Genet. Res. Camb. (1996), **67**, *pp.* 249–256 *With 2 text-figures*

An additional type of male sterility and inherited urinary obstruction in mice with the *t*-haplotype t^{h7}

MARY F. LYON
Mammalian Genetics Unit, Medical Research Council, Harwell, Didcot, Oxon OX11 0RD, UK

(*Received 5 December 1995 and in revised form 15 January 1996*)

Summary

The *t*-complex on mouse chromosome 17 results in transmission ratio distortion in males heterozygous for complete haplotypes, and sterility in those homozygous for semi-lethal or doubly heterozygous for complementing lethal haplotypes. This sterility is due to inability of spermatozoa to fertilize. The haplotype t^{h7} is an unusual laboratory-derived haplotype, postulated to carry a small duplication of *t* chromatin. Males heterozygous for t^{h7} show a new form of sterility, apparently due to failure to form copulation plugs during mating. This is accompanied by a strong propensity to acute urinary obstruction. It is suggested that both the failure to form copulation plugs and the urinary obstruction are due to some abnormality in function of the accessory sex glands, and are the result of incorrect dosage of a gene in the postulated duplication. The symbol *Msu* for male sterility and urinary obstruction is suggested for the locus concerned. Previously a recessive form of abnormal behaviour had also been attributed to this duplication.

1. Introduction

The *t*-complex is a naturally occurring variant form of mouse proximal chromosome 17 distinguished from the normal form by four non-overlapping inversions (Silver, 1985, 1993, 1996; Hammer *et al.* 1989). These inversions result in strong suppression of crossing over in the region they occupy, but rare crossovers do occur, resulting in partial *t*-haplotypes in which a portion of the *t*-complex has been replaced by wild-type chromatin. Among the unusual set of effects produced by haplotypes of the *t*-complex is that of sterility in males homozygous for semi-lethal haplotypes or doubly heterozygous for two complementing lethal haplotypes (Lyon, 1991). Mature sperm are produced, but these show abnormal motility and are unable to penetrate oocytes (Olds-Clarke & Johnson, 1993; Johnson *et al.* 1995).

The underlying genetic basis for the sterility is thought to be complex. It has been elucidated by study of partial *t*-haplotypes, most of which are characterized by loss of some properties of the *t*-complex and retention of others. The interpretation of the data is that sterility results from homozygosity for genes which when heterozygous contribute to the transmission ratio distortion in favour of the *t*-complex that is seen in heterozygous males (Lyon, 1984, 1986). There are thought to be three or more distorter genes which act in trans additively to produce a harmful effect on a cis-acting responder gene. The *t*-allele of the responder is postulated to be relatively resistant to the action of the distorter genes, with the result that in heterozygous males the functional sperm mainly carry the resistant *t* form of the responder, thus producing the ratio distortion. When the distorters are homozygous, however, the harmful effect is more severe, the *t*-allele of the responder is affected and the males are sterile (Lyon, 1986, 1991). A strong candidate gene for the responder function has been intensively studied (Bullard & Schimenti, 1990, 1991; Cebra-Thomas *et al.* 1991; Snyder & Silver, 1991), but although some candidate genes for distorters have been suggested (Rappold *et al.* 1987; Mazarakis *et al.* 1991), none has been firmly identified.

Although most partial *t*-haplotypes show loss of some properties of the *t*-complex, a few exhibit altered or new characteristics. In particular, in a few cases the modifying effect of *t* on the expression of the brachyury gene *T* is reversed. Typical *t*-haplotypes enhance the effect of *T*, so that T/t heterozygotes are tailless, rather than short-tailed as in $T/+$. By contrast, the haplotype t^{h7} suppresses the effect of *T*, so that T/t^{h7} heterozygotes are normal-tailed. In rare mutant or crossover haplotypes derived from t^{h7} the original enhancing effect on *T* is regained. This has led to the suggestion that t^{h7} involves a small duplication including the *T*-modifying locus, with the mutants being due to loss of this duplication (Lyon & Meredith,

1964; Lyon & Bechtol, 1977). However, molecular evidence of duplication has not yet been obtained.

We report here a new phenotypic effect of t^{h7} in producing male sterility in $+/t^{h7}$ or T/t^{h7} heterozygotes. Furthermore, such heterozygous males appear prone to fatal urinary obstruction. The t^{h7} haplotype arose from t^6, a partial haplotype lacking the most proximal region of the *t*-complex, including the proximal distorter gene *Tcd1* (Herrmann *et al.* 1987; Howard *et al.* 1990). Thus, t^{h7} also is thought to lack *Tcd1*, but it carries other *t*-complex genetic factors, including the t^6 recessive lethal factor. Thus t^{h7} homozygotes die *in utero*. Investigation of male sterility in t^{h7} heterozygotes began when a number of males, mated to normal females to maintain the stock, failed to breed.

