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An *XXY* mouse, the result of a rearrangement between one *X* and a *Y* chromosome

EDWARD P. EVANS[1*], GEORGE BRECKON[2] AND JOSEPHINE PETERS[2]
[1] *Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK*
[2] *Medical Research Council, Radiobiology Unit, Chilton, Didcot, Oxon OX11 0RD, UK*

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Summary

A male mouse with irregular white spotting, typical of piebald, *s*, arose during an experiment designed to search for mutations induced in spermatogonial cells by ethylnitrosourea (ENU). On being examined cytologically it was found to carry 40 chromosomes but was effectively *XXY* since one of the two *X* chromosomes present was distally fused to a *Y* chromosome. In common with the previously described *XXY* mice, all of which carried 41 chromosomes, the mouse was sterile with a total absence of germ cells. Because of this, it was not possible to determine if the white spotting was inherited. The spotting could not be related to any observable abnormality of chromosomes known to carry spotting genes, nor could it be linked in any way with the *X* and *Y* fusion. It was concluded from the cytological considerations and the time interval (6 months) that had elapsed between mutagen treatment and birth of the offspring, that whereas the spotting was probably the result of ENU damage in a spermatogonial stem cell, the *XY* fusion was probably a later and spontaneous event.

1. Introduction

Reports of the addition of non-pseudoautosomal chromatin from one of the heteromorphic sex chromosomes of the mouse to the other have so far been limited to two examples in which, by meiotic crossover, widely different-sized segments of the *Y* become attached distally to the *X* (Eicher, 1982; Evans, Burtenshaw & Cattanach, 1982). The frequency of spontaneously occurring *XXY* mice is also low (see Searle, 1981; Breckon, 1981; Beechey, 1983) with all the examples described being sterile males but generally of normal size and viability. In this paper we describe a sterile male mouse which carried both these chromosomal conditions because a rearrangement of the sex chromosomes rendered it essentially *XXY*.

The male was derived from an ethylnitrosourea (ENU) induced mutation experiment and first came to our notice because of irregular white spotting of the coat. Although subsequent cytological examination provided no explanation for the spotting, it revealed that the male was carrying 40 chromosomes but effectively had an *XXY* constitution, most of a *Y* chromosome being attached distally to one of the two *X* chromosomes present.

* Corresponding authors.

2. Materials and methods

(i) *Origin and description*

The mouse was an offspring of a (C3H/HeH × 101/H) F_1 male which had been given an intraperitoneal injection of ENU at a dose of 250 mg/kg. ENU (Fluka) was dissolved in 0·2 M phosphate buffer pH 6·0 as described by Russell *et al.* (1979). The male had subsequently been mated to a female of the outbred mutation testing stock, T, which is homozygous for seven recessive visible markers, *a*, *b*, *p*, c^{ch}, *d*, *se* and *s*. The offspring was born six months after the male parent had been treated and thus derived from an ENU treated spermatogonial stem cell. Since, in this case, the offspring showed irregular white spotting which looked like piebald (*ss*), it seemed possible that it carried a mutation at the piebald locus (*s*). This was investigated in test crosses with six females. No offspring were obtained, however, even with several changes of female but it was shown that the male was able to copulate as indicated by the presence of vaginal plugs. Male sterility was further indicated by the finding that each testis weighed less than 10 mg and was devoid of germ cells.

Externally the mouse looked like a normal male and when killed, had a body weight of 17·5 g. Although at dissection, both mesenteric and the inguinal lymph

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nodes were found to be enlarged and the also enlarged spleen was subsequently considered to have the histological appearance of a reticular cell sarcoma, no positive evidence of malignancy associated chromosomal changes were observed in preparations obtained from this organ nor from the other lymphomyeloid tissues which were examined (see Results).

(ii) *Methods*

Air-dried preparations of mitotic metaphases were obtained directly from femoral bone marrow and the spleen and also from Concanavalin A stimulated blood and spleen cultures. The preparations were examined after conventional staining with Brevans stain mountant (Breckon, 1983), after G-banding using a modification of the technique of Gallimore & Richardson (1973), after Q-banding with Atebrin and also after C-banding using the method of Sumner (1972). Attempts at obtaining fibroblast cultures from the ears failed due to a massive and uncontrollable bacterial infection.

3. Results

All the chromosome information from the mouse was obtained from the lymphomyeloid tissues. This, in part, is summarized in Table 1 and of a total of 90 metaphases scored after conventional staining, 88 contained 40 chromosomes which included a long one approximately 20% longer than the longest normal mouse chromosome. The remaining two metaphases scored also contained this long chromosome and counted 39 and 41 chromosomes respectively. The latter single, hyperdiploid count represented the only possible evidence of malignancy associated aneuploidy in the mouse.

