

Mechanism of infertility in *t*-complex mice

BY J. R. ARCHER

Searle Research Laboratories, High Wycombe, Bucks

S. J. SELF AND B. G. WINCHESTER

Department of Biochemistry, Queen Elizabeth College, London

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SUMMARY

Sperm from mice heterozygous (*t*/+) for many *t*-lethal haplotypes show segregation distortion. More offspring inherit the *t*-haplotype than the normal wild haplotype. In contrast males heterozygous (*t^x/t^y*) for two *t*-lethal haplotypes are sterile although they produce normal-looking sperm. The experiments were undertaken to show whether sperm from *t^x/t^y* mice are the same as *t*-fertile sperm from *t*/+. Female albino mice were persuaded to mate first with coloured sterile *t^x/t^y* heterozygotes and then with normal white +/+ males. With the exception of one litter (from a *t*-heterozygous male which proved to have been misidentified) all the progeny were derived from the mating with the normal father. We conclude that *t*-bearing sperm from *t*-lethal (*t^x/t^y*) heterozygotes are inviable, and different from *t*-sperm in *t*/+ heterozygotes.

1. INTRODUCTION

The *t*-complex in the mouse is a group of genes on chromosome 17 which is unusual for the diversity of the effects shown by its constituent parts and the persistence with which these genes are associated in the wild (reviewed by Gluecksohn-Waelsch & Erickson, 1970; Bennett, 1975). The lethal dominant gene Brachyury (*T*), near the centromere, causes shortening or kinking of the tail in heterozygotes with a + allele. Many wild mice, however, carry a complex of *t*-haplotypes which, in combination with *T* cause mice to be tailless. The recessive *t*-haplotypes are themselves lethal in the homozygous state but haplotypes from the different localities can sometimes complement each other to give apparently normal offspring, so that *t* genes are classified by complementation into 6 or 7 groups (Bennett, 1975). A *t*-complex normally includes a modifying gene near the centromere which interacts with *T* in heterozygotes: there is a second gene (*t^h*) some units away which, when homozygous, is lethal (or at least semi-lethal) to the embryo at a defined stage and a third gene (*t^{low}*) with effects in heterozygotes on the fertility of sperm carrying it relative to associated, non *t^{low}* sperm (Lyon & Mason, 1977). The complex substantially reduces crossing over in the region between *T* and the major histocompatibility complex (*H-2*), which normally maps at about 14 units from *T*. This permits breeding of mice as if *t*-complexes were single genes. Normally

they are bred as tailless heterozygotes with T . Matings between T/t mice give rise to heterozygous tailless offspring in which both homozygous types die *in utero*. On rare occasions tailed progeny result. These are almost invariably the results of crossovers leading to the loss of t^s . As a result the $H-2$ allele associated with t becomes the same as that associated with T .

Heterozygous male mice with genotype $t/+$ (where t represents a naturally occurring lethal t -complex) show the phenomenon of segregation distortion; Mendelian rules do not apply and more of their offspring receive the t than the $+$ genes. However, male heterozygotes with complementary t -complex genes (t^x/t^y) are sterile (Bennett & Dunn, 1971, Lyon & Mason, 1977). They produce rather low numbers of normal-looking sperm which are inactive (Bryson, 1944) and fail to pass the uterotubular junction after mating, or even to penetrate ova when assisted *in vitro* (Olds, 1970, 1971*a*). Electron microscopy suggests that though many sperm fail to develop completely, the remainder are normal and might be expected to be viable (Olds, 1971*b*; Self, Underdown & Archer, unpublished). Various explanations have been offered for the effects of t in the two sorts of heterozygote. We set out to decide whether t -bearing sperm in $t/+$ mice are the same as in t^x/t^y mice, and are effective only because of assistance from the $+$ sperm. The question can be resolved by fertilizing a mouse with a mixture of t^x/t^y and $++$ sperm and examining the progeny to see if they include any offspring from the t parent. This paper describes an approach by the technique of double mating (Sharma & Hays, 1975). We found that with one exception all of the litters we obtained were progeny of the normal father. In the exceptional case the father proved, by $H-2$ typing, to have a different genotype from the one we had expected so that the litter was not relevant to the experiment.