2. Materials and methods

(i) *Animals*

The animal studies described in this paper were carried out under the guidance issued by the Medical Research Council in 'Responsibility in the Use of Animals for Medical Research' (July 1993) and Home Office Project Licence no. 30/875.

All animals were maintained under conventional conditions in the animal house of the MRC Mammalian Genetics Unit, Harwell. For maintenance of the t^{h7} stock, females heterozygous for $t^{h7}(T/t^{h7}$ or $+/t^{h7})$ were crossed to $Ttf/+tf$ males of the inbred strain TFH/H, so that the strain became partially congenic. The mutant haplotype t^{h7m2}, which arose from t^{h7}, was similarly maintained by crosses to TFH/H. Other haplotypes used included t^{h7m}, which also arose from t^{h7}. Homozygotes for this haplotype are viable and t^{h7m} was maintained by crosses of $T^{21H}+/t^{h7m}tf\times t^{h7m}tf/t^{h7m}tf$, where T^{21H} is an allele of brachyury found after treatment of a normal male mouse with 250 mg/kg ethylnitrosourea. The haplotypes t^{h2} and t^{h49} are also viable when homozygous and were maintained in closed stocks, not congenic with TFH/H. For t^{h2}, crosses of $Tt^{h18}+/t^{h2}tf\times t^{h2}tf/t^{h2}tf$ were used, where t^{h18} is a distal partial haplotype derived from t^6, and for t^{h49} crosses were $Ttf/t^{h49}tf\times t^{h49}tf/t^{h49}tf$. The origin of t^{h2} was from t^6, and it is a proximal partial, thought to carry the *t* responder gene Tcr^t but no distorter (Lyon, 1984). t^{h49} was found among the progeny of a $Tt^{lowH}tf/t^{w5}+$animal, where t^{lowH} is a central partial haplotype carrying the responder only, and t^{h49} is thought to carry the proximal distorter *Tcd1* as well as the responder Tcr^t (Lyon, 1984).

(ii) *Testis weights and sperm counts*

Males were killed at the age of 2–3 months and their body and testis weights were determined. Epididymal sperm counts were obtained using a haemocytometer, using the method of Searle & Beechey (1974). Each caput epididymis was macerated in 0·2 ml of a 1% solution of trisodium citrate. The solution was made up to 2 ml, mixed well and allowed to settle for about 1 min. A drop of suspension was run into each chamber of a Neubauer haemocytometer, after the coverslip had been pressed down to show Newton's rings. Numbers of sperm heads were counted in the four large corner squares and the large central square, and the number shown in Table 2 is the count obtained.

(iii) *Fertility tests*

To replicate the conditions in the breeding stock of t^{h7} where the impaired fertility was first noticed, initial fertility tests were carried out using females of the TFH/H strain. Heterozygous $Ttf/t^{h7}+$ or $+tf/t^{h7}+$ males and $Ttf/+tf$ or $+tf/+tf$ litter-sibs were placed with 2 females, either $Ttf/+tf$ or $tf/+tf$, at the age of 2–3 months, and left for 1 month. If neither female had littered or showed signs of pregnancy after that time the male was judged to be sterile. For tests of formation of copulation plugs the females used were F_1 hybrids of genotype C3H/HeH × 101/H, abbreviated to 3H1, as these highly fertile females provided a more sensitive test of the fertility of the male.

(iv) *Statistical tests*

For comparisons of body and testis weights and sperm counts of t^{h7} and control males results were analysed by tests of significance of variance ratio.

3. Results

(i) *Evidence concerning impaired fertility of t^{h7} males*

In the course of maintaining the breeding stock of t^{h7}, 16 males were mated to 2 TFH/H females each and 1 to a single female (Table 1, line 1). Of these only 5 proved fertile and 1 died. The fertile males sired only one or two litters each. To investigate this impaired fertility further, 4 $Ttf/t^{h7}+$ or $+tf/t^{h7}+$ males and 4 control $Ttf/+tf$ or $tf/+tf$ litter mates were tested for fertility by mating to 2 TFH/H females each (Table 1B). All 4 control males proved fertile, but all males carrying t^{h7} were sterile. This was taken as an indication that the impaired fertility was a property of t^{h7}, rather than the general genetic background of the strain.

