or *Gs* males.

The X $Sxr^b$/Y $Sxr^a$ male was derived from a cross between a T16H/X $Sxr^b$ female and an X/Y $Sxr^a$ male. It was identified as 'daughterless' (McLaren & Burgoyne, 1983) by test-mating, and was then mated to In (X)/X females kindly supplied by Paul Burgoyne, and to $X^{Gs}$ O females. In(X)/X females are heterozygous for a large X chromosome inversion: following crossing-over within the inversion, they produce some nullo-X oocytes (Evans & Phillips, 1975).

### (ii) *Identification of chromosome constitution*

When X chromosomes carried *Ta* or *Gs*, progeny were classified by coat phenotype; when *Pgk-1* was used as a marker, blood samples were typed electrophoretically for PGK-1A and B isozyme. When none of these markers was available (as with In(X)X females), XO females were identified by karyotyping cultures of tail tip tissue taken during the first week after birth; chromosome counts were confirmed on bone marrow, using standard cytogenetic procedures. X/Y and T16H/Y males were distinguished from each other and from X/X and T16H/X males by testis weights, confirmed by testis histology, as well as by X chromosome markers.

### (iii) *Classification for $Sxr^a$ markers*

X/O males were scored for *Spy* on the basis of testis weights and histology (Burgoyne *et al.* 1986).

X/X and X/O males were scored for *Hya* by testing spleen cells for H-Y antigen expression. Spleen cell suspensions were made from partial splenectomy samples, and tested *in vitro* (i) for their capacity to stimulate proliferation of H-Y-specific T-cell clones C6(H-Y/$K^k$), B9(H-Y/$A^b$) and 10·2(H-Y/$D^b$), (ii) as target cells for H-Y-specific H-2-restricted cytotoxic T cells and (iii) for the H-2 alleles expressed, using serological typing with MHC class I-specific monoclonal antibodies and H-2-specific cytotoxic T cells (H-$2^b$, H-$2^k$ and H-$2^q$) (for details, see McLaren *et al.* 1984, Simpson *et al.* 1984, 1986). Because of the heterogeneity of H-2 types segregating, predominantly H-$2^b$ and H-$2^k$, but also some H-$2^q$, each of the H-2- and H-Y-specific typing cells and antibodies were used to test each of the X/X and X/O males, in experiments in which positive and negative controls were included. Only firm diagnoses of the presence or absence of H-Y antigen were accepted; this sometimes involved assaying several successive samples of spleen cells.

The Sx1 DNA probe was supplied by CEB (Bishop *et al.* 1988), and the Zfy probe (a 1·9 kb *Hin*d III *Zfy-1* genomic fragment) by Robin Lovell-Badge (Gubbay *et al.* 1990). For both, standard methods of Southern analysis were applied, using DNA extracted from tail tips of adult mice. The Sx1 probe hybridizes to four bands in *Eco*R I-restricted DNA of X/Y $Sxr^a$ or X/X $Sxr^a$ male mice: when X/X $Sxr^b$ DNA is used, band D (1·8 kb, single copy) is absent, and when X/Y DNA is used, band C is stronger, indicating multiple copies, and band B is absent. The Zfy probe gives two bands with *Eco*R I-restricted X/Y or X/X $Sxr^a$ DNAs, corresponding to *Zfy-1* (11 kb) and *Zfy-2* (5 kb); the *Zfy-2* band is absent when X/X $Sxr^b$ DNA is analysed. Sxr regions in X/Y mice were classified by testing X/O or X/X male progeny, if necessary in more than one generation.

Many of the X/O and X/X male progeny from the first and subsequent generations were screened by CEB for *Sby* and other Y-specific DNA probes, including Y353B (Bishop *et al.* 1985) that detects



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sequences on the long arm of the Y, suggesting that a Y chromosome is present. All or part of the spleen from each male was disrupted in 0·6 ml phosphate-buffered saline. An equal quantity of 150 $\mu$g/ml proteinase-K solution was added, and after incubation at 55 °C for 1–2 h the samples were posted to Paris or Memphis for Southern analysis.