2. MATERIALS AND METHODS

(i) Mice

Mice used were bred in the SPF unit of G. D. Searle & Co., High Wycombe and are listed, with their relevant genes, in Table 1. Strains Tt^6 and Tt^{w5} were the generous gifts respectively of Dr M. F. Lyon, Harwell, and Dr K. Artzt, Cornell University Medical School. Mice of genotype t^6/t^{w5} were obtained by mating female Tt^6 with male Tt^{w5} and selecting for tailed offspring.

(ii) Antisera

Antisera were made by immunizing appropriate mice with spleen cells (Table 2) and were tested by the lymphocyte ^{51}Cr release method of Wigzell (1965) and Sanderson (1965).

(iii) Mating

Mice were kept in a cycle of 12 h light and 12 h dark. Some of our least successful attempts took place in October, when the British put their clocks back by one hour. Male mice were boxed separately for at least one week before mating. In the first of

the two experiments reported here the first-mated male was placed in a teaser box for three days before mating, separated from its chosen mate by a metal grid. Ovulation in female mice was timed by injection of gonadotropins (Fowler & Edwards, 1957). They received pregnant mare serum (Sigma, London) 2IU/mouse at 8 p.m. two days before mating, followed by 21U human chorionic gonadotropin (Sigma, London) at 12 noon on the day of the experiment. Mice were put together

Table 1. Genotypes of mice used

	Chromosome 17				Chromosome 7	
	<i>T</i>	<i>tf</i>	<i>t</i>	<i>H-2</i>	<i>C</i>	<i>P</i>
<i>A/J</i>	+	+	+	<i>a</i>	<i>c</i>	+
	+	+	+	<i>a</i>	<i>c</i>	+
<i>BALB/c</i>	+	+	+	<i>d</i>	<i>c</i>	+
	+	+	+	<i>d</i>	<i>c</i>	+
129	+	+	+	<i>bc</i>	<i>c^{ch}</i>	<i>p</i>
	+	+	+	<i>bc</i>	<i>c^{ch}</i>	<i>p</i>
<i>Tt⁶</i>	<i>T</i>	<i>tf</i>	+	<i>T(t⁶)</i>	+ or <i>c^{ch}</i>	+
	+	+	<i>t⁶</i>	(<i>tw1</i>)	+ or <i>c^{ch}</i>	+
<i>Tt^{w5}</i>	<i>T</i>	<i>tf</i>	+	<i>T(t^{w5})</i>	+	+
	+	+	<i>t^{w5}</i>	<i>tw5</i>	+	+
<i>t⁶t^{w5}</i>	+	+	<i>t⁶</i>	<i>tw1</i>	+ or <i>c^{ch}</i>	+
	+	+	<i>t^{w5}</i>	<i>tw5</i>	+	+

Data from Staats, 1976, Hammerberg *et al.* 1976 and our own observations.

Table 2. *H-2* haplotype specificity

Antiserum	H-2 antisera		H-2 haplotype specificity
	Donor	Recipient	
A215	<i>t⁶t^{w5}</i>	<i>Tt⁶</i>	<i>tw5*</i>
A216	<i>Tt⁶</i>	<i>t⁶t^{w5}</i>	<i>T</i> (from <i>Tt⁶</i>)†
A243	<i>Tt⁶</i>	<i>t⁶t^{w5}</i>	<i>T</i> (from <i>Tt⁶</i>)†
A217	<i>Tt^{w5}</i>	<i>t⁶t^{w5}</i>	<i>T</i> (from <i>Tt^{w5}</i>)†
A254	<i>Tt^{w5}</i>	<i>t⁶t^{w5}</i>	<i>T</i> (from <i>Tt^{w5}</i>)†
A218	<i>t⁶t^{w5}</i>	<i>Tt^{w5}</i>	<i>tw1</i>

* In our hands this is serologically indistinguishable from *H-2^k*.

