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Illegitimate pairing of the X and Y chromosomes in *Sxr* mice

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Summary

X/Y male mice carrying the sex reversal factor, *Sxr*, on their Y chromosomes typically produce 4 classes of progeny (recombinant X/X *Sxr* ♂♂ and X/Y non-*Sxr* ♂♂, and non-recombinant X/X ♀♀ and X/Y *Sxr* ♂♂) in equal frequencies, these deriving from obligatory crossing over between the chromatids of the X and Y during meiosis. Here we show that X/Y males that, exceptionally, carry *Sxr* on their X chromosome, rather than their Y, produce fewer recombinants than expected. Cytological studies confirmed that X–Y univalence is frequent (58%) at diakinesis as in X/Y *Sxr* males, but among those cells with X–Y bivalents only 38% showed normal X–Y pseudo-autosomal pairing. The majority of such cells (62%) instead showed an illegitimate pairing between the short arms of the Y and the *Sxr* region located at the distal end of the X, and this can be understood in terms of the known homology between the testis-determining region of the Y short arm and that of the *Sxr* region. This pairing was sufficiently tenacious to suggest that crossing over took place between the 2 regions, and misalignment and unequal exchange were suggested by indications of bivalent asymmetry. Metaphase II cells deriving from meiosis I divisions in which the normal X–Y exchange had not occurred were also found. The cytological data are therefore consistent with the breeding results and suggest that normal pseudo-autosomal pairing and crossing over is not a prerequisite for functional germ cell formation. The data support the concept that Y short arm-*Sxr* pairing and crossing over may be the mechanism responsible for the occurrence of the *Sxr* variants reported in the literature.

1. Introduction

The sex reversed condition in the mouse (*Sxr*) (Cattanach *et al.* 1971) is attributable to a chromosome rearrangement in which part or all of the short arm of the Y containing the testis determining locus (*Tdy*) has been duplicated and transposed to the distal tip of the long arm beyond the X–Y pairing region (McLaren *et al.* 1988; Roberts *et al.* 1988). In this position, because of obligatory crossing over between the two chromosomes in male meiosis, approximately half of the X/X and X/Y progeny of X/Y *Sxr* carrier males inherit *Sxr*. The X/Y and X/Y *Sxr* classes of necessity develop as males and, typically, so too does the sex reversed X/X *Sxr* class but under exceptional circumstances X/X *Sxr* mice can develop as females. Thus, when the T(X; 16)16H X-autosome translocation is present, the non-translocated X carrying *Sxr* is genetically inactive in most somatic cells and, as a consequence, a proportion of T16H/X *Sxr* mice develop as females or hermaphrodites, rather than males (Cattanach *et al.* 1982; McLaren & Monk, 1982). The females are usually fertile and in crosses with normal males transmit *Sxr* to half their progeny. X/Y males that, exceptionally, carry *Sxr* on their X, rather than their Y (X *Sxr*/Y) can therefore be generated.

Although X/Y mice that carry *Sxr* are phenotypically normal and usually fertile, their testis size is reduced relative to their X/Y non-*Sxr* sibs (Lyon *et al.* 1981) and this has been found to correlate with high frequencies of X–Y dissociation in meiosis (Cattanach and Evans, unpublished) first noted at diakinesis by Winsor *et al.* (1978), and then by Chandley and Fletcher (1980) and Evans *et al.* (1980) at pachytene. Evans *et al.* (1982) later observed that at diakinesis the univalent Y chromosomes carried *Sxr* chromatin on both of their chromatid arms and had therefore failed to undergo crossing over with the X. They further noted that only recombinant X and Y

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chromosomes were present in metaphase II cells. These data, together with the observation that *Sxr* segregates normally among the progeny of X/Y *Sxr* mice (Lyon *et al.* 1981), suggest that X–Y pairing and crossing over are essential for normal germ cell survival (see also Miklos, 1974; Burgoyne and Baker, 1984).

The cause of the high frequencies of X-Y univalence in X/Y *Sxr* males has remained something of an enigma. Chandley and Speed (1987) reported that the univalent Y in such mice frequently self-paired as a ring at pachytene and they suggested that this was due to the homology between the proximal *Tdy* and distal *Sxr* regions, this pairing competing with, or disrupting, normal X–Y synapsis. On the other hand, from the general observation that the frequencies of X–Y univalence are higher at diakinesis than at pachytene, Lyon *et al.* (1981) had earlier concluded that the cause of the univalence was a precocious separation of the sex chromosomes, rather than a failure of normal pairing. This latter possibility has recently received some support from Tease and Cattanach's (1989) finding that high frequencies of univalence and self-pairing of the Y also occur in males that carry *Sxr* on their X (X *Sxr*/Y), rather than on their Y. The self-pairing could thus be a consequence, rather than the cause of the univalence, and this conclusion would be consistent with the observation that normal Y chromosomes have been found to self-pair in other situations in which univalence occurs (Setterfield *et al.* 1988; C. Tease, pers. comm.). However, despite the tendency of univalent Y chromosomes to self-pair, there was still good indication in Tease and Cattanach's (1989) data that the presence of *Sxr* on the Y enhances the frequency of self-pairing.