Five of these DNA samples were reanalysed using the polymerase chain reaction (PCR). The PCR reaction was run with 50 ng of genomic DNA in 10 mM-Tris-HCl pH 8·4, 50 mM-KCl, 1·5 mM-$MgCl_2$, 0·001 % gelatin, 0·2 mM each dNTP and 25 pmol each primer. The cycle parameters used were 95 °C (5 min); 72 °C to add 1·25 units of Taq polymerase (Perkin-Elmer Cetus); anneal (30 s); 72 °C (90 s) for one cycle; and then 95 °C (30 s); anneal (30 s); 72 °C (30 s) for 34 cycles followed by 72 °C (15 min). Only the annealing temperature was varied in the cycling parameters except for the oMJ3/oMJ13 primer pair where the elongation step was 1 min 30 s. All primer pairs used amplified a fragment of the predicted size from X/Y male and X/X Sxr$^a$ DNA, but not X/X female or X/X Sxr$^b$ DNA. Primers derived from Sry were used as a positive control. The Zf2 primer pair was oMJ22: AAATGGGTAAGAGGCTGG and oMJ23: CCAGGAATCCACTTGTCC, the T5 primer pair was oMJ3: TACAGCTCAAGCCAGGAC and oMJ13: CCTCCTAGTCCGTATGTC, and the two Sx1 primer pairs were oMJ26: GGATCAGTG-CATTTTTAG and oMJ27: AAACTCGTATA-CATACAG: oMJ17: AGTGCTAAGCTGCCAGTT and oMJ18: ACACTGAACTGAGTGAGG. The Sry primers were as described in Gubbay *et al.* (1990*b*). The annealing temperatures were 50 °C for oMJ26/27, 55 °C for oMJ22/23 and Sry, and 60 °C for oMJ3/13 and oMJ17/18.

## 3. Results

### (i) *X/Y Sxr$^a$♂, X Sxr$^a$/Y♂, T16H/X Sxr$^a$♀*

Does crossing-over during male meiosis transfer the Sxr region more readily from the Y chromosome to the X than from the X to the Y? To address this question, X/Y Sxr$^a$ and X Sxr$^a$/Y males were both mated to T16H/X females and the progeny typed for PGK-1. T16H carried the *Pgk-1*$^b$ allele, while the normal X chromosomes in both males and females carried *Pgk-1*$^a$. Because of the preferential expression of the translocated X, T16H/X individuals (apart from rare recombinants involving the *Pgk-1* locus) produce only the PGK-1B isozyme. T16H/X females were used in the crosses as part of a separate study of the sexual differentiation of T16H/X Sxr$^a$ progeny (reported by McLaren, 1986).

No attempt was made to determine which of the X/Y and T16H/Y sons were carrying Sxr. Estimates of recombination frequency were therefore based on the relative numbers of X/X (female) and X/X Sxr$^a$ (male) among the PGK-1A progeny, and T16H/X (female) and T16H/X Sxr$^a$ (male, female or hermaphrodite) among the PGK-1B progeny (Table 1). X/X Sxr$^a$ and T16H/X Sxr$^a$ males were distinguished from Y-bearing males by testis size (less than 25 mg) and absence of germ cells in histological preparations. T16H/X Sxr$^a$ females were distinguished from T16H/X females by test-mating, and hermaphrodites were identified morphologically.

Table 1 shows that recombination of Sxr$^a$ occurred freely in both directions, with an estimated recombination frequency not significantly different from 50% in either cross.

Table 1 also lists the progeny of T16H/X Sxr$^a$ females mated to males in which the X chromosome carried either *Ta* or *Gs*. Estimates of recombination frequency are therefore based on coat phenotype rather than PGK type. Again, no attempt was made to determine which of the X/Y and T16H/Y sons were carrying Sxr$^a$. The deficiency of X/X Sxr$^a$ sons is significant at the $P < 0{\cdot}05$ level, and is unexplained. In a T16H/X female, an average of about one chiasma would be expected to form between the normal X chromosome and the translocation product that includes the distal pseudoautosomal region of the X to which Sxr would attach. Since the recombination frequency of Sxr$^a$ does not differ significantly from 50%, it seems that the presence of Sxr$^a$ does not affect crossing-over.

### (ii) *Variants due to inter-Sxr recombination*

Of 180 progeny of the X Sxr$^b$/Y Sxr$^a$ male that survived to be sexed within a few days of birth, all were male; 174 were successfully classified as X/X Sxr, X Sxr/O or X/Y Sxr, using a combination of coat phenotype, karyotyping, testis weight and histology, and breeding.