† These haplotypes cross react strongly and may be identical; they are presumably associated with the original *tf* stock and appear identical with a haplotype on *ct* mice currently held by Dr M. Adinolfi, Guys Hospital.

for mating at 9.30–12.00 midnight. The male usually began to attempt to mate immediately. If he was showing little interest after 30 min it was often useful to replace the female with a spare. Successful ejaculation was in most cases obtained in 20–30 min, but a few mice took longer, sometimes as long as 2 h. As soon as mating was complete and the male appeared quiescent the plug, still soft, was scooped from the female, an auroscope being used to check we had succeeded in removing it completely, and the female was placed with a second male. Twelve

hours later all females were checked for second plugs and the males were removed. Double mated females were checked for litters daily after 20 days for 7 days, the eye colour of offspring was noted and as many as possible were kept for confirmation of genotype when hair developed.

All matings involved female A/J mice. In the rest of this paper double matings are described with the males named in the order in which they were mated (e.g. BALB/c-129).

(iv) *Paternity determination*

A/J mice are homozygous for the albino (*c*) gene. Progeny with $t^{6t^{w5}}$ (+/+ occasionally +/ c^{ch}) and with 129 ($p-c^{ch}/p-c^{ch}$) are black-eyed. Hence we could identify the fathers of our neonatal mice. Our strain of 129 is homozygous: many others carry *c* or are kept as c/c^{ch} heterozygotes (Staats, 1976).

Table 3. *Phenotypes of progeny from double matings with A/J females*

Mating	No. of 1st matings	No. of 2nd matings	No. of litters produced	Eye-colour	
				Black	Pink
A. BALB/c-129	5	NR	4	0	9
				0	8
				2	2
				2	4
129-BALB/c	0	NR	—	—	—
BALB/c- $t^{6t^{w5}}$	3	NR	1	0	9
$t^{6t^{w5}}$ -BALB/c	5	NR	1	9	0
B. $t^{6t^{w5}}$ -BALB/c	20	10	4	0	5
				0	13 (5 dead)
				0	8 (1 dead)
				0	7 (1 dead)

3. RESULTS

Results of two experiments are summarized in Table 3. In the first (A) four groups of 5 females were used for mating: $t^{6t^{w5}}$ -BALB/c, BALB/c- $t^{6t^{w5}}$, BALB/c-129 and 129-BALB/c. The last group failed because no 129 mated between 9 p.m. and 2 a.m., when we left (although at least one mated successfully later that night). BALB/c-129 gave 4 litters. Two, including 17 mice, were all white, suggesting that either the 129 did not mate or we failed to remove the plug properly. The other litters were clearly mixed. They must, therefore, have had two fathers and are evidence that the technique works. The BALB/c- $t^{6t^{w5}}$ group gave one litter from a BALB/c mate, and it is not possible to say whether a potentially successful second mating had taken place. The ' $t^{6t^{w5}}$ '-BALB/c litter consisted entirely of black-eyed mice, suggesting that we had made the $t^{6t^{w5}}$ fertile. However, this mouse subsequently fathered two litters with A/J without assistance. None of its 13 offspring were reactive with an anti $H-2^{w1}$ antiserum and the mouse itself was subsequently shown to carry $H-2$ antigens associated with T and t^{w5} . We concluded that it was an exception, the result of a cross-over in the Tt^6 parent leading to a new, viable, fertile t -allele.

In experiment B we used t^6/t^{w5} males which had already failed to produce offspring when kept with female A/J or BALB/c mice for 6 weeks, and which we were therefore able to regard as sterile. The BALB/c father was always used second so that we had an internal control to ensure that removal of the first plug was effective. Four litters were obtained with a total of 35 mice, every one of which at birth was pink eyed (i.e. albino). We concluded that the t^6/t^{w5} sperm were inviable, and differed from those in $t/+$ mice.

