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Search for differences among *t* haplotypes in distorter and responder genes

MARY F. LYON
MRC Radiobiology Unit, Chilton, Didcot, Oxon OX11 0RD, UK

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Summary

Transmission ratio distortion due to the mouse *t* complex is thought to be due to harmful effects of *trans*-acting distorter genes acting on a responder, with the *t* complex form of the responder being relatively resistant to this harmful action of the distorters. Previous work had indicated that naturally occurring *t* haplotypes differed in their responders or in distorters lying near the responder, with the result that animals doubly heterozygous for two responder-carrying haplotypes transmitted these haplotypes unequally. In the present work *t* haplotypes could be divided into three types on the basis of their transmission when doubly heterozygous with the responder-carrying partial haplotype t^{lowH}. The majority, t^0, t^6, t^{w1}, t^{w2} and t^{w73}, were transmitted equally with t^{lowH}, a second group, including t^{w5} and two haplotypes derived from it, were transmitted less frequently than t^{lowH}, and the single member of a third group, t^{w32}, was transmitted in excess of t^{lowH}. This last result suggests that the underlying differences are in the responder itself, rather than in the distorters. Search for differences among *t* haplotypes in distorters produced some equivocal results possibly resulting from effects of genetic background. In particular, results of others suggesting presence of a fourth distorter, *Tcd-4*, were not confirmed.

1. Introduction

The mouse *t*-complex involves a variant form of the proximal part of chromosome 17, which is maintained as a polymorphism in wild populations, through the abnormally high transmission of the complex from males heterozygous for a *t* and a non-*t* chromosome (Bennett, 1975; Frischauf, 1985; Lyon, 1989; Silver, 1985).

An outline scheme for the underlying genetic basis of this distorted transmission has been put forward (Lyon, 1984, 1986, 1989). The *t* complex is thought to include three or more distorter loci, symbolized *Tcd-1, Tcd-2,* and *Tcd-3*, which exert a harmful effect on sperm carrying the wild-type allele at a responder locus, symbolized *Tcr*. The allele of *Tcr* in a *t* complex, Tcr^t, is relatively resistant to the action of the distorters, with the result that, in a male heterozygous for the *t* complex, the majority of sperm that achieve fertilization carry Tcr^t, resulting in abnormally high transmission of the *t* complex. However, Tcr^t is not completely resistant to the action of the distorters, and when the distorters are homozygous, sperm carrying Tcr^t, as well as those carrying Tcr^+, are harmfully affected, so that the males are sterile. The different factors are located some distance apart over a 15 cM stretch of chromosome 17, and are locked together by crossover suppression due to two major and two minor chromosomal inversions (Artzt *et al.* 1982; Shin *et al.* 1983; Herrmann *et al.* 1986; Hammer *et al.* 1989).

Although the basic outline of the genetics is known, the details still remain to be elucidated. The individual genes have not been cloned [although some candidates have been found (Rappold *et al.* 1987; Schimenti *et al.* 1988)], their gene products are not known and the reproductive biology of the abnormality in sperm function is not understood.

As a step towards further understanding of the *t* complex it is important to study the genetics in greater detail. Although the scheme put forward can explain most of the features of the complex some points still remain unexplained. Both the distorted transmission and the effects on male fertility are susceptible to changes in the genetic background. These background effects are at least partly due to genes on chromosome 17 (Bennett *et al.* 1983; Gummere *et al.* 1986) and these genes remain to be identified. In addition, there is evidence that different *t* haplotypes may vary in the distorters or responders they carry. Animals carrying Tcr^t on both homologues of chromosome 17 would be expected to show equal transmission of the two homologues. However, Lyon & Zenthon (1987) showed that this was not always so. Animals doubly

heterozygous for the responder in the partial *t* haplotype t^{lowH} and the complete haplotype t^{w1} showed equal transmission of the two chromosome 17 homologues, whereas males with the haplotype t^{w5} opposite t^{lowH} gave an excess of offspring carrying t^{lowH}.