Of the four markers used to distinguish Sxr$^a$ and Sxr$^b$, *Hya* was scored by typing spleen cells for the presence or absence of H-Y antigen, *Spy* by the weight and histology of the testes in X Sxr/O individuals (or for X/Y Sxr individuals, their X Sxr/O progeny), and *Zfy-2* and pSx1 by Southern analysis. The overall results are given in Table 2, from which it is clear that both the X chromosome transmitted by the male to his X/X and X/O sons, and the Y chromosome transmitted to his X/Y sons, were equally likely to carry Sxr$^a$ or Sxr$^b$, i.e. the Sxr region recombined freely in both directions.

Not all the markers could be successfully scored in all progeny. In particular, the varied H-2 haplotypes segregating in these mice sometimes prevented reliable classification for H-Y antigen. The detailed results are given in Table 3.

Three Sxr variants were identified in 159 progeny, suggesting an incidence of unequal crossing-over of nearly 2%. The variants are detailed in Table 4.

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Table 1. *Frequency of recombination of Sxr$^a$ in the progeny of X/Y Sxr$^a$ and X Sxr$^a$/Y males mated to T16H/X females, and T16H/X Sxr$^a$ females mated to X/Y males*

| Chromosome constitution of progeny | Progeny of X/Y Sxr$^a$ ♂♂* | Progeny of X Sxr$^a$/Y ♂♂† | Progeny of T16H/X Sxr$^a$ ♀♀‡ |
|---|---|---|---|
| X/X | 52 (NR) | 55 (R) | 70 (R) |
| T16H/X | 41 (NR) | 31 (R) | 82 (NR) |
| X/X Sxr$^a$ | 46 (R) | 47 (NR) | 51 (NR) |
| T16H/X Sxr$^a$ | 31 (R) | 47 (NR) | — |
| T16H Sxr$^a$/X | — | — | 75 (R) |
| X/Y (Sxr$^a$) | 84 (—) | 94 (—) | — |
| X (Sxr$^a$)/Y | — | — | 132 (—) |
| T16H/Y (Sxr$^a$) | 88 (—) | 94 (—) | — |
| T16H (Sxr$^a$)/Y | — | — | 145 (—) |
| Recombinant (R) | 77 | 86 | 145 |
| Non-recombinant (NR) | 93 | 94 | 133 |
| Recombination frequency | 45·3% | 47·7% | 52·2% |

* Data from 17 matings, some previously included in McLaren (1986), i.e. those in which all progeny were classified.
† Data from 11 matings, all previously included in McLaren (1986). The 11 sires were produced from 6 T16H/X Sxr ♀♀ mated to Ta ♂♂.
‡ Data from 26 matings.

Table 2. *Progeny of X Sxr$^b$/Y Sxr$^a$ ♂, mated to XO or In(X)/X ♀♀*

| | Sxr$^a$ | Sxr$^b$ | New Sxr variants | Not scored | Total |
|---|---|---|---|---|---|
| XX | 26 | 31 | 2 | 4 | 63 |
| XO | 14 | 12 | 0 | 1 | 27 |
| XY | 38 | 35 | 1 | 10 | 84 |
| Total | 78 | 78 | 3 | 15 | 174 |

Table 3. *Markers for which progeny of X Sxr$^b$/Y Sxr$^a$ male were successfully classified*

| Markers scored | | | | Sxr$^a$ | Sxr$^b$ | Variant | Total |
|---|---|---|---|---|---|---|---|
| XO | *Hya*, | *Spy*, | *Zfy-2*, Sx1 | 10 | 8 | 0 | 18 |
| | | *Spy*, | *Zfy-2*, Sx1 | 4 | 4 | 0 | 8 |
| XX | *Hya*, | | *Zfy-2*, Sx1 | 18 | 24 | 2 | 44 |
| | | | *Zfy-2* Sx1 | 8 | 7 | 0 | 15 |
| XY | *Hya*, | *Spy*, | *Zfy-2* Sx1 | 34 | 27 | 0 | 61 |
| | *Hya*, | | *Zfy-2*, Sx1 | 1 | 0 | 0 | 1 |
| | | *Spy*, | *Zfy-2*, Sx1 | 1 | 7 | 1 | 9 |
| | | | *Zfy-2*, Sx1 | 2 | 1 | 0 | 3 |
| Total | | | | 78 | 78 | 3 | 159 |

Unfortunately the only variant transmitted through an X/Y male was lost before a line could be established. *Zfy-1*, which was identified by the *Zfy* probe, was present in all males tested, and *Tdy* was clearly also retained, since the X Sxr$^b$/Y Sxr$^a$ male sired no daughters.

