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Cattanach's translocation [Is(7:*X*)Ct] corrects male sterility due to homozygosity for chromosome 7 deletions

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SUMMARY

Mice carrying particular deletions of chromosomal material at and around the colour (*C*) locus on chromosome 7 (c^{3H}/c^{6H}) are viable but sterile. An insertional translocation of chromosome 7 into the *X* (Cattanach's) has been previously used to rescue females carrying deletions of chromosome 7 which are ordinarily lethal. We studied the ability of this translocation to correct the sterility found in the presence of the two partially complementing deletions. We predicted that the sterility would be corrected in females who would be mosaics because of X-inactivation. The result in males was uncertain since the sterility had been shown to be due to defective spermatogenesis, and the X chromosome is inactivated early in the course of spermatogenesis. The c^{3H}/c^{6H} male and female deletional sterility were rescued by Cattanach's translocation.

1. INTRODUCTION

X-inactivation during spermatogenesis is a phenomenon less well known and studied than *X*-inactivation in female somatic cells. The evidence for *X*-inactivation in mammalian males has been primarily deduced from cytogenetic observations of sex chromosomes. *X*-inactivation in male meiosis is carried to an extreme by the creeping vole, which eliminates the *X*-chromosome in its spermatogonia (Ohno *et al.* 1963), and is probably found in all male heterogametic animals (Lifschytz, 1972). The implications of *X*-inactivation for the biochemical genetics of spermatogenesis have not been generally explored. Studies of the expression of the *X*-linked enzymes glucose-6-phosphate dehydrogenase (Erickson, 1975, 1976) and phosphoglycerate kinase-1 (Kramer & Erickson, 1981) have been inconclusive as to whether meiotic and/or post-meiotic transcription or translation occurred. However, three *X*-linked enzyme activities were increased in spermatocytes of mice rendered sterile by chromosomal alterations (Hotta & Chandley, 1982), suggesting the possibility of abnormal meiotic *X*-inactivation.

Several radiation-induced deletions at and around the albino locus on chromosome 7 of the mouse include those causing sterility (for review cf. Gluecksohn-Waelsch, 1979). Homozygotes for the deletion c^{3H} die in the newborn period and homozygotes for c^{6H} at the egg cylinder stage; however, the compound heterozygotes are viable

though sterile (Lewis *et al.* 1978). On the premise that this sterility is due to a defect in germ cells, an insertional translocation between chromosome 7 and the X-chromosome, [Cattanach's 'flecked', designated Is(7:X)Ct] measuring 21 cM and including the *C* locus (Cattanach & Isaacson, 1965), offered the opportunity to study the effects of *X*-inactivation during spermatogenesis. The question arose if this translocation could correct the sterility of c^{3H}/c^{6H} males. Previous studies have demonstrated that potentially lethal genotypes including the *C* locus were subject to varying degrees of rescue by this translocation (Gluecksohn-Waelsch *et al.* 1980).

In the present paper we report the results of breeding experiments carried out to determine whether Cattanach's translocation could correct male sterility due to the c^{3H}/c^{6H} genetic constitution. The results reveal that one definite and one probable c^{3H}/c^{6H} male and one c^{3H}/c^{6H} female were fertile.

2. MATERIALS AND METHODS

Females heterozygous for Cattanach's translocation (X^T) and a normal *X* and heterozygous for a deletion (Df) of 21 cM (equivalent to the translocated piece) on one chromosome 7 and the c^{3H} deletion on the other chromosome 7 were obtained from the matings described previously (Gluecksohn-Waelsch *et al.* 1980). They were mated to males heterozygous for the c^{6H} chromosome 7 deletion and c^{ch}, the chinchilla allele at the *C* locus:

$$X^T/X\ \mathrm{Df}/c^{3H} \times X/Y\ c^{ch}/c^{6H}$$

Surviving, fully pigmented (*C*) males from this cross can be (1) $X^T/Y\ \mathrm{Df}/c^{ch}$, (2) $X^T/Y\ \mathrm{Df}/c^{6H}$, (3) $X^T/\mathrm{Y}\ c^{3H}/c^{ch}$, or (4) $X^T/Y\ c^{3H}/c^{6H}$. Although all of these possible genotypes are phenotypically similar, being fully coloured, they may be distinguished by a test-cross to *c/c* females. Thus, the first and third classes will give rise to chinchilla male offspring half the time. The second and fourth classes will only have albino offspring but can be partially distinguished by the male/female ratio of their offspring: $X/Y\ \mathrm{Df}/c$ is lethal because of monosomy for this 21 cM section of chromosome 7. Thus, $X^T/Y\ \mathrm{Df}/c^{6H} \times X/X\ c/c$ will result in a deficiency of male albino offspring. However, the distinction between classes two and four is not essential. Although it has not been tested, Df/c^{6H} is expected to be sterile since it is deficient for the chromosome 7 material, and more, deficient in sterile c^{3H}/c^{6H}. Thus, it will equally be a test of *X*-inactivation, and fertility of males in classes 2 and 4 would indicate correction.

All offspring from the cross were kept until their coat pigmentation was evident. Full-coloured males and 'flecked'/albino females were mated to random-bred CD-1 *c/c* mice of the opposite sex (two CD-1 females for the coloured males, one CD-1 male per 'flecked'/albino female). Their offspring were similarly reared to weaning age and their coat colours recorded.

3. RESULTS

Four of 11 fully pigmented male offspring from the X^T/X Df/$c^{3H} \times X/Y\ c^{6H}/c^{ch}$ cross were fertile when tested for at least 4 female-months, (Table 1). Two of the fertile males were heterozygotes for chinchilla but two were not. Thus, lethal albino sterility (DF/c^{6H} or c^{3H}/c^{6H}) was corrected by Cattanach's translocation in at least

Table 1. *Classes of progeny from* X^T/Y *?/? fertile males from the cross of* X^T/X *Df*/$c^{3H} \times X/Y\ c^{ch}/c^{6H}$

Male	No of litters*	Average size litters	No. of progeny/ month†	Classes of progeny: Albino	Dilute chinchilla	Flecked	Not typed	Presumed genotype at colour locus
8785‡	2	7·5	1·58	7	0	8	—	Df/c^{6H} or c^{3H}/c^{6H}
9062	5	5·4	4·5	0	8	17	2	DF/c^{ch}
9063	5	9	5·6	25	0	20	—	c^{3H}/c^{6H}
9064	7	9	10·5	9	10	25	19	c^{3H}/c^{ch}

* Since the two females often have litters at the same time, this is a minimal estimate.
† Males caged with two females continuously which were not rotated.
‡ Pedigree number.

Table 2. *Fertility and classes of progeny from 'flecked'/albino* (X^T/X *DF*/c^{6H} *or* $X^T/X\ c^{3H}/c^{6H}$) *compared to 'flecked'/chinchilla* (X^T/X *Df*/c^{ch} *or* $X^T/X\ c^{3H}/c^{6H}$ *females*

Female	No. of litters	Average litter size	No. of progeny/ month	Classes of progeny: Albino	Dilute chinchilla	Flecked	Albino	Coloured	Dilute chinchilla	Not typed
'Flecked'/ Albino										
8789*	0†	0	0	—	—	—	—	—	—	—
8790	2	2·5	1	2	—	2	—	—	—	1
'Flecked'/ Chinchilla										
8787	4	7·0	7·0	—	3	11	—	6	4	4
9059	3	7·3	7·3	—	5	8	—	5	4	—

* Pedigree number.
† Tested for 5 months with a random-bred CD-1, *c*/*c* male.

one male, and probably two (Table 1). Male 8785 was of low fertility. His seven male offspring were all albino. While it is possible that he was heterozygous for c^{ch} and did not have dilute chinchilla offspring by chance ($\chi^2 = 3{\cdot}5$, $P < 0{\cdot}1$), it is more likely that he was Df/c^{6H} or c^{3H}/c^{6H}, which are ordinarily sterile. Male 9063 showed average fertility, for these four males. With 25 albino sons and no dilute chinchilla son ($\chi^2 = 12{\cdot}5$, $P < 0{\cdot}01$), there is little doubt that he did not carry the chinchilla allele from his father. Since he had slightly more male than female offspring, it is unlikely that he was Df/c^{6H}, as he would then have been expected to have half as many sons as daughters. Thus, Cattanach's translocation was able

to correct male sterility due to compound heterozygosity for lethal albino deletions in at least one case.