In this communication we present evidence of a novel X–Y pairing in X *Sxr*/Y mice which could be consistent with the enhanced levels of Y self-pairing in X/Y *Sxr* mice being at least partly due to short arm-*Sxr* homology. Breeding and cytological data are described which indicate that in X *Sxr*/Y males the Y frequently pairs with the X, not, as is usual, by its long arm X–Y pairing region, but by its short arm. Moreover, this association is shown to fulfil the requirement of X–Y pairing for normal, functional germ cell development.

2. Materials and Methods

The X *Sxr*/Y males that comprised the subject matter of the present study were derived by the standard procedure (Cattanach *et al.* 1982; McLaren & Monk, 1982) of first crossing females heterozygous for the T(X; 16)16H X-autosome translocation with X/Y *Sxr* males to produce T16H/X *Sxr* offspring, and then crossing those that developed as females with normal X/Y males to produce X/Y sons, half of which should carry *Sxr* on their X chromosome. The X *Sxr*/Y class was then identified in breeding tests by its ability to produce X/X *Sxr* sons, this associated with a corresponding shortage of daughters and a consequent skewed sex ratio.

The identification of the various classes of offspring generated throughout the series of crosses was facilitated by the use of the X-chromosome marker genes, blotchy (Mo^{bl_o}) and tabby (*Ta*), and it should be noted that the normal X/Y males mated with the T16H/X *Sxr* females were F_1 hybrids derived from the cross of C3H/HeH females and 101/H males. They, and all their X/Y descendants therefore carried a Y of 101/H strain origin in contrast to mice of the standard *Sxr* strain which carries a Y of RIII origin.

The genetic investigation of the X *Sxr*/Y males comprised the analysis and ascertainment of the frequency of occurrence of each of the expected 4 classes of young (recombinant X/X ♀♀, non-recombinant X/X *Sxr* ♂♂, recombinant X/Y *Sxr* ♂♂, and non-recombinant X/Y *non-Sxr* ♂♂) produced in crosses with normal females. The presence of a marker gene in the X *Sxr*/Y fathers allowed the phenotypic identification of the X/X *Sxr* and X/X classes. The X/Y *Sxr* and X/Y non-*Sxr* groups were distinguished either on the basis of breeding tests with females carrying marked X chromosomes (about 20 progeny per male) or by testis weight. X/Y male sterility was attributed to the presence of *Sxr* but, in samples of animals, conclusive proof was provided by the cytological observation of *Sxr* chromatin (Evans *et al.* 1982).

For the cytological studies, air-dried preparations of the condensed meiotic stages were made by a standard method (Evans *et al.*, 1964). The slides were aged for a minimum of 3 days before G-banding with a combined treatment of 2 × SSC for 1·5 h at 65 °C, followed by 0·025 % trypsin in normal saline for 5 secs at room temperature. The treated slides were stained in Giemsa in pH 6·8 buffer.

3. Results

The genetic data derived from the X *Sxr*/Y males differed from those of their 'standard' X/Y *Sxr* counterparts in two respects. First, the 4 classes of young did not appear in equal frequencies and, second, almost all of the X/Y *Sxr* young were sterile. The inequality was initially observed among the genetically-marked X/X progeny (Table 1); only 88 X/X females (20 %) were detected, as opposed to 357 X/X *Sxr* males (80 %), and this distribution differs from the 50:50 expected with obligatory crossing between the X and Y in the pseudo-autosomal region at a very high level of significance ($\chi^2_1 = 162{\cdot}6$; $P = 3{\cdot}0 \times 10^{-7}$). The skew was found with each of the 9 X*Sxr*/Y males tested, although there was a marginally significant heterogeneity between animals in this respect ($\chi^2_8 = 15{\cdot}23$; $P = 0{\cdot}055$).

