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Sex chromosome configurations in pachytene spermatocytes of an XYY mouse

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Summary

Karyotypic investigation of a phenotypically normal but sterile male mouse showed the presence of an XYY sex chromosome constitution. The synaptic behaviour of the three sex chromosomes was examined in 65 pachytene cells. The sex chromosomes formed a variety of synaptic configurations: an XYY trivalent (40%); an XY bivalent and Y univalent (38·5%); an X univalent and YY bivalent (13·8%); or X, Y, Y univalence (7·7%). There was considerable variation in the extent of synapsis and some of the associations clearly involved nonhomologous pairing. These observations have been compared with previously published information on chromosome configurations at metaphase I from other XYY males.

1. Introduction

The XYY sex chromosome constitution occurs infrequently in mammals. In man, for example, approximately 0·5% of newborn babies have this aneuploid karyotype (see Jacobs *et al.* 1989). Similarly, in the laboratory mouse, Ford (1970) found only 1 XYY male among 5460 mainly male mice screened cytogenetically. Male mice with an XYY karyotype are phenotypically normal but almost invariably sterile; one exceptional male has been described that was initially fertile but thereafter became sterile (Evans *et al.* 1978). XYY mice have generally been found fortuitously as a result of cytogenetic analysis of males with unexpected sterility. Their rarity has been the principal factor limiting study of the influence of this type of sex chromosome aneuploidy on meiotic chromosome behaviour. To date, only 7 XYY male mice have been examined (Cattanach & Pollard, 1969; Rathenberg & Muller, 1973; Evans *et al.* 1978; Das & Kar, 1981) although additional data have been obtained from an X0/XYY mosaic male (Evans *et al.* 1969) and from X0/XY/XYY mosaic animals (Das & Behera, 1984; Palmer *et al.* 1990). In XYY spermatocytes, 4 types of sex chromosome configuration at metaphase I have been found: an XYY trivalent; an XY bivalent and Y univalent; an X univalent and YY bivalent; and X, Y, Y univalents. The relative frequencies of these different configurations varied considerably between males. For example, the proportion of spermatocytes with XYY trivalence ranged from 3·2% (Evans *et al.* 1978) to 33·6% (Rathenberg & Muller, 1973). In addition, there was disagreement between reports as to whether the sex chromosomes were randomly associated. Rathenberg & Muller (1973) and Das & Kar (1981) presented data indicating random involvement of the X and Y chromosomes in metaphase I configurations. In contrast, Evans *et al.* (1978) concluded the X chromosome had an overall pairing advantage as it was present as a univalent on fewer occasions than expected of random association. Das & Behera's (1984) data also indicated a paucity of X chromosome univalence.

Recently, Palmer *et al.* (1990) analysed 39 pachytene spermatocytes with an XYY chromosome constitution from 2 X0/XY/XYY mosaic mice. Of these cells, 19 had an XYY trivalent, 18 had an X univalent and YY bivalent, 1 had an XY bivalent and Y univalent, and 1 had X, Y, Y univalents. There is an intriguing contrast between these observations which indicated preferential involvement of the Y chromosomes in synapsis and the metaphase I data which suggested either random X and Y association (Rathenberg & Muller, 1973; Das & Kar, 1981) or preferential X chromosome involvement (Evans *et al.* 1978; Das & Behera, 1984). This brief report describes the synaptic behaviour of the sex chromosomes in pachytene spermatocytes of another XYY male. The information obtained may provide some further insights as to possible causes of the inter-animal variability in metaphase I sex chromosome association patterns and also whether there is any preferential involvement in chromosome pairing.

2. Materials and methods

The XYY male described here was from a stock of mice homozygous for the translocation T(14;15)6 Ca (hereafter abbreviated to T6); this translocation is maintained on the CBA/H inbred genetic background. None of the females to which he was mated became pregnant. To investigate the cause of his unexpected sterility the male was killed when 6 months old for cytogenetic analysis. The testes were removed and weighed: the left was found to be 22 mg and the right, 26 mg. One testis was used to make standard meiotic chromosome preparations (Evans *et al.* 1964), the other for synaptonemal complex preparations for electron microscopy (see Tease & Cattanach, 1989).