A further 20 metaphases were karyotyped from G-banded cells and the chromosomes classified according to the standard ideogram (Nesbitt & Francke, 1973). All these metaphases showed an apparently normal karyotype (Fig. 1 *a*) apart from the long marker which was considered to represent most of one *X* chromosomes with its distal end attached to the distal end of most a *Y* chromosomes. Further banding studies confirmed this conclusion regarding the *Y* in that the distal third of the long marker showed the typical appearance of the mouse *Y* chromosome, uniformly brightly fluorescing after Q-banding (Fig. 1 *b*) and dark staining but showing some structure after C-banding (Fig. 1 *c*). In some of the cells, in both conventionally stained and in banded preparations, the typical bifurcating short arm of the mouse *Y* chromosome (Ford, 1966) could also be seen (Fig. 1 *d*), a feature which indicated the presence of a centromeric constriction and so suggested that the long chromosome was a dicentric. A comparison of the G-band patterns of the long chromosome with the standard ideogram for the *X* and *Y* chromosomes showed that all the recorded bands seemed present (Fig. 1 *e*) although, as has previously been pointed out (Evans *et al.* 1982), the *Y* frequently reveals more bands than portrayed in the standard ideogram and the *X* has an additional band distal to XF4 (Sawyer, Moore & Hozier, 1987; Evans, 1989) which has previously regarded as the terminal band.

Table 1.

Chromosome sample source	Chromosome counts 39	40	41	Total meta-phases
Direct bone marrow	0	25	0	25
Direct spleen	0	14	1	15
48 h spleen culture	1	24	0	25
48 h blood culture	0	25	0	25
Totals	1	88	1	90

4. Discussion

The presence of the long chromosome incorporating most of *X* and *Y* (hereafter referred to as X^Y) in all of the mitotic cells observed is consistent with the conclusion that it was not associated with malignancy but that the mouse carried the chromosome from an early stage of development. This conclusion is supported by the testicular size and contents which were typical of the testes previously described in *XXY* mice (Breckon, 1981; Beechey, 1983).

The X^Y rearrangement could have occurred in early embryogenesis or in a paternal germ cell. If formed during embryogenesis, the zygote would initially have had to be of an *XXY* constitution and the *Y* chromosome translocated on to one of the *X* chromosomes. Unless the translocation occurred at the pronuclear stage or at an early cleavage division so that selection of the new line could alone give rise to the embryo proper, the *XXY* cell line could also have persisted but was not observed in the sampling. Although requiring the occurrence of two rare and unusual events in the same cell, it seems more feasible to consider that both X^Y and a mutation which gave rise to the irregular white spotting were induced by ENU in a paternal germ cell. Since 6 months elapsed between treatment and the birth of the carrier, such induction would have had to occur in a spermatogonial stem cell, thus giving the X^Y chromosome the potential to form a clone of germ cell descendants and also necessitating its passage through the meiotic division to produce a functional gamete. Burgoyne & Baker (1984) have argued strongly that completing the meiotic process requires successful pachytene pairing and it is difficult to envisage how this could be accomplished with the X^Y chromosome if the pairing segments were already fused before pairing