This effect of t^{h7} on male fertility was different from the typical effects of the *t*-complex, in that it occurred in single heterozygotes for t^{h7}, rather than in double heterozygotes, putatively homozygous for distorter genes. In earlier work it had been shown that under certain circumstances impaired fertility could occur in males heterozygous (rather than homozygous) for distorter genes (Lyon, 1987) and that this effect could be alleviated by introduction of a *t* responder gene Tcr^t on the homologous chromosome. A question to

Table 1. *Fertility tests of males of various genotypes*

Genotype of male	Number of males	Number of females	Number of fertile males	Litters	Young born	Young/female/month
A. General breeding tests of males						
1. $t^{h7}+/+tf$ or $t^{h7}+/Ttf$	16*	31	5	8	29	0·9
2. $t^{h7}+/t^{h2}tf$	10†	19	1	2	10	1·7
3. $t^{h7}+/t^{h49}tf$	5‡	10	0	—	—	—
4. $t^{h7m}tf/t^{h7m}tf$	12	24	10	122	616	5·8
5. $t^{h7m}tf/Ttf$	13	22	12	116	696	4·7
6. $t^{h7m2}+/+tf$ or $t^{h7m2}+/Ttf$	20	29	17	108	477	3·2
B. Tests of t^{h7} males and control sibs						
7. $t^{h7}+/+tf$ or $t^{h7}+/Ttf$	4	8	0	—	—	—
8. $Ttf/+tf$ or $+tf/+tf$	4	7	4	8	47	4·3

* One with urinary obstruction, 2 died of unknown cause.
† One with urinary obstruction, 3 died of unknown cause and 1 additional male died before completing the fertility test.
‡ Two with urinary obstruction.

Table 2. *Body and testis weights and sperm counts of t^{h7} males and control litter-sibs*

	$t^{h7}/+$ or t^{h7}/T males			$+/+$ or $T/+$ males		
Litter number	Body wt (g)	Testis wt (mg)	Sperm count	Body wt (g)	Testis wt (mg)	Sperm count
356·1	26·2	60·8; 61·4	93; 85	26·7	78·9; 68·8	89; 100
339·4	26·2	58·8; 60·1	62; 53	31·0	71·3; 74·4	135; 104
				30·7	68·8; 70·6	85; 76
357·2	26·1	64·9; 66·1	81; 97	29·8	68·7; 67·9	73; 65
	20·7	56·3; 55·4	60; 71			
375·1	25·8	62·8; 63·2	28; 33	27·9	61·7; 62·0	54; 63
	24·1	59·7; 57·9	127; 122	27·3	70·5; 68·8	96; 82
	22·3	59·6; 56·6	86; 62	28·4	66·2; 69·7	94; 105

be considered was whether the sterility in t^{h7} was due to an unusual set of distorter genes, caused by the presumed duplication in t^{h7}. If this were so, then the introduction of Tcr^t on the homologous chromosome might lessen the adverse effect on fertility. A cross was therefore made of t^{h7} to t^{h2}, which carries Tcr^t but no known distorters. As a control, t^{h7} was also crossed to t^{h49}, which carries $Tcd1^t$ and Tcr^t. Because of the presence of $Tcd1^t$, t^{h49} would not be expected to alleviate the sterility. Males of genotype $t^{h7}+/t^{h2}tf$ and $t^{h7}+/t^{h49}tf$ were tested for fertility by mating to 2 TFH/H females each. Only 1 of 10 $t^{h7}+/t^{h2}tf$ males was fertile, and he sired only two litters during 3 months of mating. Similarly, none of 5 $t^{h7}+/t^{h49}tf$ males was fertile (Table 1, lines 2 and 3). These tests were contemporaneous with those of the 4 t^{h7} and control males described above. Thus, there was no evidence that the introduction of a t responder, resistant to the effects of the of the distorters, reduced the adverse effects of t^{h7} on fertility, and thus no evidence that distorter genes were involved.

To investigate the underlying basis of the sterility further, body and testis weights and sperm counts were obtained from 7 t^{h7} and 7 control litter-sibs from four litters (Table 2). The results showed that t^{h7} males were significantly smaller than their sibs ($P = 0{\cdot}0037$), and their testis weights were lower ($P = 0{\cdot}0019$). There was also significant variation among sibs of the same genotype in testis weight ($P = 0{\cdot}0022$). By contrast, sperm counts did not differ between t^{h7} and normal sibs ($P = 0{\cdot}52$). There was, however, marked variation among sibs of the same genotype, which remained after log transformation. In view of the lower body weight of the t^{h7} males, a somewhat lower testis weight was to be expected, and the difference in testis weight observed between t^{h7} and control sibs was thought to be roughly in line with expectation. Since sperm counts varied so markedly between individuals within a litter, it is not clear whether these values are biologically meaningful. None of the males had been mated before sperm counts were obtained. However, clearly t^{h7} males do have sperm and there is no evidence that their sterility is due to oligospermia.

Studies were next made of the mating behaviour of t^{h7} males. Individual t^{h7} males and normal litter-sibs were placed with 2 TFH/H or 3H1 females each, and the females examined for copulation plugs each morning for 7 days. The males were of two groups: firstly, 3 of the 4 pairs of t^{h7} and control males previously tested for fertility as shown in Table 1*B*

https://doi.org/10.1017/S0016672300033735 Published online by Cambridge University Press

Table 3. *Tests of formation of copulation plugs by $t^{h7}/+$ males and control sibs*

Genotype of male	Number of males	Number of females	Plugs	Pregnancies
A. Males previously tested for fertility				
$t^{h7}/+$ or t^{h7}/T	3*	10	—	—
$T/+$ or $+/+$	3	12	11	7
B. Males not previously tested for fertility				
$t^{h7}/+$ or t^{h7}/T	12†	28	5	3(+1)‡
$T/+$ or $+/+$	10	24	15	15(+1)‡

* Two with urinary obstruction.
† Two with urinary obstruction, 1 died of unknown cause.
‡ In this female no plug was seen.