3. Results

(i) *Karyotype*

There were very few dividing cells on the slides made by the standard method, and those that were present tended to be of poor quality. For these reasons, no systematic analysis of meiotic chromosome behaviour was undertaken with these preparations. However, they were of value for establishing the animal's karyotype. Seven spermatogonial metaphases were located in which chromosome numbers could be counted unambiguously, and all contained 41 chromosomes. This situation could have arisen through primary autosomal trisomy, tertiary trisomy or sex chromosome aneuploidy. Primary autosomal trisomy almost invariably causes death during embryonic development or very shortly after birth (Gropp, 1982), and is therefore an unlikely explanation for the aneuploid karyotype. Tertiary trisomy for the T6 marker chromosome is a viable condition but as far as is known is produced only by T6 heterozygotes. It also has a diagnostic phenotype of head shaking and jerky movements (Cattanach, 1967) which was not found in the animal under study. Furthermore, analysis of the few clear metaphase I spermatocytes indicated both T6 marker chromosomes of the homozygous T6 mouse were present and that the extra chromosome was larger, and more similar in size to the Y chromosome (Fig. 1). The observations described in detail below for pachytene spermatocytes likewise indicated the additional chromosome to be a Y, as it was found to associate only with the sex chromosomes and never with autosomal elements.

(ii) *E.M. analysis of pachytene spermatocytes*

Sixty five pachytene spermatocytes were found in which sex chromosome synaptic behaviour could be analyzed (Table 1). Four general categories of sex chromosome behaviour were observed: XYY trivalents; XY bivalent with Y univalent (XY, Y); X univalent with Y bivalent (X, YY); and, univalence of all three chromosomes (X, Y, Y).

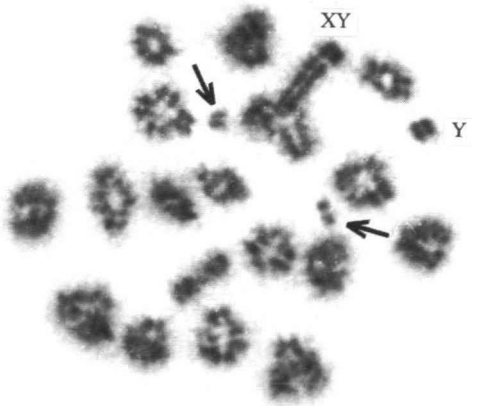

Fig. 1. A metaphase I spermatocyte containing 18 autosomal bivalents, two univalent T6 chromosomes (arrows), an XY bivalent and a Y univalent.

Table 1. *The numbers (and per cent) of pachytene spermatocytes with an XYY trivalent, an XY bivalent and Y univalent (XY, Y), an X univalent and Y bivalent (X, YY), and asynapsis of the sex chromosomes (X, Y, Y)*

Configuration				Total number of cells
XYY	XY, Y	X, YY	X, Y, Y	
26 (40)	25 (38·5)	9 (13·8)	5 (7·7)	65

(*a*) *XYY*

In 26 cells, the sex chromosomes were associated as a trivalent. However, the actual configuration adopted and the extent of XY synapsis varied considerably. The most common type, in 18 of the 26 cells, was a trivalent with association of the distal ends of each chromosome (Fig. 2*a*,*b*). In 7 cells, the X was associated with one Y chromosome proximally and the other distally (Fig. 2*c*); the proximal association was presumably non-homologous and therefore unlikely to permit chiasma formation. In 1 cell, the two Y chromosomes were associated proximally, with one Y axis paired distally with the X (Fig. 2*d*).

(*b*) *XY, Y*

Twenty five cells were found to have an XY, Y configuration. The extent of XY synapsis varied from terminal association through to approximately three-quarters of the Y paired with the X; this variability was not unexpected as it is a feature of normal XY pairing (e.g. Tres, 1977). Four of the cells contained an unusual XY configuration: a hairpin Y; uneven ended pairing; interstitial, hairpin pairing in the X axis (Fig. 2*e*) and a ring Y (Fig. 2*f*). These curious configurations are reminiscent of those seen in males carrying the sex-reversal (*Sxr*) factor in which the normal pattern of XY pairing is disrupted (Chandley