The same type of skew was then found among the X/Y progeny. Among 91 such males subjected to

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Table 1. *Results of crossing X Sxr/Y males with normal females*

Male no.	Progeny X/X♀	X/X *Sxr* ♂	X/Y *Sxr* ♂	X/Y non-*Sxr* ♂	Unclassified XY ♂	XO ♀
2	11	55	11	25	27	3
4	11	37	7	35	16	3
5	10	42	11	27	20	3
6	18	47	8	21	16	1
7	6	27	1	10	11	1
8	12	43	15	16	27	3
9	5	8	8	12	21	2
10	11	34	9	17	13	5
11	4	64	2	21	36	6
Total	88	357	72	184	187	27

breeding 68 were fertile and proved not to carry *Sxr* (non-recombinants). Only one fertile male clearly carried *Sxr* (recombinant), while one other showed only a transient fertility, becoming sterile before it could be classified for *Sxr*. All of the remaining 21 males were sterile. Testis weights were taken on most of these animals and it can be seen from the data presented in Fig. 1 that all the sterile males, the single X/Y *Sxr* recombinant and the incompletely tested male had small testes, while all the fertile X/Y non-*Sxr* males had large testes. As sterility and small testis size is known to be associated with the X/Y *Sxr* genotype (Lyon *et al.* 1981), it is likely that all the small testis animals carried *Sxr*. This conclusion was further supported for the partially tested, fertile male by the finding that both of 2 sons examined also had small testes. In total, therefore, only 23 (25%) of the

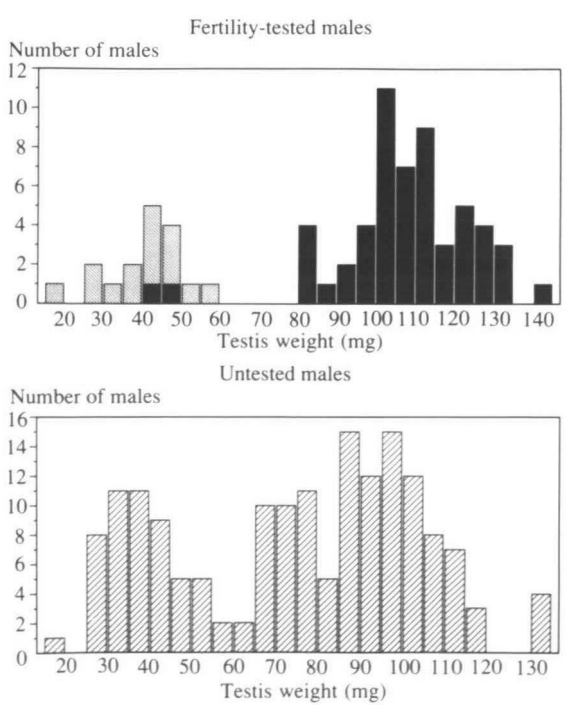

Fig. 1. Distributions of testis weights among fertility-tested and untested progeny of X *Sxr*/Y males. ▩ sterile males ■ fertile males ▨ untested males.

91 fertility-tested X/Y males may be deduced to be recombinants. Testis weight data were, however also obtained on a further 165 X/Y males that had not been fertility-tested. Being a younger group (age 8 wks) their gonads were generally smaller but nevertheless their testis weights also showed a clear bimodal distribution (Fig. 1). If a 55 mg weight is taken as the dividing point between the two distributions, 116 animals (70%) might be deduced to be X/Y non-*Sxr* non-recombinants and 49 (30%) X/Y *Sxr* recombinants. These frequencies are in reasonable accord with those from the fertility tested group ($\chi^2_1 = 0{\cdot}567$; $P = 0{\cdot}45$) and have been combined to provide the X/Y *Sxr* and X/Y non-*Sxr* data shown in Table 1.

Cytological confirmation of the presence of *Sxr* was established in 13 of the sterile/small testis animals taken randomly. Two others were independently shown to be X/Y *Sxr* in molecular studies (Bishop, pers. comm.). These findings, together with the fact that all the fertile sons of the X *Sxr*/Y males, apart from the 2 exceptions discussed above, did not carry *Sxr*, effectively confirm the X/Y *Sxr* genotype of this class. The reason for the high incidence of sterility among the X/Y *Sxr* group is not clear but it may reflect the substitution of the original Y of the *Sxr* stock by one of the 101/H strain origin (Tease and Cattanach, 1989). Meiotic analyses and histological studies of the animals revealed that, as observed with sterile presumptive X/Y *Sxr* males from the original *Sxr* stock, (Cattanach, Pollard & Hawkes, 1971) the male sterility was associated with disturbances in spermatogenesis such that few post-meiotic cells were present.