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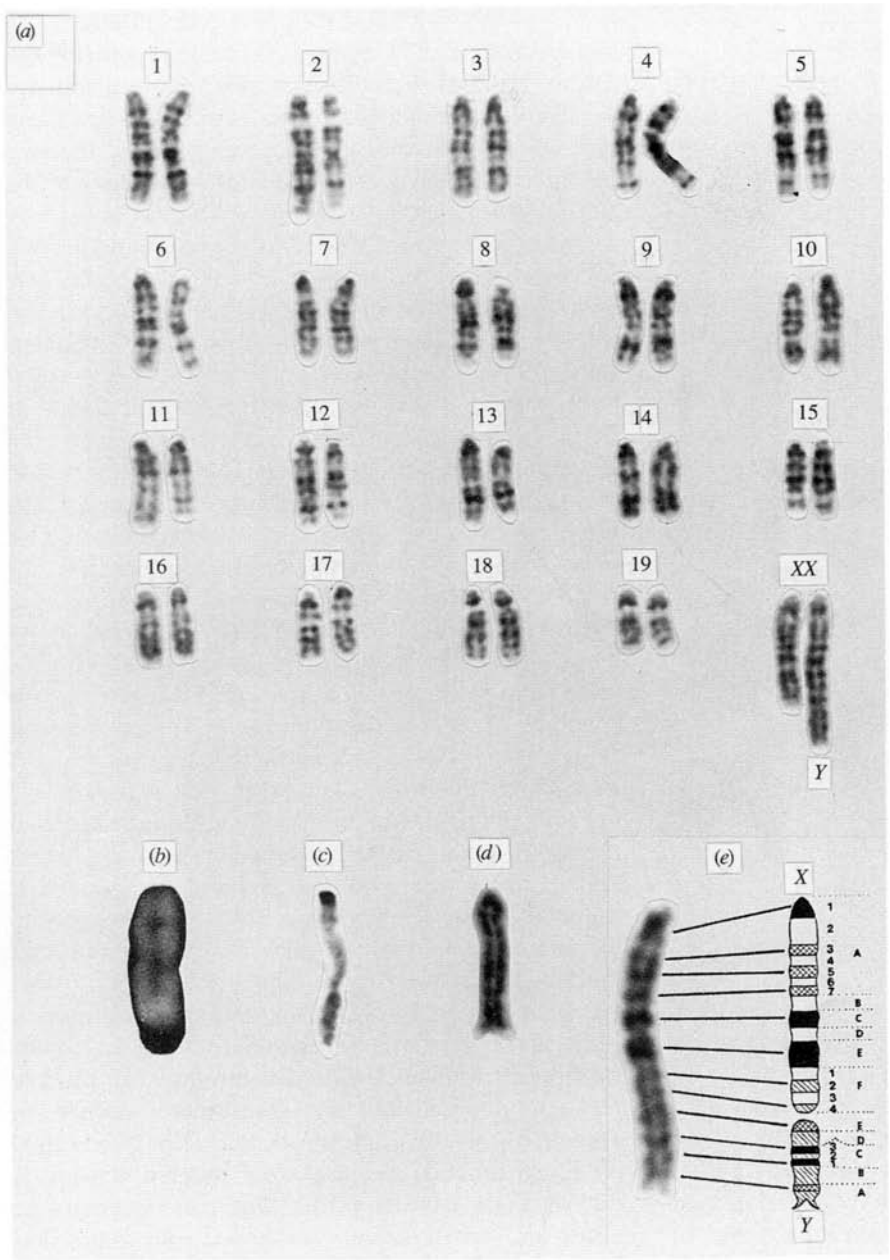

Fig. 1. (*a*) G-banded karyotype, normal apart from the X^{Y}-chromosome. (*b*) Q-banded X^{Y} showing the uniformly, brightly fluorescing distal third. (*c*) C-banded X^{Y} showing the darker staining and some structure in the distal third. (*d*) Conventionally stained X^{Y} with the distal terminus showing the bifurcating short arm of the mouse *Y* chromosome. (*e*) A comparison of X^{Y} with the standard ideogram for *X* and *Y* showing the presence of all the major bands.

commenced. However, since the pairing segments are near terminal (Evans *et al.* 1982), fusion might ensure that no pairing segments remained unsaturated (Miklos, 1974) and possibly enable the spermatocyte to proceed through the meiotic division.

Irrespective of this possibility, the morphology presented by X^{Y} is reminiscent of the configuration presented by the *XY* bivalent at diakinesis of meiosis in which the two chromosomes are terminally associated at their distal ends. It has been suggested (Eicher, 1982; Hansmann, 1982; Burgoyne, 1982) and demonstrated in the case of 'sex-reversed' (*Sxr*) mice that, prior to this, the *X* and *Y* partake in an obligatory crossover which may be near terminal (Evans *et al.* 1982). An error during crossing over, in which the distal chromatin is not exchanged between *X* and *Y* in the normal manner but in which the centromeric chromatid of the *X* rejoins with the centromeric chromatid of the *Y*, would subsequently present a dicentric chromosome of similar morphology to X^{Y} (Fig. 2*a*). Alternatively, the same type of chromatid could be produced if the *Y* chromosome paired with the *X* in a reverse or inverted manner and a crossover followed (Fig. 2*b*). Either mechanism could be responsible and it is of interest that examples of similar chromatids have recently been observed at