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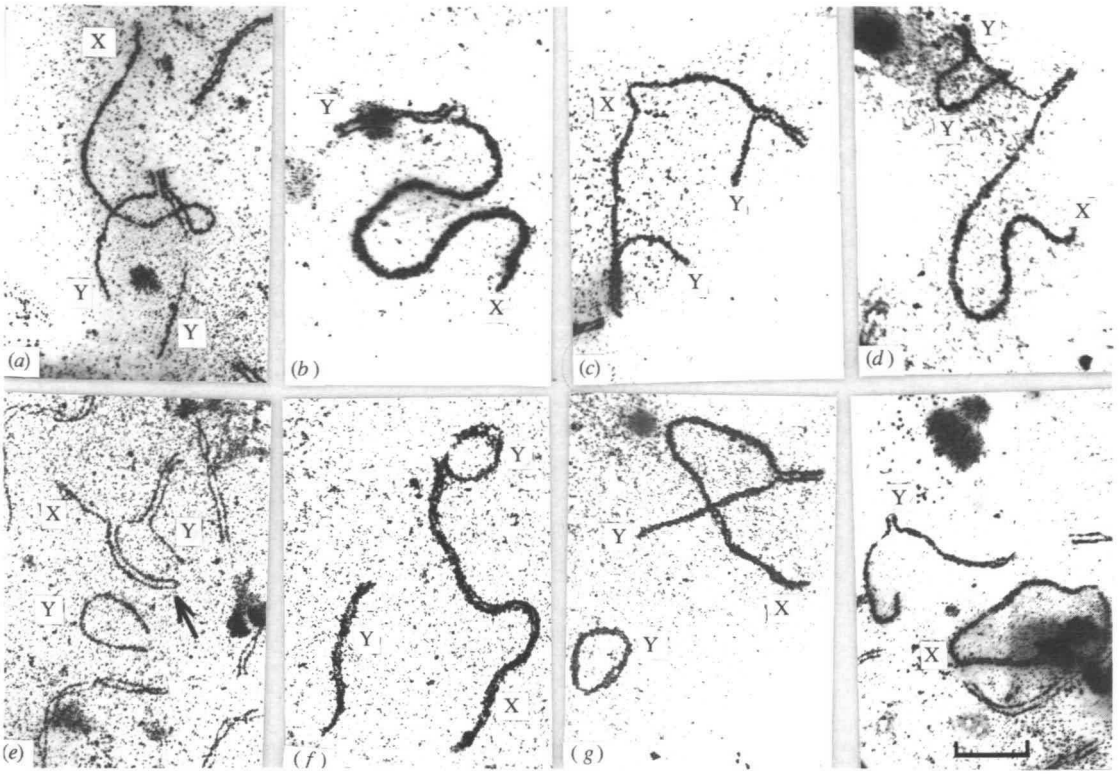

Fig. 2. Sex chromosome configurations in pachytene spermatocytes. *a*. An XYY trivalent. *b*. An XYY trivalent with paired Y axes. *c*. An XYY trivalent in which the Y axes are separately associated with the proximal and distal ends of the X. *d*. An XYY trivalent with the Y axes associated proximally but only one Y associated with the X. *e*. Foldback pairing of the X axis (arrow) of an XY bivalent; a rod-like Y univalent is present. *f*. An XY bivalent with a ring Y, the Y univalent is present as a rod-like structure. *g*. An XY bivalent and a ring-like Y univalent. *h*. A ring-like X univalent and partial pairing of the presumptive Y bivalent. The bar represents 2 μm, except in *b* and *d* where it represents 1·25 μm.

& Speed, 1987; Tease & Cattanach, 1989). The Y univalent was present as a rod-like structure in 17 cells and as a ring-like structure in 8 cells (Figs. 2*f*,*g*).

(*c*) *X, YY*

A univalent X axis was found in 9 cells. Six of these cells contained 20 fully paired SCs among which the Y bivalent could not be unambiguously identified. In 3 cells, however, a small bivalent, located adjacent to the X, was part paired (Fig. 2*h*). Although the identity of this bivalent could obviously not be definitely established, its location close to the X suggested it may have been the Y chromosome pair. The univalent X was present as a rod-like structure in 4 cells and a ring-like structure in the other 5.

(*d*) *X, Y, Y*

Five cells with univalence of all three sex chromosomes were found. The univalent X was present as a rod-like structure in 2 cells and a ring-like structure in 3 cells. The univalent Y axes were rod-like on 7 occasions, ring-like once and in 2 instances had self-paired to form hairpin structures. In cells with sex chromosome univalence, the identity of the Y axes could not absolutely be confirmed; for example, it is possible that the Y pair had formed a bivalent and the unpaired axes were autosomal. However, as the axes were of the size anticipated of Y univalents it seems reasonable to propose that they were unpaired Y chromosomes.

Discussion

The sterility of the male under study has been found to be the result of an XYY chromosome constitution. Examination of pachytene spermatocytes from this male has shown that the X and Y chromosomes synapsed in a non-uniform fashion giving rise to a diverse array of associations, some of which clearly involved nonhomologous pairing.

Overall, the pattern of sex chromosome synapsis in this male differed considerably from that described by Palmer *et al.* (1990). The principal difference lay in the relative compositions of the bivalent plus univalent categories. In the present male, 25/65 (36·5%) cells were of XY, Y type and 9/65 (13.8%) of X, YY. Palmer *et al.* (1990) reported 1/39 (2·6%) to be XY, Y and 18/39 (46·2%) to be X, YY. They speculated that the bias towards Y chromosome involvement in synapsis in the latter, mosaic males might be related to the fact they were (CXBH × BALB/c) F_1 hybrids. The X chromosome inherited from the CXBH strain

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might therefore be genetically distinct in the distal pairing region from the BALB/c Y chromosomes. Consequently, the similarity of the 2 Y chromosomes may have favoured their association. As the male described in the present report was from an inbred strain, no genetic differences might be expected in the X and Y chromosomes' pairing regions. This lack of distinction may be responsible for the different pattern of sex chromosome association found here compared to that of Palmer *et al.* (1990).