The 465 DNA samples successfully subjected to Southern analysis by CEB were mostly derived from progeny and grand-progeny of the X/Y sons of the original X Sxr$^b$/Y Sxr$^a$ male. The Sxr$^b$ deletion breakpoints have been shown to lie within the Zfy genes, placing Zfy-1 and Zfy-2 at opposite ends of the deleted DNA (Simpson & Page, 1991). By chromosome walking, 270 kb of the deleted DNA has been isolated from three distinct loci, termed Zf2, T5 and Sx1 (Mitchell & Bishop, 1992). The Zf2 contig was initiated from the 3′ end of the Zfy-2 gene, while the T5 contig, which contains the spermatogenesis gene *Sby* (Mitchell *et al.* 1991), is linked to the 5′ end of the Zfy-1 gene. These contigs, therefore, are positioned at either end of the deleted DNA. The Sx1 contig lies between the Zfy-2/Zf2 and Zfy-1/T5 loci. Probes from these three loci, pZf.1.8.B2 (Zf2), pSx.2.6.C (Sx1), pSx.D.1.B2 (Sx1) and pT-5.1.1.PH (T5), essentially define both ends and a central point of the Sxr$^b$ deleted DNA. The results obtained with these probes by Southern analysis were concordant for all mice successfully tested, suggesting that the Sxr DNA deleted in the formation of the Sxr$^b$ region was either wholly present or wholly absent in each case. DNAs from six mice that failed to give clear results by Southern analysis were re-analysed using PCR. The primers used were derived from the Zf2, Sx1 and T5 loci and the results were again concordant among these three loci. The Sxr regions of three of these latter mice are represented as Sxr$^f$, Sxr$^g$ and Sxr$^h$ in Table 4. As the molecular data are in disagreement with the H-Y typing, it is necessary to postulate that an interstitial insertion and deletion of Hya created the Sxr$^f$ and Sxr$^g$ regions respectively.

One mouse classified as X/X on coat phenotype and testis size proved positive for the Y353B probe, so

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Table 4. *Sxr variants transmitted to one XY and two XX sons of an X Sxr$^b$/Y Sxr$^a$ male. The Sxr region of the XY son was analysed by progeny testing. For nomenclature of Sxr variants, see McLaren* et al. *(1988)*

| Variant | Chromosome constitution of F1 bearer | *Zfy-2* | *Spy* | *Sby* | Sx-1 (1·8 kb band) | *Hya** |
|---|---|---|---|---|---|---|
| Sxr$^f$ | XX | — | ND | — | — | + |
| Sxr$^g$ | XX | + | ND | + | + | — |
| Sxr$^h$ | XY | — | — | — | + | ND |
| Sxr$^a$ | . | + | + | + | + | + |
| Sxr$^b$ | . | — | — | — | — | — |

* Sxr$^f$ was carried by an XX male which was an *H-2$^{kb}$* heterozygote: *Hya*-positive typing was established with T cells specific for each *H-2* haplotype.

Sxr$^g$ was carried by an XX male which was an *H-2$^{kb}$* heterozygote: he was likewise typed *Hya*-negative. He had two *H-2$^{kb}$* brothers, one of which was *Hya*-positive and had inherited all other Sxr$^a$ markers; the other was *Hya*-negative and had Sxr$^b$ markers.

Table 5. *Variants resulting from illegitimate pairing of an Sxr$^b$ region with the short arm of the Y. For nomenclature of Sxr variants, see McLaren* et al. *(1988)*

| Variant | *Zfy-2* | Sx1 (band D) | *Sby* | Sx1 (band C, multiple copies) | Y-353B | H-Y |
|---|---|---|---|---|---|---|
| Sxr$^i$ | — | — | — | + | — | — |
| Sxr$^i$ | — | — | — | + | — | — |
| Sxr$^j$ | + | + | + | + | — | ND |
| Sxr$^f$ | — | — | — | — | — | + |
| XY$^{p\text{-}del}$ | — | — | — | — | + | ND |
| XY$^{p\text{-}del}$ | — | — | — | — | + | ND |
| Sxr$^a$ | + | + | + | — | — | + |
| Sxr$^b$ | — | — | — | — | — | — |
| Y | + | + | + | + | + | + |

presumably carried an intact Y chromosome; it was probably an X/X/Y male.

