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Daughterless X *Sxr*/Y *Sxr* mice

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SUMMARY

McLaren & Monk (1982) and Cattanach *et al.* (1982) reported that T(*X*; 16)16H/*X Sxr* mice, in which the *X* chromosome bearing *Sxr* is the inactivated *X* chromosome, can develop as fertile females. By mating such females to *X*/*Y Sxr* males it has been possible to produce mice homozygous for *Sxr*. Two *X Sxr*/*Y Sxr* males were identified which together fathered 141 sons and 1 daughter. The single daughter proved to be *XO*, indicating a non-disjunctional event with neither paternal sex chromosome being transmitted. It is concluded that *X Sxr*/*Y Sxr* mice are viable and fertile, and that all their progeny, provided they receive a paternal sex chromosome, develop as males.

1. INTRODUCTION

In 1971, Cattanach and his colleagues described a spontaneous mutation which made chromosomally *X*/*X* mouse embryos develop as phenotypic males (Cattanach, Pollard & Hawkes, 1972). Sex-reversed (*Sxr*), as the mutation was termed, has recently been shown to be located on the distal end of the *Y* chromosome in *X*/*Y Sxr* carrier males, and on the distal end of the *X* in *X*/*X Sxr* males (Singh & Jones, 1982; Evans, Burtenshaw & Cattanach, 1982). It appears to have arisen as a duplication of the testis-determining region that is normally situated proximally, adjacent to the centromere of the *Y* chromosome. By virtue of its distal location, *Sxr* is transferred to the *X* chromosome when the *X* and *Y* chromosomes pair and undergo crossing-over during male meiosis (Burgoyne, 1982; Eicher, 1982). Since *X*/*X* germ cells in a testis degenerate soon after birth (Burgoyne, 1978; McLaren, 1983), *X*/*X Sxr* males are always sterile.

The usual random pattern of *X* inactivation in *X*/*X* individuals can be altered by introducing the *X*-autosome translocation T(*X*; 16)16H. In females heterozygous for this translocation, the normal *X* is seldom if ever expressed in the adult (Lyon *et al.* 1964).By producing T(*X*; 16)16H/*X Sxr* mice it is possible to ensure that it is the *X* carrying *Sxr* which is inactivated. With *Sxr* inactivated such mice would be expected to be female. Surprisingly it was found that these mice developed sometimes as males, sometimes as females, and occasionally as hermaphrodites (McLaren & Monk, 1982; Cattanach *et al.* 1982). The variable sexual phenotype suggests that there is variable spreading of inactivation into the attached *Sxr* segment, as is the case with autosomal regions attached to the *X* (Cattanach, 1974).

Presumably the sex of the T(X; 16)H/X *Sxr* embryo is determined by the relative proportion of cells in the gonad primordia that have *Sxr* inactivated (and hence are female-determining) or expressed (and hence are male-determining). In X/X *Sxr* mice the combination of random X inactivation, together with the frequent

Table 1. *Progeny classes expected when T(X; 16)16H* Pgk-1^b/X Pgk-1^a Sxr *females are mated to* (X/Y) Sxr *males*

(Since *Sxr* shows a recombination frequency of 50% in both male and female gametes, the 16 classes are expected to occur with equal frequency.)

Male gametes		Female gametes: T(X; 16)16H *Pgk-1^b* +	Female gametes: T(X; 16)16H *Pgk-1^b* *Sxr*	Female gametes: X *Pgk-1^a* +	Female gametes: X *Pgk-1^a* *Sxr*
X*	+	PGK-1B ♀ (1)	PGK-1B Sterile ♂ (5)	PGK-1A or AB ♀ (9)	PGK-1A or AB sterile ♂ (13)
	Sxr	PGK-1B ♀ or sterile ♂ (2)	PGK-1B sterile ♂ (6)	PGK-1A or AB sterile ♂ (10)	PGK-1A or AB sterile ♂ (14)
Y	+	PGK-1B sterile ♂ (3)	PGK-1B sterile ♂ (7)	PGK-1A fertile ♂ (1♂:1♀)† (11)	PGK-1A fertile ♂ (3♂:1♀)† (15)
	Sxr	PGK-1B sterile ♂ (4)	PGK-1B sterile ♂ (8)	PGK-1A fertile ♂ (3♂:1♀)† (12)	PGK-1A fertile ♂ (all ♂ progeny)† (16)

* Some of the paternal X chromosomes carried *Pgk-1^a* and some *Pgk-1^b*, but in the presence of the T(X; 16)16H *Pgk-1^b* chromosome, *Pgk-1^a* was not expressed.

† Expected sex ratio in progeny.

expression of *Sxr* on the 'inactive X', will increase the proportion of cells expressing *Sxr* (which are hence male-determining) to above 50%, which is consistent with the observation that X/X *Sxr* mice are invariable male.

The female T(X; 16)16H/X *Sxr* mice are fertile, and pass on *Sxr* to 50% of their offspring. By mating T(X; 16)16H/X *Sxr* females to X/Y *Sxr* males, it should be possible to obtain homozygous X *Sxr*/Y *Sxr* males. The present investigation was undertaken to ascertain (a) whether such individuals were viable, (b) if so, whether they were fertile, and (c) if so, what would be the sex ratio of their progeny.