Of 28 offspring born to the X^T/X Df/$c^{3H} \times X/Y$ c^{ch}/c^{6H} cross, two were 'flecked'/albino (X^T/X Df/c^{6H} or c^{3H}/c^{6H}) females (3·5 expected). Although the fertility of these mice was decreased in comparison to 'flecked'/chinchilla sibs, in one case the female sterility associated with compound heterozygosity for lethal albino deletions was corrected, (Table 2). This female was more likely to be X^T/X c^{3H}/c^{6H} than X^T/X Df/c^{6H} since only 8% of c^{6H}/c^{6H} were 'rescued' by X^T (Gluecksohn-Waelsch *et al.* 1980).

4. DISCUSSION

The pairing of heteropycnotic X and Y chromosomes to form the sex bivalent has been studied in various species. Although in the mouse the sex bivalent at pachytene is associated with a nucleolus (Geyer-Duszynsska, 1963; Ohno *et al.* 1959; Sachs, 1954), such an association with the bivalent is variable in man (Moses *et al.* 1975; Solari & Tres, 1970). In both mouse and human spermatocytes, nucleoli are organized by autosomal loci during leptotene-zygotene (Kierszenbaum & Tres, 1974; Tres, 1975). Nucleolar masses detach from autosomal organizers and then migrate during pachytene towards the condensed sex bivalent, where they segregate into granular and fibrillar masses (Kierszenbaum & Tres, 1974). RNA synthesis in the sex chromosomes is not detectable by autoradiography (Geyer-Duszynska, 1963) at a time when the autosomal bivalents are engaged in both ribosomal and non-ribosomal RNA synthesis. This absence of RNA synthesis is characteristic also of the inactive X in female somatic cells. In addition, the Y and most of the X chromosome are late replicating during the male meiotic prophase (Kofman-Alfaro & Chandley, 1970; Odartchenko & Pavillard, 1970) as is the inactive X of somatic female cells. The sex bivalent disjoins at diplotene-diakinesis and the X and Y chromosomes show pre-reductional separation during anaphase I. The small amount of post-meiotic RNA synthesis cannot be attributed to specific chromosomes (Monesi, 1965). However, both the X and Y may be condensed during spermiogenesis, and condensed chromatin masses in spermatids are not labelled with [^{3}H]uridine (Kierszenbaum & Tres, 1975). Thus we wondered if autosomal material inserted into the X would be active during spermatogenesis.

The series of radiation-induced deletions at the albino locus of mice, singly or in combination, provide a spectrum of phenotypic effects (Gluecksohn-Waelsch, 1979). Compound heterozygotes for c^{3H} and c^{6H} are viable but runted and sterile (Lewis *et al.* 1978). Testes of c^{3H}/c^{6H} males are characterized by morphological defects in germ cells but not somatic cells: nuclear condensation is abnormal and there is a deficiency in numbers of maturing spermatids (Lewis *et al.* 1978). Such defects occur after the time at which X-inactivation occurs. Most of the spermatozoa which are produced by c^{3H}/c^{6H} males are immotile and grossly abnormal in morphology. On the other hand, oogenesis and mating are normal in c^{3H}/c^{6H} females but their foetuses, even though genotypically normal (by outcrosses to $+/+$ males), fail to survive (Lewis *et al.* 1978). It seemed probable that Cattanach's X:7 translocation would correct c^{3H}/c^{6H} female sterility since it had