(iii) *Variants due to illegitimate pairing*

A few X/X male mice appeared to have gained DNA sequences by illegitimate pairing of an Sxr$^b$ region with the short arm of the Y (Table 5). Two were males from the F1 generation, scored as Sxr$^b$ on H-Y typing. Southern analysis showed that they lacked *Zfy-2* and band D of Sx1, but carried the repeated band C of Sx1, which was presumably acquired from Yp by illegitimate pairing and crossing-over. We term this variant Sxr$^i$. The other example, Sxr$^j$, was an X/X male from the F2 generation whose father was classified as X/Y Sxr$^b$ on the basis of 5 X/O and X/X Sxr$^b$ sons. This male, however, proved positive for all Sxr loci normally deleted in Sxr$^b$, and carried in addition the repeated band C of Sx1 characteristic of the Y. Since hybridization with Y353B showed that a complete Y was not present, we presume that the male had acquired Y short-arm material as a result of illegitimate pairing. Another X/X male (from the F3 generation) was indistinguishable from Sxr$^b$ on Southern analysis, as expected, but tested positive for H-Y antigen, as in the variant Sxr$^f$ that was seen in the F1 generation (Table 4).

A more interesting example of presumed illegitimate pairing is illustrated in Fig. 3. An X/Y son of the original male was mated to an X/O ♀ to determine, by progeny testing, whether he had inherited Sxr$^a$ or Sxr$^b$. Of 3 X/X and 3 X/O male progeny in his first 3 litters, all gave results consistent with an Sxr$^a$ constitution on testing with Zfy and Sx1 DNA probes. In addition, one of the X/X males was shown to express H-Y antigen, and all 3 of the X/O males proved to carry *Spy*. The X/Y male of the F1 generation was therefore classified as having inherited a non-recombined Sxr$^a$ region from his father. However, in a subsequent litter two males were born that had small ( < 25 mg) testes and 40 chromosomes, hence were classified as X/X, but gave results on

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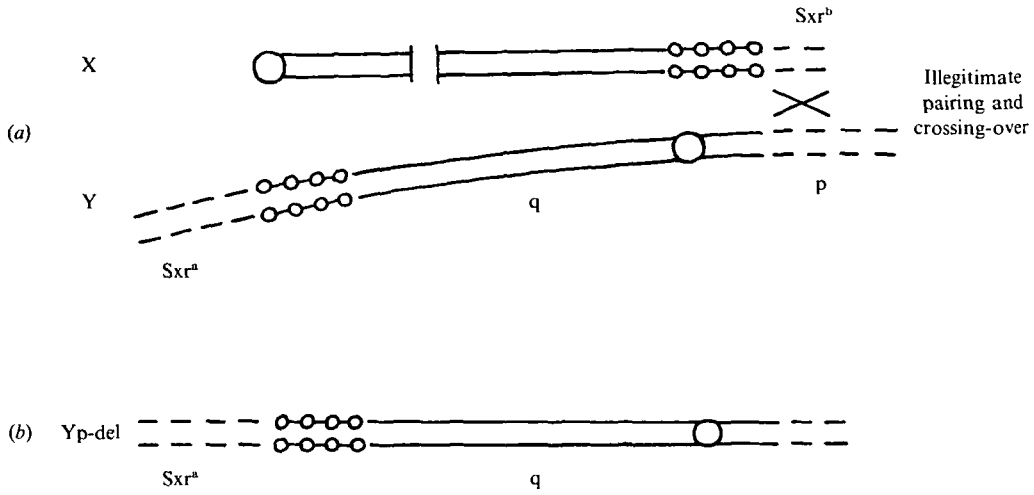
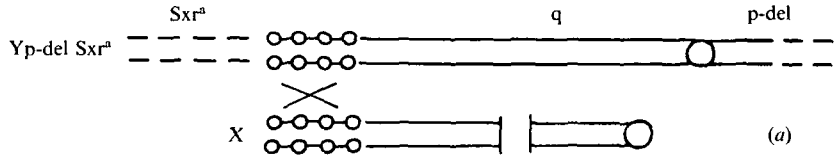
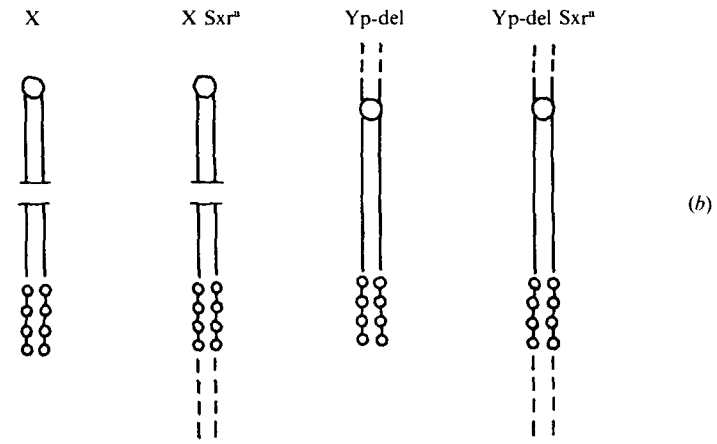