A further result of note in the breeding data is the large number of presumptive XO females. Cytological confirmation of the chromosome constitution was obtained in 10 of these and it is therefore likely that they were all of the same genotype. The frequency of their occurrence among all progeny (2·95%) greatly exceeds ($\chi^2_1 = 14{\cdot}75$; $P = 12 \times 10^{-4}$) that found (0·96%) in a large survey of young from X/Y *Sxr* males (Lyon *et al.* 1981).

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Because failure of pairing and crossing over are normally associated with a failure of germ cell survival (Miklos, 1974; Burgoyne & Baker, 1984), the skewed distribution of progeny from the X *Sxr*/Y males suggested that some novel form of pairing and crossing over that satisfied the requirement for germ cell survival was occurring in these animals. One possibility was that the proximal short arm of the Y (Yp) might pair and cross over with its homologous region represented by *Sxr* on the distal tip of the X beyond the pseudo-autosomal region. Cytological investigations of 2 of the 9 X *Sxr*/Y males provided confirmation of such illegitimate pairing.

After G-banding, the short arm of the Y chromosome stains darkly as a single or twin body at diakinesis and often displays a pinched-in appearance. With normal X–Y pairing, this dark-staining region is visualized at the free, unpaired end of the Y (Fig. 2*a*). In X/Y *Sxr* males, the *Sxr* region on the distal end of the long arm of the Y stains in a similar way to the short arm, and at diakinesis, the *Sxr* bodies typically lie close together to one side of the end-to-end paired sex bivalent, this association providing the classic evidence of crossing over between the X and the Y. The free end of the Y, however, still shows the darkly-staining pinched-in characteristic of the short arm. This type of pairing was also found in the present cytological study (Fig. 2*b*), but a second type of pairing was also recognized. In such cells the end-to-end pairing of the X and Y chromosomes showed up to 4 dark-staining bodies in close association, while the free end of the Y was generally blunt-ended, rather than pinched-in (Figs. 2*c*, *d*, *e* and *f*). An illegitimate pairing of the short arm of the Y with the *Sxr* region on the X was therefore indicated. In some cells, the disposition of the dark-staining bodies was symmetrical (Figs. 2*c*, *d* and *e*) while, in the others there was asymmetry (Fig. 2*f*). Of 754 cells scored, 26%

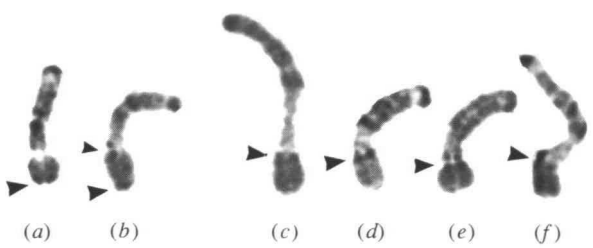

Fig. 2. X–Y pairing at diakinesis in X *Sxr*/Y males. Normal pairing. (*a*) Normal pairing of the X and Y chromosomes at diakinesis with the Y short arm stained darkly as twin bodies and 'pinched-in' (arrowhead) at the free unpaired end. (*b*) X and Y pairing in a X *Sxr*/Y male. The Y short arm stained darkly as a single body and 'pinched-in' (arrowhead) at the free unpaired end, and the two similarly stained *Sxr* bodies lying adjacent at the paired end of the bivalent (smaller arrowhead) following crossing over of one body from X to Y. (*c–f*). Examples of pairing of the short arm of Y to the distal, *Sxr* bearing end of X. Up to four darkly staining bodies are evident at the pairing junction (arrowhead) while the free end of the Y is blunt-ended and not 'pinched-in'. (*c*) Symmetrical disposition of the dark staining bodies with the pairing resisting sex chromosome stretching. (*d*) Symmetrical disposition with the tight merger of the Y short arm and *Sxr*. (*e*) Symmetrical disposition but with four dark staining bodies clearly visible, two representing the Y short arm (larger) and two *Sxr* (smaller). (*f*) Asymmetrical disposition suggesting the transposition of one Y short arm chromatid to the X (larger body, left side) and *Sxr* (smaller body, right side) from X to Y.