Table 4. *Behaviour in response to oestrous females of t^{h7} males and control sibs previously tested for plug formation*

	$t^{h7}/+$ or t^{h7}/T	$+/+$ or $T/+$
Number of males	14	13
No. that previously formed plugs	5	11
Activity		
Sniffing	14	12
Mounting	10	10
Thrusting	4	3
Plugs formed	0	4

(the fourth t^{h7} male died) and, secondly, a group of previously untested males. Twelve of 13 control males made copulation plugs; 21 of the 26 females had vaginal plugs and 18 of these became pregnant (Table 3). Of 15 t^{h7} males, placed with 38 females, only 5 made plugs, but 3 of the plugged females became pregnant, together with 1 other female in which a plug was not seen, although the male had caused a plug and pregnancy in the other female to which he was mated. It thus appeared that the sterility of the t^{h7} males was due to failure to produce copulation plugs.

Failure to produce plugs could be due either to failure to mate with oestrous females, or to some anatomical or physiological defect in the mechanism for production of a plug during mating. The mating behaviour of t^{h7} and control males placed with known oestrous females was then studied. Males which had been tested for plug formation were left singly in cages for some days, to establish a territory. The cycles of hybrid 3H1 females aged 8–12 weeks were synchronized by treatment with 5 iu pregnant mare's serum (PMS) at 1600–1700 hours, followed by 5 iu human chorionic gonadotrophin (hCG) 48 h later. At 0900–1000 hours the following morning, when they were expected to be in late oestrus, 2 females were introduced into each male's cage, and the behaviour of the animals was observed for 35–50 min. Four types of interaction between the males and females were noted (Table 4). The males began by sniffing at the genitalia of the females, who reacted by squeaking. This was followed by attempted mounting of the females, then by mounting and thrusting, and finally by plug formation. Of 14 t^{h7} and 13 control males observed, all except 1 control male showed at least some sniffing and squeaking (Table 4). Ten males of each type showed mounting behaviour, and 4 t^{h7} and 3 control males also exhibited thrusting. In the 3 control males this was accompanied by plug formation, and a fourth control male also produced a plug, although thrusting behaviour had not been noted. All 4 of these plugs resulted in pregnancy of the females. By contrast, none of the t^{h7} males produced plugs and no pregnancies occurred. Thus, no difference from normal was noted in the behaviour of t^{h7} males towards oestrous females. Rather it seemed that normal mating behaviour did not result in plug formation.

(ii) *Genetic basis of the sterility*

The t^{h7} haplotype arose from t^6 and the type of male sterility seen in t^{h7} is not found in t^6 nor in any other partial haplotypes derived from it. As previously mentioned, t^{h7} is postulated to involve a small duplication, and it could be this unusual change that has resulted in the novel effect on male fertility. However, no such effect was noted when t^{h7} was first discovered (Lyon & Meredith, 1964). Its presence later might be due to a change in genetic background, or it might be that a new mutation had occurred in the t^{h7} chromosome, distinct from the duplication. If the duplication event itself were responsible for the male sterility, then sterility should not be present in mutant haplotypes derived from t^{h7}, putatively by loss of the duplication. Two such mutant haplotypes were available, and their male fertility was studied. The two mutants were of the contrasting types previously obtained from t^{h7}. Both had lost the brachyury-suppressing effect of t^{h7} and regained the enhancing effect, so that heterozygotes with T were tailless. One, t^{h7m}, was found in a tufted offspring of a cross of $t^{h7}+/Ttf \times Ttf/+tf$. Homozygotes for t^{h7m} were viable, the transmission ratio from heterozygous males was normal, and crossing over between T and tf was not suppressed. Thus t^{h7m} was a typical proximal partial haplotype. Both heterozygous and homozygous males appeared normally fertile. Of 13 $T^{21H}/t^{h7m}tf$ males tested 12 were fertile, and of 12 $t^{h7m}tf/t^{h7m}tf$ males 10 were fertile (Table 1, lines 4 and 5).