2. MATERIALS AND METHODS

The stocks of mice used and the production of T(X; 16)16H/X *Sxr* females are as detailed in McLaren & Monk (1982). The T(X; 16)16H/X *Sxr* females, in which the normal X was marked by the variant allele *Pgk-1^a* that codes for phosphoglycerate kinase 1A (PGK-1; E.C. 2.7.2.3), were mated to X/Y *Sxr* males. Such a

cross is expected to yield 16 genotypic classes in equal proportions, as set out in Table 1. PGK-1 phenotypes (A, B or AB) of the progeny were determined by gel electrophoresis (Bucher *et al.* 1980) of blood samples taken from the retro-orbital sinus. The *Pgk-1*^{a} allele on the normal *X* chromosome is not expressed in the

Table 2. *Progeny obtained from mating T*(X; *16*)*16H* Pgk-1^b/X Pgk-1^a Sxr *females to* X/Y Sxr *males*

	♀	♂ sterile	♂: fertile, progeny sex ratios*		
			1 : 1	3 : 1	all ♂
PGK-1B	3	19	0	0	0
PGK-1A or AB	8	11	3	3	2

* See Table 3.

Table 3. *Progeny obtained from mating the fertile PGK-1A males listed in Table 2 to normal* X/X *females*

	Mean litter size	♂	♀	Presumed genotype
♂ A	8·1	89	0	*X Sxr/Y Sxr*
♂ B	10·6	52	1*	
♂ C	13·3	37	16	*X Sxr/Y* or *X/Y Sxr*
♂ D	7·4	30	7	
♂ E	12·7	30	8	
♂ F	9·5	19	19	*X/Y*
♂ G	13·3	23	17	
♂ H	10·6	42	32	

* This female proved on karyotyping to have a diploid chromosome number of only 39, and hence was presumably *X/O*.

presence of T(*X*; 16)16H, so PGK-B progeny were assumed to be carrying the translocation (recombinants are rare, see McLaren & Monk, 1982). PGK-B progeny were discarded, since both T(*X*; 16)16H/*Y* and T(*X*; 16)16H/*X* males are sterile and their *Sxr* status can therefore not be determined. PGK-AB males (in cells 10, 13 and 14) were assumed to be *X/X Sxr* and were also discarded. PGK-A males (in cells 10–16 in Table 1) were then test-mated to randomly bred females (MF1 from Olac).

3. RESULTS

Table 2 shows the progeny obtained by mating female *Sxr* carriers to *X/Y Sxr* males. Of the fertile males (Table 3), 3 produced approximately equal numbers of males and females (*X/Y*, cell 11 of Table 1), 3 produced progenies consistent with a 3:1 ratio of males to females (*X Sxr/Y* and *X/Y Sxr*, cells 12 and 15), and 2 produced all males, with the exception of a single daughter which proved on chromosomal examination to have only 39 chromosomes and hence was presumably *X/O*. Mean litter size was similar for all males. We concluded that males A and B in Table 3 were *Sxr* homozygotes, corresponding to cell 16 of Table 1.

4. DISCUSSION

Of the two males that we have identified as *Sxr* homozygotes, one had 89 sons and no daughters, the other 52 sons and 1 daughter. This single female was the 'exception that proved the rule', since she had a diploid chromosome number of 39 and hence was presumably *X/O* in chromosome constitution, due to paternal non-disjunction leading to loss of the *Sxr*-bearing *X* or *Y* chromosome.

If the event that generated *Sxr* had involved the loss of any chromosomal material from the distal end of the *Y* chromosome, a homozygous *Sxr* embryo would be nullisomic for the deleted region and hence might be non-viable, or at least infertile. Two *X/Y* homozygotes in a total of 49 progeny, with an *a priori* expectation of 1 in 16, is consistent with normal viability for the homozygous condition, while the litter sizes that we observed give no indication of reduced fertility. We therefore conclude that no chromosomal material of significance to viability or fertility has been lost.

In insects, genetical systems exist whereby all progeny produced are male, since females carry lethal genes (see Strunnikov, 1981, for an example from silkworm genetics). Such lethality results of course in reduced fecundity. In mice, an *X*-linked lethal such as brindled, which kills carrier males before weaning, leads to a 2:1 sex ratio in favour of females at weaning. Large shifts in sex ratio brought about otherwise than by differential mortality are less common. In amphibia, where germ cell sex is determined not by the chromosomal constitution of the germ cells themselves but by the phenotypic sex of the gonad in which they develop, sex reversal may be brought about by germ cell transplantation. Blackler (1965), by grafting pieces of tissue from one *Xenopus* embryo to another, produced fertile female (ZW) toads in which the germ cells were entirely male (ZZ) in chromosome constitution. When mated to normal ZZ males, all-male progenies were produced.

The system for generating all-male progenies described in this paper makes use of two genetic aberrations: (*a*) an abnormal *Y* chromosome carrying a masculinizing segment in a position such that it can be transferred to the *X* chromosome by crossing-over during male meiosis, and (*b*) an *X*-autosome translocation which suppresses expression of the normal *X* chromosome and hence of the attached masculinizing segment, allowing it to be transmitted through the female line.

If such a system could be devised in cattle, it would be of economic value in the beef cattle industry. Translocation of a masculinizing segment to a position on the *X* and *Y* chromosome where it can be exchanged between the *X* and *Y* by crossing-over during male meiosis, as occurs in *Sxr*, is presumably a very rare event. An *X* chromosome with a masculinizing segment translocated to any other position would normally fail to be transmitted, since the *X/X* carriers would develop as sterile males. However, if such an *X* chromosome were to arise in a male, either spontaneously or as a result of irradiation, it might be rescued if the male were mated to a female heterozygous for a differentially active *X*-autosome translocation. The translocation provides the potential for suppressing the 'masculinizing *X*' in *X/X* carriers, thus overcoming the block to further transmission. This approach is feasible, because *X*-autosome translocations have been identified in cattle (Gustavsson *et al.* 1968; Eldridge, 1980) and there is evidence that the translocated *X* is indeed differentially active (Gustavsson, 1971).

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