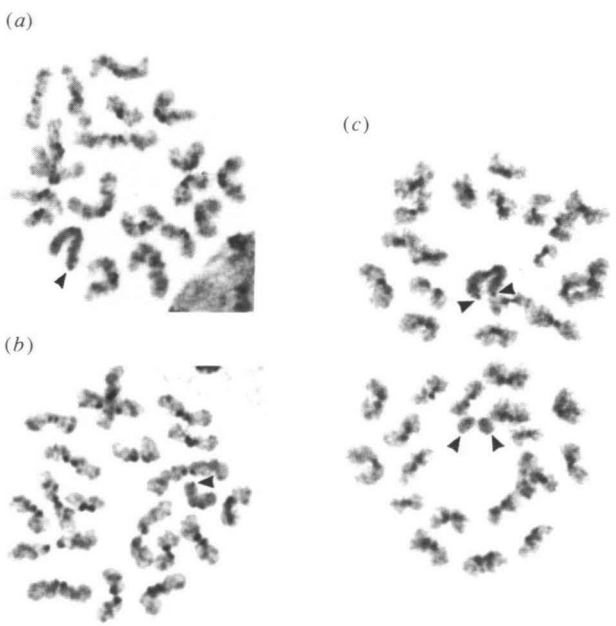

Fig. 3. Location of *Sxr* bodies in metaphase II cells. (*a*) Metaphase II cell with the X chromosome carrying an *Sxr* body on one chromatid (arrowhead). (*b*) Metaphase II cell with the Y chromosome carrying an *Sxr* body on one chromatid (arrowhead). (*c*) Sister metaphase II divisions with the retention of *Sxr* bodies on both chromatids of the X (upper cell, arrowheads) and their absence from the chromatids of the Y (lower cell, arrowheads).

Table 2. *Diakinesis analysis of X Sxr/Y males*

	Sex chromosome pairing			
Male no.	Pseudo-autosomal	Yp – *Sxr*	X–Y univalence	Total cells
5	65	119	248	432
6	56	76	190	322
Total	121	195	438	754
Frequency	16%	26%	58%	

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Table 3. *Metaphase II analysis of X Sxr/Y males*

Male no.	*Sxr* and chromatid classes				
	X *Sxr Sxr*	X *Sxr*	Y *Sxr*	Y	Total
5	73	116	78	83	350
6	10	19	16	9	54
Total	83	135	94	92	404
Frequency	21%	33%	23%	23%	

X *Sxr Sxr* = X with *Sxr* on both chromatids; X *Sxr* = X with *Sxr* on only one chromatid; Y *Sxr* = Y with *Sxr* on only one chromatid; Y = Y with neither chromatid carrying *Sxr*.

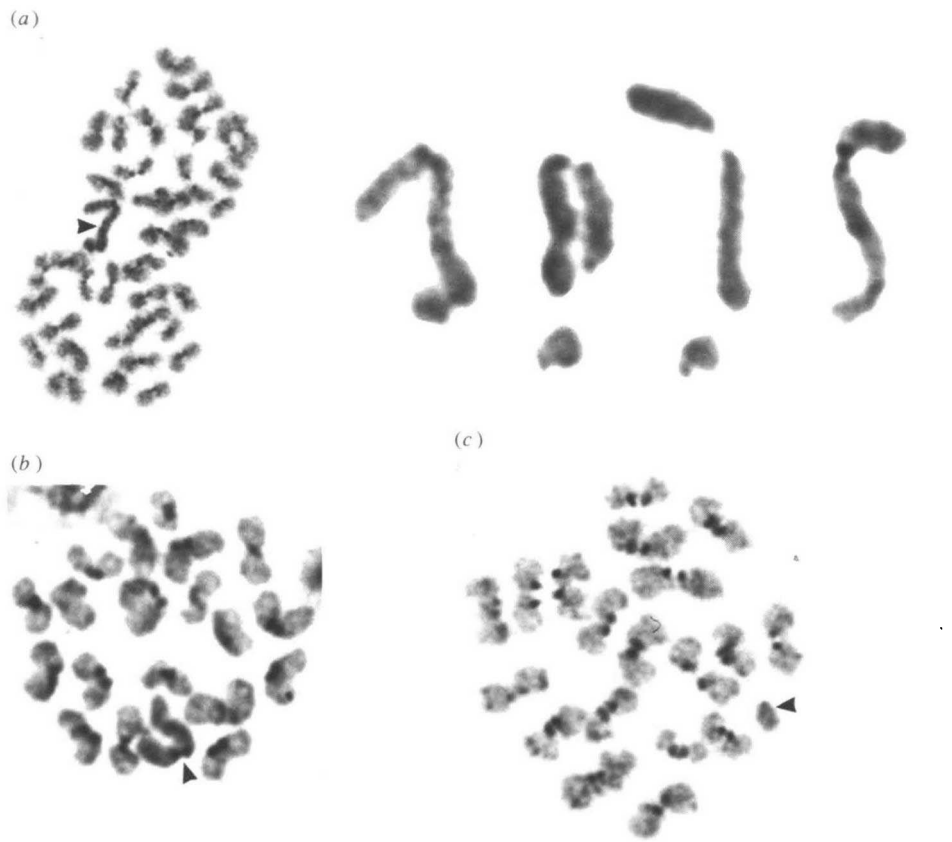

Fig. 4. Bridge formation in metaphase II cells. (*a*) Two sister metaphase II cells with a bridge formed by joined X and Y chromatids (arrowhead). Right, enlarged examples of joined chromatids with X uppermost and joined distally to the distal end of a Y chromatid (arrowhead), the other Y chromatid has separated in the middle two examples. Clearly, the central component is a dicentric composed of most of one chromatid from X and one from Y. (*b*) Joined X and Y chromatids (arrowhead) in a haploid metaphase II cell. (*c*) Single Y chromatid (arrowhead) in a haploid metaphase II cell.