The second mutant haplotype, t^{h7m2}, was found when a typical normal-tailed non-tufted female from a cross of $t^{h7}+/Ttf \times Ttf/tf$ produced several tailless non-tufted offspring when crossed to $Ttf/+tf$. The conclusion was that she was genetically $t^{h7m2}+/+tf$, where t^{h7m2} was derived from t^{h7} by a change in the tail-modifying factor. In genetic tests t^{h7m2} proved to be indistinguishable from t^6, from which t^{h7} itself

https://doi.org/10.1017/S0016672300033735 Published online by Cambridge University Press

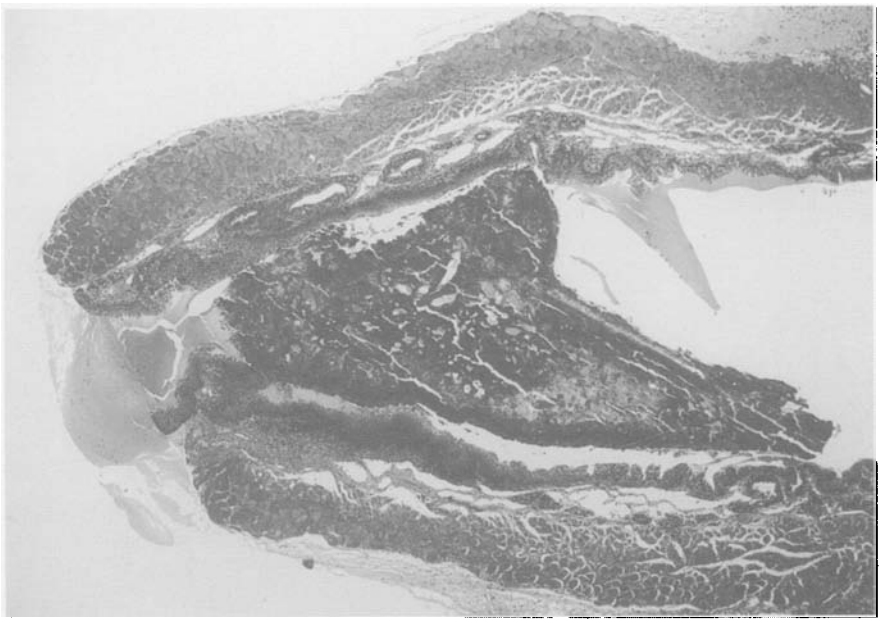

Fig. 1. Section through the neck of the bladder of a t^{h7} male found acutely ill with urinary obstruction. The lumen is filled with an amorphous material. Magnification ×21.

originally arose. It was lethal when homozygous, suppressed crossing over between *T* and *tf* and showed a moderately high transmission from heterozygous males. As this haplotype presumably retains most of the *t*-chromatin present in t^{h7} it seemed important to know whether it also retained the harmful effect on male fertility. t^{h7m2} was maintained on the same genetic background as t^{h7}, i.e. the stock was maintained by crosses to TFH/H or by mating together heterozygotes. In crosses of *Ttf*/+*tf* or +*tf*/+*tf* TFH/H females to t^{t7m2}+/+*tf* or *Ttf*/t^{h7m2}+males, 17 of 20 males tested were fertile and produced a mean of 3·2 young per female per month – a value within the normal range for a stock congenic with TFH/H (Table 1, line 6). In tests of copulation plug formation 7 t^{h7m2} heterozygotes and 3 *Ttf*/+*tf* control males all made plugs when left with 2 3HI females each for 1 week, and 6 of 13 t^{h7m2} plugs and all of 6 control plugs resulted in pregnancy. It is not clear why several plugs from t^{h7m2} males did not result in pregnancy, but clearly these males were able to form plugs normally. Thus, it appears that the genetic change by which t^{h7m2} arose from t^{h7} led not only to a change in the tail-modifying effect but also to loss of the harmful effect on male fertility.

(iii) *Urinary obstruction in* t^{h7} *males*

In the course of this work it became clear that males heterozygous for t^{h7} were susceptible to premature death. In the breeding tests and mating tests 19 t^{h7}/+, t^{h7}/t^{h2} or t^{h7}/t^{h49} males died or were found ill and killed. In all 9 cases where the cause of illness or death could be established the animals had a grossly distended bladder, apparently due to some urinary obstruction. In some cases the ureters and seminal vesicles also were distended. No control males died during the comparable test periods, nor did any males carrying the two mutant haplotypes derived from t^{h7}, t^{h7m} and t^{h7m2}.

Several t^{h7}/+ or t^{h7}/T males were observed daily until their illness or death. From this it became clear that the urinary obstruction occurred suddenly. Some animals were found dead, having appeared normal the previous day. Others were found acutely ill, having been normal the day before, and in all these cases the male was killed and found to have a grossly distended bladder. The bladders of two of these males were histologically sectioned. The neck of the bladder was found to be blocked by some amorphous material which stained pink with haematoxylin and eosin (Fig. 1). All the males under observation died when only a few months of age.