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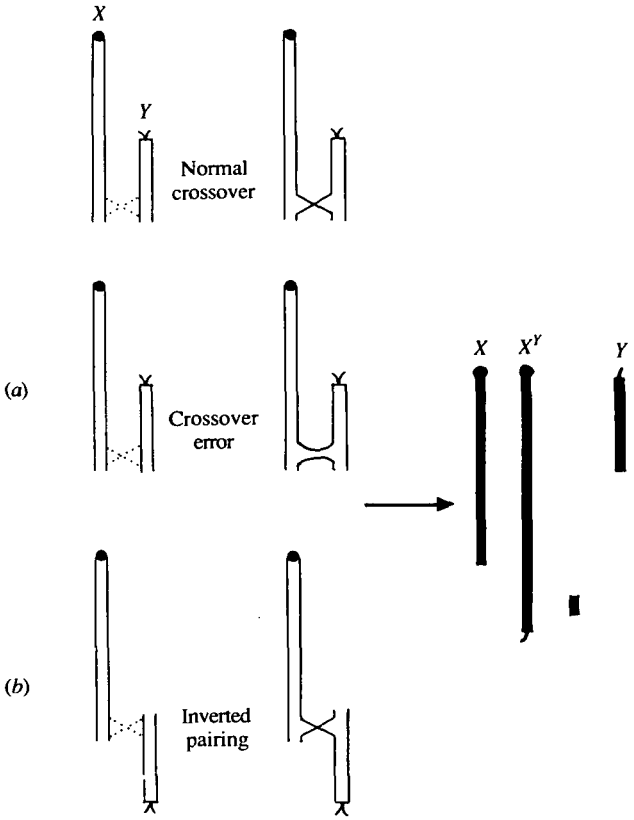

Fig. 2. The possible mode of origin of X^Y. (*a*) By crossover error at the pachytene stage of meiosis. (*b*) By inverted or reversed pairing followed by crossover at pachytene.

the second meiotic division in three untreated male mice (Evans, unpublished) which implies that errors in pairing or crossing over can arise.

The spontaneous formation of the rearrangement at a later germ cell stage conflicts with the suggestion that both X^Y and the irregular white spotting resulted from concomitant ENU damage in a spermatogonial stem cell. If not, it would require the occurrence of two separate and rare events in a cell but taking place several months apart. Although seemingly unusual, it is not without precedence and it is known for a mouse, or a cell, carrying one mutational event to acquire a further unrelated spontaneous mutation at a later time (see Searle, 1981).

Unfortunately, since the carrier was male sterile, the mutation responsible for the irregular white spotting could not be tested. Examination of the distal segment of chromosome 14 to which *s* maps revealed no departure from the normal G-band pattern (Fig. 2*a*) and neither did the examination of the other autosomes.

Burgoyne (unpublished) observed a similar X^Y chromosome in a runted male which was the offspring of a cross, $In(X)1H/X \times X^{Ta}/Y^{Sxr}$. This male, however, was a mosaic containing cell lines with $39X/O$ and a $39X^Y/O$ constitution and, since the phenotype was hemizygous tabby (*Ta*), it was concluded that both sex chromosomes (that is *X* and *Y*) had been inherited from the father and at some point in development, X^Y had reverted to *X* by discarding the *Y*. It is feasible to speculate that this occurred because both centromeres were active at one stage and in attempting to distribute themselves at anaphase to opposite poles, the two chromosomes separated with subsequent loss of the *YO* daughter cells, a genotype known to be inviable in the mouse (Morris, 1968; Burgoyne, unpublished). Such breakage, however, according to the classic concepts of telomere conservation (Muller, 1940) would require the broken *X* chromosome to undergo some form of distal repair before a cell line could become established. We saw no sign of the fission of the X^Y, nor of the appearance of anaphase bridges in our material and it seems reasonable to conclude that although the chromosome had the morphology of a dicentric, only one centromere was functional. This concept of active and inactive centromeres in mammalian dicentric chromosomes has previously been offered as an explanation for the successful maintenance of Robertsonian chromosome systems during fusion and fission (e.g. see Imai, 1978), as also has the deletion of the centromere as a mechanism for achieving stability in a dicentric (Vianna-Morgante & Rosenberg, 1986).

From the evidence presented, it is concluded that the X^Y-probably arose spontaneously in a primary spermatocyte and was not induced in a spermatogonial stem cell by the ENU treatment. Unfortunately, the two examples of X^Y chromosomes described here were carried by infertile males, ours because of its *XXY* constitution and that of Burgoyne because of runting. Consequently, the intriguing question of whether such a chromosome can proceed through meiosis and be transmitted cannot be answered at this time. However, the recent observation of X^Y chromatids at the second meiotic division in further male mice confirms that meiotic errors do occur and additional informative carrier males may yet be found.

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