showed some such evidence of Yp – *Sxr* pairing, 16% showed normal pseudo-autosomal pairing, and in the remaining 58% cells the X and Y lay unpaired (Table 2). The high frequency of cells with unpaired sex chromosomes is typical of animals carrying *Sxr* on their X or Y or both sex chromosomes (Lyon *et al.* 1981; Tease and Cattanach, 1989).

To investigate the frequency of crossover transfer, metaphase II cells from the same 2 males were scored for the presence of *Sxr* bodies on the chromatid arms of the X and Y. With normal diakinesis pairing and obligatory crossing over, all metaphase II cells should possess either an X or Y, each with only one chromatid carrying an *Sxr* body at its distal end (Figs. 3*a* and *b*, respectively). In the absence of this crossover transfer, metaphase II cells with an X bearing *Sxr* bodies on both chromatids, or with a Y lacking an *Sxr* body on either chromatid might be found. Such cells were detected in the X *Sxr*/Y males (Fig. 3*c*) in relatively high frequencies (Table 3), although never observed in X/Y *Sxr* animals (Evans *et al.*, 1982). It therefore seems likely that they derived from meiosis I cells in which the Yp – *Sxr* pairing had occurred.

During the metaphase II scoring, numerous diploid

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cells were observed and in 8% of these (8 in 100) the X and Y chromatids were joined (Fig. 4) and frequently formed bridges between their two haploid nuclei (Fig. 4*a*). Rare examples (< 1%) of joined X and Y chromatids were also observed in haploid cells (Fig. 4*b*) or indicated evidence of their presence by cells carrying a single Y chromatid (Fig. 4*c*). Bridge formation appeared to derive from meiosis I cells that had undergone the normal, pseudo-autosomal type of X–Y pairing.

4. Discussion

Cytological evidence of crossing over is usually not obtainable in normal mice. However, in *Sxr* carrier males, the additional dark-staining body serves as a visible crossover marker and this has allowed us to conclude that an obligate crossover between the X and the Y in the pseudo-autosomal region is a prerequisite for the production of functional spermatozoa (Evans *et al.* 1982). The deduction was based on a series of observations in X/Y *Sxr* mice, thus; 1) the two *Sxr* bodies were regularly found in an adjoining position to one side of the sex bivalent at diakinesis, so providing evidence of crossing over between the two chromosomes; 2) in probably all spermatocytes with unpaired X and Y chromosomes both chromatids of the Y carried the *Sxr* bodies, indicating an absence of crossing over with the X; 3) only crossover X and Y chromosomes were found in haploid metaphase II cells, suggesting a loss of meiosis I cells in which crossing over had NOT occurred; and 4) the latter conclusion was confirmed by the genetic data which indicated that progeny carrying recombinant and non-recombinant paternally-derived X and Y chromosomes appeared in equal frequencies (Lyon *et al.* 1981).

The observations made with X *Sxr*/Y mice in the present study differ from those made with X/Y *Sxr* mice in two important ways; (1) progeny carrying recombinant and non-recombinant X and Y chromosomes did not appear in equal frequencies, but rather, there was a marked shortage of detectable X–Y recombinants; and (2) non-crossover X and Y chromosomes were found in some haploid metaphase II cells. Both observations suggest that normal pairing and crossing over in the pseudo-autosomal region is not a prerequisite for functional germ cell survival. This seeming discordance between the findings with X/Y *Sxr* and X *Sxr*/Y mice can be accounted for by the additional observation in X *Sxr*/Y animals, namely that Yp – *Sxr* pairing (probably with crossing over) occurs in a high proportion of cells. It may be concluded that this illegitimate Yp – *Sxr* pairing satisfies the requirement for sex chromosome pairing, and this suggests that it is the formation of bivalents and perhaps their retention through meiosis by crossing over that is necessary for germ cell viability through meiosis, rather than a need to saturate pairing sites (Miklos, 1974) in the normal pairing regions. It may be noted that the frequencies of the 4 classes of progeny detected in the breeding data are in remarkably good accord with those expected from the diakinesis and metaphase II data (Table 4).