The present paper describes further investigation of transmission ratio in animals carrying Tcr^t on both chromosome 17 homologues, together with studies into possible differences among *t* haplotypes in distorters or sterility factors, and possible additional distorters. In particular, Silver & Remis (1987) postulated the existence of a fourth distorter gene *Tcd-4*, in the proximal part of the complex, on the basis of results with a partial *t* haplotype, t^{h45}, which carries a duplication of a small part of the proximal region (Herrmann *et al.* 1987). Furthermore, some results of Hammerberg (1982) on male sterility in mice with various combinations of two *t* haplotypes suggested that the haplotype t^0 might differ from others in its sterility factors. Such a difference if real might be valuable in cloning the various factors involved.

2. Materials and methods

All mice were bred in this laboratory. The haplotype t^{w73} was kindly given by Dr Dorothea Bennett and Dr Karen Artzt, and t^{Lub2} by Dr Heinz Winking.

The genetic structure of the various partial *t* haplotypes used is shown diagrammatically in Fig. 1 and Table 1. The complete haplotypes t^0, t^{w1}, t^{w2}, t^{w5},

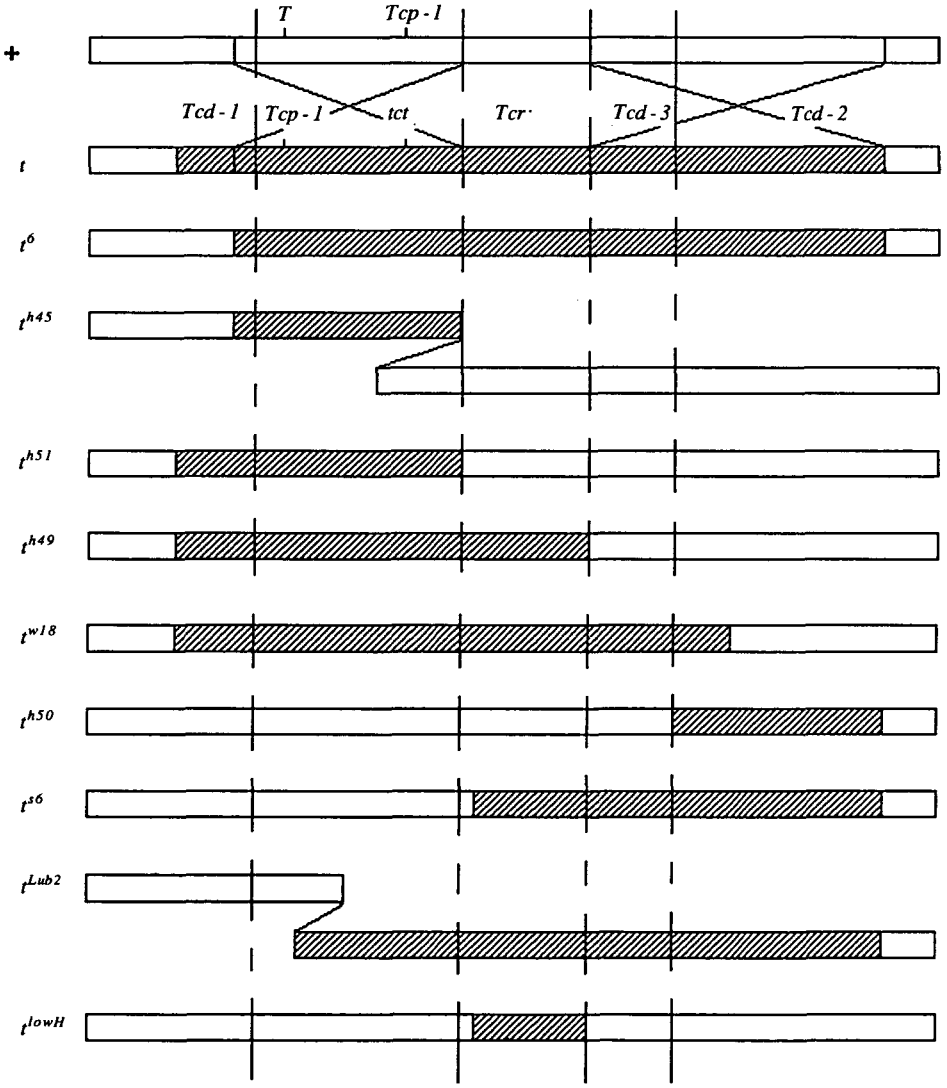

Fig. 1. Diagrammatic representation of the genetic structure of the various complete and partial *t* haplotypes used. Normal chromatin is shown white and *t* chromatin hatched. The two major inversions by which *t* complexes and normal chromatin differ are shown in the top two lines; the top line gives the wild type and the second line the *t*-complex orientation. The complete haplotypes t^0, t^{w1}, t^{w2}, t^{w5}, t^{w32} and t^{w73} all have a structure as in the top hatched line labelled *t*. The extent of *t* chromatin in the partial haplotypes is not drawn to scale, but merely indicates the various genetic factors carried. The duplications in t^{h45} and t^{Lub2} result in both *t* and wild alleles of *Tcp-1* in t^{h45}, and of *t* and + alleles of *T* (or *tct*) in t^{Lub2} (and also no copies of *Tcp-1*). *T* = brachyury (short tail); *Tcp-1* = *t*-complex protein 1; *tct* = *t*-complex tail modifier (thought to be allelic with *T*).

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t^{w32} and t^{w73} all have a structure as indicated by the first hatched line in Fig. 1.