4. Discussion

Fertility in male mice heterozygous for t^{h7} was clearly severely impaired, apparently due to failure of the males to form copulation plugs. There was no evidence of any abnormality in sperm formation. Testis weights were normal and spermatozoa were present in normal numbers. Furthermore, in the few cases in which vaginal plugs were observed, the chance of pregnancy was quite high. The sexual behaviour of males confronted with oestrous females was apparently normal, and thus the absence of copulation plugs must have been due to some anatomical or physiological abnormality of plug formation.

There was no evidence that the effect was due to the already known distorter genes of the *t*-complex. On the one hand this type of sterility has not been previously reported as an effect of the *t*-complex. On the other hand fertility was not altered by introduction of the *t*-complex responder gene on the homologous

https://doi.org/10.1017/S0016672300033735 Published online by Cambridge University Press

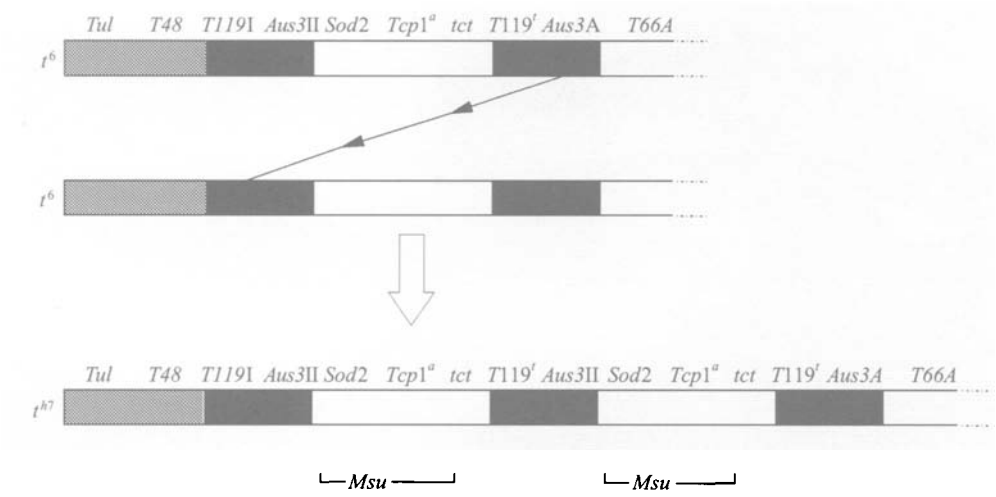

Fig. 2. Diagram of the structure of the proximal part of the haplotype t^6, and a possible means of origin of t^{h7} from it by misalignment of sister chromatids. Grey, wild-type chromatin; white, *t*-chromatin; black, region duplicated in t^6. Abbreviated symbols for some of the loci present are shown. The region within which the *Msu* locus is postulated to lie is indicated by a line.

chromosome, nor by the combination of a distorter and responder in males of the genotype t^{h7}/t^{h49}. Conversely, there is evidence that the genetic change responsible for the sterility accompanied that which led to the altered tail-modifying effect of t^{h7}. Two contrasting mutants derived from t^{h7}, in which the effect on the tail had reverted to that typical of most *t*-haplotypes, also lost the effect on male fertility. This does not imply that the tail-modifying factor itself is involved. The nature of the genetic change which gave rise to t^{h7} is unknown. It is postulated to involve a duplication of the tail factor, but it is not known how much material is duplicated. Moreover, in cases where the molecular basis of changes giving rise to new *t*-haplotypes is known there is sometimes deletion as well as duplication of material (Sarvetnick *et al.* 1986; Howard *et al.* 1990).

There are already a number of known loci involved in male fertility lying in the region of chromosome 17 occupied by the *t*-complex, in addition to the *t*-complex distorters. Pilder *et al.* (1991, 1993) found three loci involved in hybrid sterility (*Hst4* to *Hst6*) in addition to the locus *Hst1*, found earlier (Forejt & Ivanji, 1975; Forejt *et al.* 1991). None of these loci is likely to underlie the defect in t^{h7}. A recessive form of male sterility present in the mutant quaking, *qk* (Bennett *et al.* 1971), is again unlikely to be involved. The defect in t^{h7} appears to be a new form of male sterility, inherited in a dominant manner.

The sterility was not noticed when t^{h7} was originally found (Lyon & Meredith, 1964). The genetic background of the strain has since changed because the TFH/H strain, with which t^{h7} is now partially congenic, was not then available. Thus, the penetrance and expressivity of the defect may depend on the genetic background of the strain. However, both the sterility and the susceptibility to urinary obstruction persisted when t^{h7} was crossed with the haplotypes t^{h2} and t^{h49}, neither of which is maintained on a TFH/H background, and thus the effect is not limited to the TFH/H background alone. Even on the TFH/H background the defect is 'leaky' in that a few t^{h7} males made copulation plugs, which resulted in pregnancy, and sired young in breeding tests. However, the fertility of these males was transitory. This suggests that the penetrance of the defects may be complete, but with variation in expression.