previously rescued females homozygous for potentially lethal genes when they were made heterozygous for the translocation-carrying *X*-chromosome (Gluecksohn-Waelsch *et al.* 1980). The rescue was more efficient with perinatal lethal than with early embryonic development lethal deletions (Gluecksohn-Waelsch *et al.* 1980). However, the outcome was uncertain in the case of males because of the above-described *X*-inactivation during spermatogenesis. The sterility of c^{3H}/c^{6H} (or Df/c^{6H}) is apparently due to homozygosity for the deletion of a small stretch of chromosome 7 (see Gluecksohn-Waelsch, 1979 for map of these deletions). The presence of chromosomal 7 genes on an active *X*-chromosome would be expected to correct the sterility. Correction of sterility was found in at least one male and one female. This result cannot be explained by variability in fertility of c^{3H}/c^{6H} males. Lewis *et al.* (1978) found no offspring in more than 100 female-months (Lewis, S. E., personal communication), while we have found none in about an additional hundred female-months (unpublished observations).

There are two classes of possible explanations for the low fertilities of the progeny of the X^T/X $Df/c^{3H} \times X/Y$ c^{ch}/c^{6H} cross. The first class of explanation concerns the effects of trisomy versus disomy for the 21 cM piece of chromosome 7 inserted into the *X* in Cattanach's translocation. It has been known for some time that X^T males trisomic for the fragment of 7 (two normal 7s) are usually runts but fertile, while the X^T males with Df are normal in appearance but usually sterile (Eicher, 1967; the degree of the effect is dependent on strain background). Similarly, the unbalanced females have decreased fertility because they frequently have imperforate vaginas (Eicher, 1967). The decreased fertility of balanced males could be due to chromosomal imbalance (monosomy fragment 7) after *X*-inactivation. The effect of Cattanach's on male fertility should be due to incorrect gene dosage for products outside the c^{3H}/c^{6H} deleted region since we herein show fertility when there is only one copy of this region. The second class of explanations concerns the degree of correction that Cattanach's provides for c^{3H}/c^{6H} sterility. The correction may be of low degree, a question which our data are insufficient to answer.

A possible explanation for the rescue of c^{3H}/c^{6H} male sterility by Cattanach's translocation despite normal *X*-inactivation would involve a mechanism allowing the autosomal insertion into the *X* to be expressed despite being part of the sex bivalent. We have confirmed earlier data (Tres, L. L. & Erickson, R. P., unpublished observations) that no autosome arm was seen protruding from the condensed chromatin mass of the sex bivalents (Eicher & Kirkland, 1969).

Other alternative explanations for the rescue of c^{3H}/c^{6H} male sterility by Cattanach's translocation despite *X*-inactivation include: (1) sterility is due to lack of a gene product(s) which is transcribed and/or translated early in meiosis, i.e. before the *X* is inactivated; (2) the defect causing sterility in c^{3H}/c^{6H} males is intrinsic to the somatic cells of the testes (where *X*-inactivation does not occur); or (3) the *X* is re-activated after the meiotic divisions and *X*-chromosomal genes are transcribed post-meiotically, as has been found with several autosomal messages (Fujimoto *et al.* 1983). With regard to the first of these possibilities, we have used two-dimensional gel electrophoresis of c^{3H}/c^{6H} and control testes extracts in order to determine if protein spot 5, which is not expressed in c^{3H}/c^{6H}

livers (Baier *et al.* 1983) was expressed in testes. Since it is not expressed (unpublished data), this gene product cannot be studied in this regard. With regard to the second of these possibilities, the morphological appearance of the supporting cells is normal (Lewis *et al.* 1978) and somatic cells, especially Sertoli cells, have little genetic influence on the germ cells (see Erickson *et al.* 1981 for review). None the less, this is a reasonable alternative explanation. With regard to the third of these possibilities, the *X*-chromosome of spermatozoa (paternal) and the paternal *X* in extra-embryonic membranes (where preferential paternal *X*-inactivation occurs; Takagi & Sasaki, 1975; West *et al.* 1977) is not inactivated to the same extent as is the inactive *X* in female somatic cells, as measured by the ability of *X*-linked DNA to transfer cells missing an X-linked function (Kratzer *et al.* 1983). Thus, partial expression immediately before, after, or during the cytologically apparent spermatogenic *X*-inactivation may be possible.

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