The pairing of the Yp with the *Sxr* region on the X is in itself of considerable interest. Homology of some genetic sequences in the two regions has been clearly established (Roberts *et al.* 1988) and this no doubt is

Table 4. *Observed and expected frequencies of progeny from X Sxr/Y males based on breeding and cytological data*

	Frequencies of expected classes of progeny			
Source of data	X/X ♀	X/X *Sxr* ♂	X/Y *Sxr* ♂	X/Y ♂
Breeding study				
From X/X classes[a]	10·0%	40·0%	10·0%	40·0%
From tested X/Y classes[b]	14·2%	35·8%	14·2%	35·8%
Overall	12·8%	37·2%	12·8%	37·2%
Diakinesis pairing[c]				
Pseudo-autosomal	9·5%	9·5%	9·5%	9·5%
Yp – *Sxr*	—	31·0%	—	31·0%
Total	9·5%	40·5%	9·5%	40·5%
Metaphase II cells[d]				
X *Sxr Sxr*	—	21·0%	—	—
X *Sxr*	16·5%	16·5%	—	—
Y *Sxr*	—	—	11·5%	11·5%
Y	—	—	—	23·0%
Total	16·5%	37·5%	11·5%	34·5%

[a] X/X data from males 5 and 6, Table 1.
[b] X/Y data from males 5 and 6, Table 1.
[c] Data from Table 2, excluding cells with unpaired sex chromosomes.
[d] Data from Table 3.

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responsible for their tendency to pair during meiosis. The situation is novel, however, in the fact that the Y short arm normally does not undergo any form of pairing. That it can pair with *Sxr* suggests that no special structures or pairing sites (Miklos, 1974) may be needed for pairing; homology alone appears to be adequate and this is emphasized by the observation that this novel homologous pairing was found to occur more frequently in X *Sxr*/Y mice than the conventional pseudo-autosomal pairing (62% cf. 38%, respectively, Table 2). The Yp – *Sxr* homology may also be expected to be responsible for the high levels of end-to-end self-pairing of the univalent Y in X/Y *Sxr* mice (Chandley and Speed, 1987; Tease and Cattanach, 1989) and for the much rarer X–Y pseudo-autosomal plus Yp – *Sxr* double pairing that has been observed in some meiotic prophase cells (Chandley & Speed, 1987). In both X *Sxr*/Y and X/Y *Sxr* mice, however, it may be that a disturbance of pseudo-autosomal pairing due to the presence of *Sxr* on one or other sex chromosome enhances the illegitimate pairing of the Y short arm and the *Sxr* region. However, the cause of the high frequencies of X–Y univalence in X *Sxr*/Y, X/Y *Sxr* and X *Sxr*/Y *Sxr* mice (Tease and Cattanach, 1989) remains unclear. That univalent Y chromosomes that do not carry *Sxr* sometimes also self-pair, as in X *Sxr*/Y mice (Tease and Cattanach, 1989), thus suggests that there could be some as yet undetected homology between the telomeric regions of the two arms of the Y, and this may have been a key factor in the origin of the *Sxr* mutation (Roberts *et al.* 1988). Some hint of such an homology is in fact evident in their *in situ* hybridization patterns of Roberts *et al.* (1988).

While the evidence of Yp – *Sxr* pairing is substantial, there is also some indication from the cytological work of crossing over between the two regions. It may be seen from Figs 2*c*, *d*, *e* and *f* that the illegitimate pairing of the X and Y is not a 'loose association' but sufficiently tenacious to resist extensive stretching of the sex bivalent (Fig. 2*c*). If bivalent survival to diakinesis is determined by the same constraints that apply to conventionally paired sex bivalents, a meiotic prophase crossover between the Yp and *Sxr* on the X would be required to preserve the association. Unfortunately, both segments after staining are cytologically similar and, in the bivalent, are so closely paired that they merge (Fig. 2*d*), or appear as 4 dark staining bodies, with the two representing the short arms of the Y occasionally appearing as the larger and more prominent pair (Fig. 2*e*). Consequently, a reciprocal exchange would be difficult to recognize unless there was appreciable misalignment and unequal exchange that resulted in marked asymmetry; and asymmetrical bivalents were in fact observed. Thus, rare (< 1%) examples were found in which most of one Yp chromatid arm appeared to be transposed to the X, while the smaller *Sxr* body was transposed from the X to the Y (Fig. 2*f*). Other less obvious examples were also recorded.