The other abnormality found in t^{h7} heterozygous males was urinary obstruction, leading to death. The inheritance of this paralleled that of the sterility. It was present, as was the sterility, in t^{h7}/t^{h2} and t^{h7}/t^{h49} males, and not present in males carrying the mutant haplotypes derived from t^{h7}, t^{h7m} and t^{h7m2}. Bendele & Carlton (1986) and Wojcinski *et al.* (1992) recognized two types of urinary obstruction in mice: acute and chronic. In the chronic type the bladder was filled with crystalline material and there were external signs of skin ulceration and inflammation in the preputial area, apparently due to dribbling of urine. In the acute type the mice died without signs of illness, the bladder was distended with clear fluid, and the neck of the bladder and the urethra were blocked with amorphous proteinaceous material. Clearly the obstruction in t^{h7} mice was of the acute type. In animals observed daily the time between onset of illness and death was apparently less than 1 day. Moreover, the obstruction appeared due to amorphous material in the neck of the bladder. Silverstein *et al.* (1961) suggested that this amorphous material was formed from the seminal fluid mixed with the secretion of the male sexual accessory glands. Urinary obstruction due to such material did not occur in females or in castrated males. Similar suggestions for the origin of the amorphous material from the accessory glands were made by Bendele & Carlton (1986) and Wojcinski *et al.* (1992). In the case of t^{h7} the grounds for such a speculation are strengthened by the abnormality in formation of copulation plugs. It is reasonable to

https://doi.org/10.1017/S0016672300033735 Published online by Cambridge University Press

attribute both the urinary obstruction and the failure of plug formation to a single cause. It is not known whether this cause is some anatomical abnormality in the ducts of the various glands, or whether the defect is in the physiology or biochemistry of plug formation. On the present genetic background of the t^{h7} stock, the abnormality is inherited in a dominant manner. The expression seems to be variable in that some males showed transitory fertility. The penetrance appears to be high in that all males tested showed at least some impairment of fertility and the incidence of acute urinary obstruction also appeared to be high. Thus, there must be a locus situated near the *T* locus in the proximal region of chromosome 17 which is involved in the function or anatomy of the male accessory glands, and which is dosage sensitive. A suggested symbol for this locus is *Msu*, for male sterility and urinary obstruction.

Howard *et al.* (1990) studied the structure of t^6, from which t^{h7} arose, and showed that this haplotype carried two homologous short stretches including the *D17Leh119* and *D17Aus3* loci separated by a stretch of *t*-chromatin including the tail-modifying locus, *tct* (Fig. 2). If there were misalignment of sister chromatids at pairing of t^6 this could result in duplication of the short segment of *t*-chromatin including the tail-modifying factor, as is postulated to have occurred at the origin of t^{h7} (Fig. 2). Thus, there would be a number of genes duplicated in t^{h7} and *Msu* would be among them. Lyon & Bechtol (1977) described a recessive type of 'drunken' behaviour in animals putatively homozygous for the duplicated region. Thus, the dominant male sterility and urinary obstruction described here constitutes a second phenotypic effect of wrong dosage of this small region.

The author is grateful to K. Whitehill for animal care, K. Glover for Fig. 1, P. H. Glenister for Fig. 2, and D. Papworth for statistical tests.

References

Bendele, A. M. & Carlton, W. W. (1986). Incidence of obstructive uropathy in male B6C3F$_1$ mice on a 24-month carcinogenicity study and its apparent prevention by ochratoxin A. *Laboratory Animal Science* **36**, 282–285.

Bennett, W. I., Gall, A. M., Southard, J. L. & Sidman, R. L. (1971). Abnormal spermiogenesis in quaking, a myelin deficient mutant mouse. *Biology of Reproduction* **5**, 30–58.

Bullard, D. C. & Schimenti, J. C. (1990). Molecular cloning and genetic mapping of the *t-complex responder* candidate gene family. *Genetics* **124**, 957–966.

Bullard, D. C. & Schimenti, J. C. (1991). Molecular structure of *Tcp-10* genes from the *t* complex responder locus. *Mammalian Genome* **1**, 228–234.

Cebra-Thomas, J. A., Decker, C. L., Snyder, L. C., Pilder, S. H. & Silver, L. M. (1991). Allele- and haploid-specific product generated by alternative splicing from a mouse *t complex responder* locus candidate. *Nature* **349**, 239–241.

Forejt, J. & Ivanyi, P. (1975). Genetic studies on male sterility of hybrids between laboratory and wild mice (*Mus musculus* L.). *Genetical Research* **24**, 189–206.

Forejt, J., Vincek, V., Klein, J., Lehrach, H. & Loudova-Mickova, M. (1991). Genetic mapping of the *t*-complex region on mouse chromosome 17 including the *Hybrid sterility-1* gene. *Mammalian Genome* **1**, 84–91.