Fig. 3. (*a*) Illegitimate pairing and crossing-over between the Sxr$^b$ region on an X Sxr$^b$ chromosome, and the testis-determining region on the short arm of its Y Sxr$^a$ partner. (*b*) The Yp-del Sxr$^a$ chromosome that could result from the type of pairing and crossing-over illustrated in (*a*).




| | XO, XX ♀ | XO, XX Sxr$^a$ ♂ | XYp-del ♂ | XYp-del Sxr$^a$ ♂ |
|---|---|---|---|---|
| Observed | 4 | 6 | 2 | 6 |
| Expected | 6 | 6 | 3 | 3 |

(*c*)

Fig. 4. (*a*) Illegitimate pairing and crossing-over between a Yp-del Sxr$^a$ chromosome (see Fig. 3*b*) and a normal X chromosome. (*b*) The four types of chromosome (2X, 2Y) that should be produced in equal numbers by the pairing and crossing-over event illustrated in (*a*). (*c*) The four classes of progeny expected when an X/Yp-del Sxr$^a$ male is mated to an X/O female, and the numbers of each that were observed.

Southern analysis consistent with an Sxr$^b$ constitution. Testing with probe Y353/B revealed that each of these males carried a Y chromosome. Evidently this Y lacked the relevant portion of the short arm. The same deleted Y (Yp-del) must have been present in their fertile father, but in him and in the 6 sons scored as

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X/Y on account of their large testes, the short-arm deletion must have been compensated for by a pseudoautosomally located $Sxr^a$ region (Fig. 3). We therefore presume that an illegitimate meiotic pairing (Fig. 3*a*) must have taken place in the original male, between the $Sxr^b$ region on the X and the short arm of the $Sxr^a$-carrying Y chromosome, giving rise to a Yp-del $Sxr^a$ chromosome (Fig. 3*b*). An X/Yp-del $Sxr^a$ male would be expected to produce four types of sperm in equal numbers, namely X, X/$Sxr^a$, Yp-del and Yp-del $Sxr^a$ (Fig. 4*a*, *b*). When the presumed X/Yp-del $Sxr^a$ male was mated to an X/O female, the $F_2$ progeny obtained did not depart significantly from expectation (Fig. 4*c*). Unfortunately the deleted Y chromosome was not examined cytologically, and has not been preserved.

## 4. Discussion

When either the X or the Y chromosome carries an Sxr region at its distal end, exchange of the Sxr region from one sex chromosome to the other during male meiosis may take place as a result of normal pseudo-autosomal pairing and crossing-over. Alternatively, the Sxr region may pair with the homologous region of the Y short arm. If the Sxr region is complete and not rearranged (i.e. $Sxr^a$) and pairing is correctly aligned, no redistribution of genetic material will be observed; but if the Sxr region has undergone a deletion ($Sxr^b$) or if mismatched pairing and crossing-over takes place, new Sxr variants may be generated. If both the X and the Y chromosome carry an Sxr region, legitimate pairing between the Sxr regions as well as two types of illegitimate pairing with Yp become feasible.

Cattanach *et al.* (1990) found that attachment of $Sxr^a$ to the X chromosome appeared to inhibit normal pseudo-autosomal recombination during male meiosis. Only 38% of spermatocytes with X-Y bivalents showed pseudo-autosomal pairing, and the frequency of Sxr recombination on to the Y chromosome was much below the expected 50%. In the remaining 62% of X-Y bivalents, pairing was between $Sxr^a$ and the short arm of the Y chromosome, and some cytological evidence for mismatched pairing and crossing-over was reported.