In total, the genetic and cytological data provide direct supporting evidence for the hypothesis that the Yp and *Sxr* can pair and undergo crossover exchange (Chandley & Speed, 1987; Roberts *et al.* 1989). The consequent reduction in X–Y pairing in the pseudo-autosomal region accounts for the observed shortage of normal X–Y recombinants. Without appropriate markers e.g. *Zfy* polymorphisms, recombination between the Yp and *Sxr* regions cannot be detected. But, the observations of asymmetry in diakinesis bivalents clearly suggest that the Yp *Sxr* pairing may be imprecise and, as a result, crossing over could generate duplications and deficiencies in the *Sxr* and Y short arm regions. As such, unequal crossing over between the Yp and *Sxr*, whether located on the X or Y, and also between 2 *Sxr* regions in *Sxr* homozygotes is very likely responsible for the *Sxr* variants reported by Maclaren *et al.* (1988) and Roberts *et al.* (1988), and also for an exceptional T16H/Y female derived from an X *Sxr*/Y male and an X/Y non-*Sxr* male derived from an X *Sxr*/Y *Sxr* homozygote (Cattanach, unpublished). Genetic studies using molecular markers to investigate this possibility are in progress.

The joined X and Y chromosomes (Fig. 4*a*, *b*) observed in several diploid and a few haploid metaphase II cells present an enigma and cannot easily be understood in terms of normal meiotic crossing over between the two chromosomes. In most of the examples recorded the two chromosomes appeared pyknotic but, from the morphology and the fact that in some cases they formed a bridge between two haploid cells (Fig. 4*a*), it is evident that the central component was a dicentric chromatid composed of most of one chromatid of the X and most of the chromatid of the Y. As the normal X and Y chromatids in all cases lay at each end of the configuration it may be concluded that the responsible crossover events must have occurred distally in both chromosomes. Such dicentrics could have occurred either by crossing over between pseudo-autosomal regions paired in an inverted fashion (Fig. 5), or by correct pairing with crossover error (Fig. 5). The potential of the dicentrics unequally to disjoin the chromatids of the sex chromosomes at anaphase I could account for the very high frequency of XO females detected in the genetic study. It would seem probable that the presence of *Sxr* is responsible for this meiotic error but it is difficult to understand how the XO frequency should be so much higher among the progeny of X *Sxr*/Y males (2·95%) than among those of X/Y *Sxr* animals (0·96%, Lyon *et al.*, 1983). The substitution of the original Y for one of 101/H origin may be a pertinent factor. Male mice carrying joined X and Y chromosomes have recently been found in both *Sxr* and normal mouse stocks (Evans *et al.* in preparation).

The sterility of most recombinant X/Y *Sxr* males in the present experiment, as detected directly or by

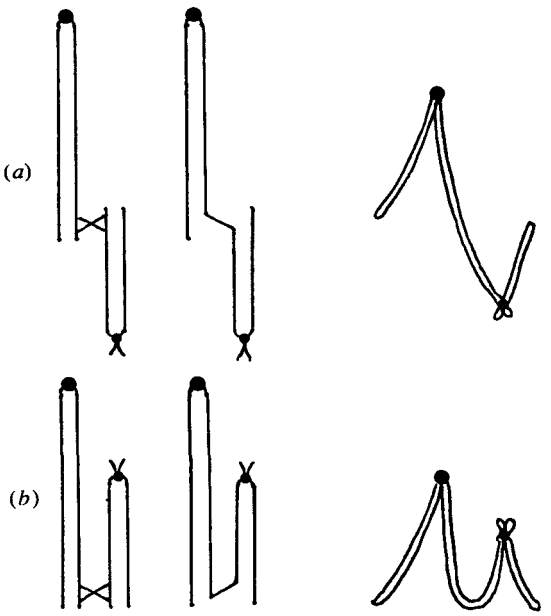

Fig. 5. Possible modes of origin of the joined X and Y chromatids. (*a*) Reversed or inverted pairing of the Y long arm pairing segment and subsequent crossing over. (*b*) Conventional pairing of X and Y followed by a cross-over error.

reduced testis weight, is somewhat surprising insofar as a fertile crossover of this kind had been detected in a previous experiment (Cattanach & Kirk, 1983) with the 101/H strain Y chromosome again being involved. Almost all of this male's X/Y *Sxr* descendants were also fertile, although they typically had greatly reduced testis weights. A level of biological variability is indicated. Consistent with this is the recent finding of varying proportions of fertile and sterile X/Y *Sxr* males derived by recombination from X *Sxr*/Y mice carrying Y chromosomes of different strain origins (Cattanach, unpublished).

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