4. DISCUSSION

If t^6/t^{w5} sperm merely required assistance from normal sperm we would have expected some offspring from t^6/t^{w5} fathers and in fact they might reasonably have been expected to be in the majority, by analogy with $t/+$ litters. The experiments therefore show that infertility in t^x/t^y heterozygotes results from an event which takes place before mating.

It should be noted that the deceptive result obtained in our first group of matings was not particularly improbable. Cross-overs resulting in exceptions occur in about 1/500 individuals (Lyon & Phillips, 1959). However the phenotype of such an exception is expected to be the same as that for the genotype for which we were selecting, which was obtained in about 1/4 individuals. The probability of a selected tailed animal carrying an exception gene from one or other of its parents is therefore nearer 4/500 and the probability of finding 1 amongst 21 individuals is of the order of 20%. The result illustrates both the danger of assuming that tailed mice are of the t^6/t^{w5} genotype, and one advantage of the double mating technique which leaves the male in a fit state for confirmatory experiments.

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REFERENCES

- BENNETT, D. & DUNN, L. C. (1971). Transmission ratio distorting genes on chromosome IX and their interactions. *Proc. Symp. Immunogenetics of the H-2 system*, pp. 90-103. Karger-Basel.
- BENNETT, D. (1975). The T-locus of the mouse. *Cell* 6, 441-454.
- BRYSON, V. (1944). Spermatogenesis and fertility in *Mus musculus* as affected by factors at the T-locus. *Journal of Morphology* 74, 131-179.
- FOWLER, R. E. & EDWARDS, R. G. (1957). Induction of superovulation and pregnancy in mature mice by gonadotropins. *Journal of Endocrinology* 15, 374-384.
- GLUECKSOHN-WAELSCH, S. & ERICKSON, R. P. (1970). The T-locus of the mouse: implications for mechanisms of development. *Current Topics in Developmental Biology* 5, 281-316.
- HAMMERBERG, C., KLEIN, J., ARTZT, K. & BENNETT, D. (1976). Histocompatibility-2 system in wild mice. II. H-2 haplotypes of *t*-bearing mice. *Transplantation* 21, 199-212.
- LYON, M. F. & MASON, I. (1977). Information on the nature of *t*-haplotypes from the interaction of mutant haplotypes in male fertility and segregation ratio. *Genetical Research* 29, 255-266.
- LYON, M. F. & PHILLIPS, R. J. (1959). Crossing-over in mice heterozygous for *t*-alleles. *Heredity* 13, 23-32.

- OLDS, P. J. (1970). Effect of the *T*-locus on sperm distribution in the house mouse. *Biology of Reproduction* **2**, 91–97.
- OLDS, P. J. (1971*a*). Effect of the *T*-locus on fertilization in the house mouse. *Journal of Experimental Zoology* **177**, 417–434.
- OLDS, P. J. (1971*b*). Effect of the *T*-locus on sperm ultrastructure in the house mouse. *Journal of Anatomy* **109**, 31–37.
- SANDERSON, A. R. (1965). Quantitative titration, kinetic behaviour and inhibition of cytotoxic mouse isoantisera. *Immunology* **9**, 287–300.
- SHARMA, O. P. & HAYS, R. L. (1975). Heterospermic insemination and its effect on the offspring ratio in rats. *Journal of Reproduction and Fertility* **45**, 533–535.
- STAATS, J. (1976). Standardized nomenclature for inbred strains of mice: sixth listing. *Cancer Research* **36**, 4333–4377.
- WIGZELL, H. (1965). Quantitative titrations of mouse *H*-2 antibodies using Cr⁵¹ labelled target cells. *Transplantation* **3**, 423–431.