The previous studies on metaphase I spermatocytes from XYY males described the occurrence of trivalents, bivalents plus univalents, and complete sex chromosome univalence (Cattanach & Pollard, 1969; Rathenberg & Muller, 1973; Evans *et al.* 1969; Evans *et al.* 1978; Das & Kar, 1981; Das & Behera, 1984). The observations made here on pachytene spermatocytes suggest the variability in metaphase I configurations could result from an earlier variability of sex chromosome synapsis. However, failure of chiasma formation between associated chromosomes may also contribute to the relative frequency of the different configurations at metaphase I. Evans *et al.* (1978) found that the incidence of XYY trivalents declined from early diakinesis to metaphase I with a correlated increase in univalence. The relative contribution of variations in synapsis and chiasma failure will only become clear by comparison of all stages of meiosis from pachytene to metaphase I within individual XYY males.

Evans *et al.* (1978) assessed the relative involvement of X and Y chromosomes in synapsis by comparing the ratios of XY, Y to X, YY configurations in the published cases of XYY males. Excluding the males described by Cattanach & Pollard (1969) and Evans *et al.* (1969) in which only 15 and 17 metaphase I cells were analysed respectively, 5 XYY males were then available for comparison (Rathenberg & Muller, 1973; Evans *et al.* 1978). One male had a ratio of 1·6:1, the remainder had ratios larger than 2:1, namely 2·4:1, 2·5:1, 3·2:1 and 12·9:1. This tendency to larger ratios led Evans *et al.* (1978) to suggest the X chromosome had an overall pairing advantage. Although Das & Kar's (1981) subsequent data provided a ratio of 2·04:1 which obviously did not support this suggestion, Das & Behera's (1984) data were supportive in providing a ratio of 11·7:1. In the XYY male presented here, 25 XY, Y and 9 X, YY configurations were found giving a ratio of 2·8:1. However, the actual numbers observed did not differ significantly from expectation ($\chi_1^2 = 0{\cdot}70$, $P = 0{\cdot}4$). Thus although there was a slight excess of bivalents involving the X chromosome, it was not sufficiently large to demonstrate a significant pairing advantage at pachytene. The description of ratios significantly in excess of 2:1 (Evans *et al.* 1978) indicates that either sex chromosome pairing behaviour may vary considerably, or chiasma formation is generally favoured between X and Y chromosomes over that between Y chromosomes in some, but not all, XYY males.

Very little information is available on the synaptic behaviour of sex chromosomes at early prophase I in XYY spermatocytes of species other than the mouse. In Sitka deer mice, Hale & Greenbaum (1986) identified 7 XYY cells among a sample of 422 zygotene and pachytene spermatocytes from 3, chromosomally normal males. Of these 7 cells, 1 had an XYY trivalent, 2 had an X univalent and a loosely associated Y bivalent, and the remaining 4 had X, Y, Y univalents. Although the cells examined were at late zygotene/early pachytene in which sex chromosome synapsis might not necessarily have been completed, nevertheless the observations made are akin to those described here in showing variability in sex chromosome associations. In contrast to these data are those of Berthelsen *et al.* (1981) who undertook serial reconstructions of ultrathin sections of 8 pachytene spermatocytes from 3 human XYY males. In 7, the Y chromosomes were paired via their short arms and although the X chromosome was adjacent it did not form a synaptonemal complex with the Y chromosomes. In the eighth cell, an XY bivalent was present; however, the second Y chromosome appeared to have a deletion of the short arm which might have precluded its involvement in synapsis. One zygotene cell was reconstructed and this was found to contain a Y bivalent and an X univalent. Overall, this limited sample of human XYY spermatocytes showed a remarkably consistent pattern of sex chromosome association with an apparent preferential pairing of Y chromosomes to the exclusion of the X. Recent observations on another XYY human male have confirmed this remarkable consistency. Of 16 pachytene XYY spermatocytes which could be unambiguously analysed, 13 contained an X univalent and YY bivalent, 1 had an XY bivalent and Y univalent, and 2 had X, Y, Y univalence (R. M. Speed and M. Faed, personal communication). This relative consistency is markedly different to the situation in the laboratory mouse and in Sitka deer mice and may indicate a species-specific variation in sex chromosome pairing behaviour at pachytene in XYY aneuploids.

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