Hammer, M. F., Schimenti, J. & Silver, L. M. (1989). Evolution of mouse chromosome 17 and the origin of inversions associated with *t* haplotypes. *Proceedings of the National Academy of Sciences, USA* **86**, 3261–3265.

Herrmann, B. G., Barlow, D. P. & Lehrach, H. (1987). A large inverted duplication allows homologous recombination between chromosomes heterozygous for the proximal *t*-complex inversion. *Cell* **48**, 813–825.

Howard, C. A., Gummere, G. R., Lyon, M. F., Bennett, D. & Artzt, K. (1990). Genetic and molecular analysis of the proximal region of the mouse *t*-complex using new molecular probes and partial *t*-haplotypes. *Genetics* **126**, 1103–1114.

Johnson, L., Pilder, S., Bailey, J. & Olds-Clarke, P. (1995). Sperm from mice carrying one or two *t* haplotypes are deficient in investment and oocyte penetration. *Developmental Biology* **168**, 138–149.

Lyon, M. F. (1984). Transmission ratio distortion in mouse *t*-haplotypes is due to multiple distorter genes acting on a responder locus. *Cell* **37**, 621–628.

Lyon, M. F. (1986). Male sterility of the mouse *t*-complex is due to homozygosity of the distorter genes. *Cell* **44**, 357–363.

Lyon, M. F. (1987). Distorter genes of the mouse *t*-complex impair male fertility when heterozygous. *Genetical Research* **49**, 57–60.

Lyon, M. F. (1991). The genetic basis of transmission ratio distortion and male sterility due to the *t*-complex. *American Naturalist* **137**, 349–358.

Lyon, M. F. & Bechtol, K. B. (1977). Derivation of mutant *t*-haplotypes of the mouse by presumed duplication or deletion. *Genetical Research* **20**, 63–76.

Lyon, M. F. & Meredith, R. (1964). Investigations of the nature of *t*-alleles in the mouse. II. Genetic analysis of an unusual mutant allele and its derivatives. *Heredity* **19**, 313–325.

Mazarakis, N. D., Nelki, D., Lyon, M. F., Ruddy, S., Evans, E. P., Freemont, P. & Dudley, K. (1991). Isolation and characterisation of a testis-expressed developmentally regulated gene from the distal inversion of the mouse *t*-complex. *Development* **111**, 561–571.

Olds-Clarke, P. & Johnson, L. R. (1993). *t* haplotypes in the mouse compromise sperm flagellar function. *Developmental Biology* **155**, 14–25.

Pilder, S. H., Hammer, M. F. & Silver, L. M. (1991). A novel mouse chromosome 17 hybrid sterility locus: implications for the origin of *t* haplotypes. *Genetics* **129**, 237–246.

Pilder, S. H., Olds-Clarke, P., Phillips, D. M. & Silver, L. M. (1993). *Hybrid sterility-6*: a mouse *t* complex locus controlling sperm flagellar assembly and movement. *Developmental Biology* **159**, 631–642.

Rappold, G. A., Stubbs, L., Labeit, S., Crkvenjakov, R. B. & Lehrach, H. (1987). Identification of a testis-specific gene from the mouse *t*-complex next to a CpG-rich island. *EMBO Journal* **6**, 1975–1980.

Sarvetnick, N., Fox, H. S., Mann, E., Mains, P. E., Elliott, R. W. & Silver, L. M. (1986). Nonhomologous pairing in mice heterozygous for a *t* haplotype can produce recombinant chromosomes with duplications and deletions. *Genetics* **113**, 723–734.

Searle, A. G. & Beechey, C. V. (1974). Sperm-count, egg-fertilization and dominant lethality after X-irradiation of mice. *Mutation Research* **22**, 63–72.

Silver, L. M. (1985). Mouse *t* haplotypes. *Annual Review of Genetics* **19**, 179–208.

Silver, L. M. (1993). The peculiar journey of a selfish chromosome: mouse *t* haplotypes and meiotic drive. *Trends in Genetics* **9**, 250–254.

Silver, L. M. (1996). Maps of the mouse *t* complex. In *Genetic Variants and Strains of the Laboratory Mouse*, 3rd edn (ed. M. F. Lyon, S. Rastan & S. D. M. Brown), pp. 925–928. Oxford: Oxford University Press.

Silverstein, E., Sokoloff, L., Mickelsen, O. & Jay, G. E., Jr (1961). Primary polydipsia and hydronephrosis in an inbred strain of mice. *American Journal of Pathology* **38**, 143–159.

Snyder, L. C. & Silver, L. M. (1991). The mouse *t* complex responder locus. *Genome Analysis*, vol. 3, *Genes and Phenotypes*, pp. 37–57. Cold Spring Harbor: Cold Spring Harbor Press.

Wojcinski, Z. W., Renlund, R. C., Barsoum, N. J. & Smith, G. S. (1992). Struvite urolithiasis in a B6C3F1 mouse. *Laboratory Animals* **26**, 281–287.