Our results, in contrast, show no reduction in recombination frequency when $Sxr^a$ is attached to an X chromosome. The rate of exchange did not differ significantly from 50%, whether the Sxr region was being transferred from a Y to an X, from an X to a Y, or from an X-autosome translocation to a normal X (Table 1). Presumably, therefore, normal pseudo-autosomal pairing and crossing-over was taking place, though illegitimate pairing could have occurred at the same time (Fig. 1*d*). This double pairing has been observed by Chandley & Speed (1987), but only very rarely. The difference between our results and those of Cattanach *et al.* (1990) may reflect the genetic origin of the Y chromosomes. Cattanach *et al.*'s X Sxr/Y males carried a Y chromosome derived from strain 101, in contrast to X/Y Sxr males of the standard Sxr strain, which carries a Y of RIII origin. Cattanach *et al.* do not provide information on the frequency of exchange in X/Y Sxr males with a Y chromosome from strain 101. In our crosses the X Sxr/Y males were derived by crossing T16H/X Sxr females with males from a tabby stock (Falconer, 1953), derived originally from crosses in which the Y chromosomes came from strains RIII and A. Perhaps the tabby stock Y chromosome was of the RIII type, similar to that of the standard Sxr strain, rather than the A type. Perhaps Sxr recombines freely between X chromosomes and RIII Y chromosomes, in either direction, but 101 Y chromosomes do not undergo pseudo-autosomal pairing and crossing-over so readily. In any case, since we cannot be certain that the Y chromosome in the tabby stock that we used was one of the original tabby stock Y chromosomes, this explanation must remain speculative.

The strain of origin of the Y chromosome is known to affect the proportions of fertile and sterile X/Y Sxr males derived by recombination from X Sxr/Y fathers (Cattanach, personal communication).

When both the X and the Y chromosome carry an Sxr region, the opportunity arises for Sxr/Sxr as well as pseudo-autosomal pairing. Crossing-over could then take place between Sxr regions as well as, or even instead of, pseudo-autosomally. Our X $Sxr^b$/Y $Sxr^a$ male transmitted as many X $Sxr^a$ and Y $Sxr^b$ as X $Sxr^b$ and Y $Sxr^a$ chromosomes (Table 2); if the single obligatory crossover ever strayed into the Sxr region, it must therefore have remained proximal to the deletion in $Sxr^b$.

We had predicted that mismatched pairing and crossing-over between Sxr regions, facilitated by the abundance of repeated DNA sequences, might generate recombination among the four markers present in $Sxr^a$ but missing in $Sxr^b$. In particular, if *Spy* had recombined with either *Hya* or *Zfy-2*, it would have ruled out H-Y antigen or the *Zfy-2* gene product as the protein required for normal spermatogonial differentiation that is lacking in XO $Sxr^b$ males (Sutcliffe & Burgoyne, 1990). Unfortunately no such recombinant was found: *Spy* can only be scored in XO males, only 26 XO males were available for analysis in the $F_1$ generation, and the rate of recombinants detected in XY males by progeny testing was very low (Table 3).

The frequency of variants seen overall (Table 3) was somewhat less than the frequency of restriction fragment-length polymorphisms at one specific locus detected by Epplen *et al.* (1988) in our $Sxr^a$ and $Sxr^b$ back-crossing programme (about 3%). An even higher rate of unequal recombination (7%) has been reported for the pseudo-autosomal region of the mouse Y chromosome (Harbers *et al.* 1987). Probably the high

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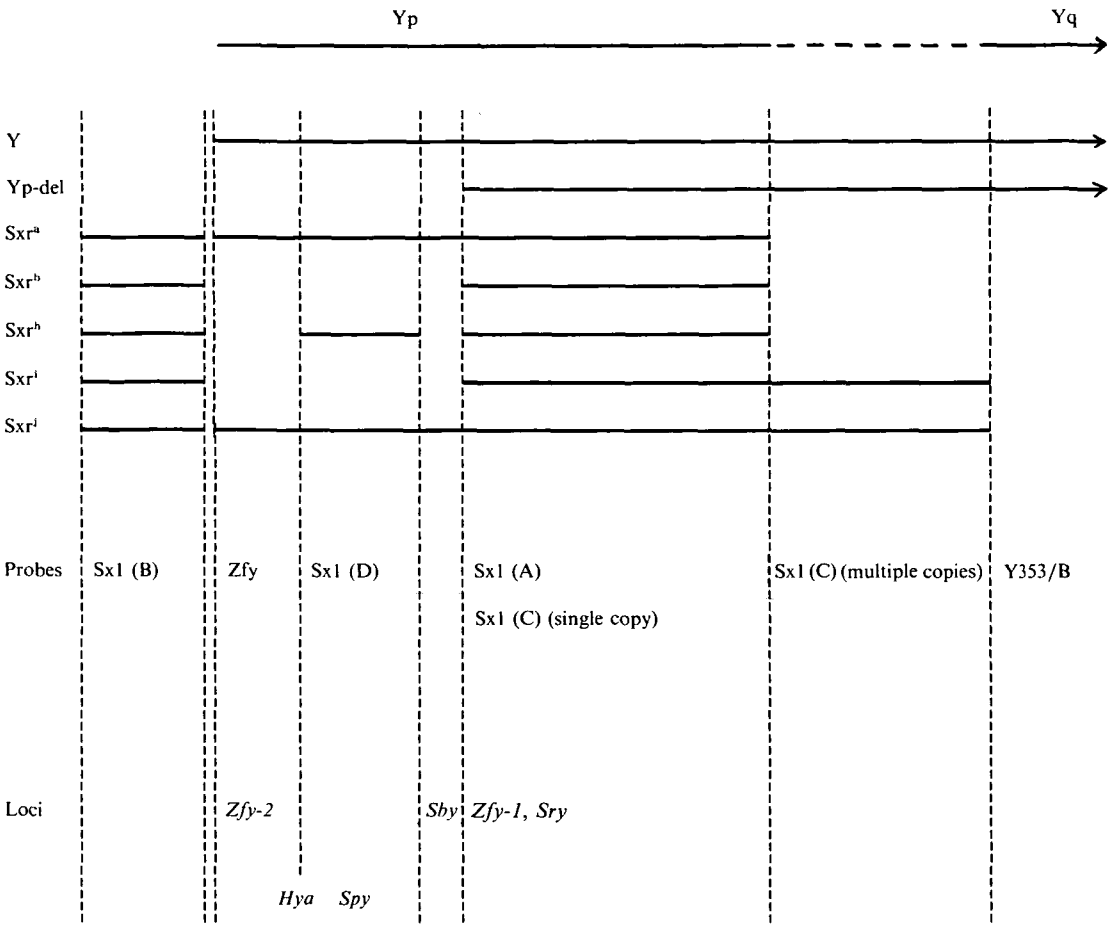


Fig. 5. The distribution of loci (*Hya*, *Spy*, *Sby*, *Sry*, *Zfy-1*, *Zfy-2*) and sites of hybridization of the DNA probes Sx1, Zfy and Y353/B, among the Y chromosomes and Sxr variants reported in this paper. The order of *Zfy-1* and *Zfy-2* within Yp is not known; in this diagram, *Zfy-1* and *Sry* have been assumed to be proximal. With *Eco*R 1-restricted DNA of various Sxr variants, the Sx1 probe hybridizes to a total of four bands: A (7 kb), B (5 kb), c (2·8 kb) and D (1·8 kb). For further information on the DNA probes, see Materials and Methods.

proportion of repeated DNA sequences (e.g. GATA/GACA) in the mouse Y generates a high mutation rate.

Some other illegitimate pairing events were observed in our study. The presumed exchange between the short arm of the Y chromosome and the Sxr$^b$ region attached to the X would have generated a Y chromosome that lacked *Spy*. In that lineage X/Y males were only fertile if they carried Sxr$^a$ (see Figs 3 and 4). The three other illegitimate pairing events detected all occurred in later generations, when the Sxr region in X/Y males was always attached to the Y; they must therefore have resulted from 'hairpin' pairing of the distally located Sxr with the short arm of the Y. The recombinant males had all acquired the repeated 2·8 kb band of pSx1 characteristic of the Y but normally lacking from Sxr. This suggests that the Sxr region does not duplicate the whole of the Y short arm.

The distribution of loci and sites of hybridization of the various DNA probes among Y chromosomes and Sxr variants is shown in Fig. 5. Since the 5 kb Sx1 band (band B) is only seen with *Eco*R I-restricted X/X Sxr DNA, not with X/Y DNA, it is likely to represent an Sxr-specific polymorphism.

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