For tests of transmission ratio, each male was placed with two females of the inbred strain TFH/H and allowed to breed. The TFH/H strain is genetically *Ttf*/+*tf*, *T* (brachyury) and *tf* (tufted) both being markers of chromosome 17 affecting tail length and fur structure respectively. Females were inspected for pregnancy weekly, and pregnant females were examined for births daily on Monday to Friday. In most tests the offspring were diagnosed as carrying one homologue or other of chromosome 17 on the basis of tail-length, using brachyury, *T*, as a marker. In a few tests the marker tufted, *tf*, was used. Tail length was classified at birth, and tufted at age 4–5 weeks. The aim was to classify 40 young from each of five males in each test.

For tests of fertility, each male was again placed with two females of the inbred strain TFH/H. Females were inspected for pregnancy weekly. If neither female had become pregnant after one month, then the females were dissected and further examined for signs of pregnancy.

3. Results

(i) *Transmission ratio from males heterozygous for the responder in* t$^{\text{lowH}}$ *and a complete haplotype*

Males were bred which carried t^{lowH} on one chromosome 17 (together with brachyury, *T* as a marker) and a complete haplotype on the other. The haplotypes chosen included several naturally occurring ones not already studied by Lyon & Zenthon (1987) and also the recombinant haplotype t^{hr15}. This arose by crossing-over between the partial haplotypes t^{w18} and t^{h50} and thus has its proximal and central region derived from t^{w5} and its distal region from t^{6}. In addition, the haplotype t^{6} was included as a complete haplotype although in fact its *t*-chromatin does not extend as far proximally as that of the true complete haplotypes (Fox *et al.* 1985) (Fig. 1).

The various complete haplotypes could be divided into three groups on the basis of the transmission ratio of the doubly heterozygous males. Five haplotypes, t^{0} t^{6}, t^{w1}, t^{w2} and t^{w73} gave ratios that did not differ significantly from 50% (Table 2). The second group of haplotypes included t^{hr15}, together with t^{w5} and t^{hr1}, which were studied by Lyon & Zenthon (1987). These gave a statistically significant excess of t^{lowH} offspring, ranging from 62·3 to 66·1%. The $Tt^{lowH}tf/t^{w32}$+ males, however, gave a statistically significant deficiency of offspring carrying t^{lowH}, with a mean of 34·9% t^{lowH} and χ^2 for departure from 1:1 of 23·6 ($P < 0{\cdot}001$), thus forming a third group.

A notorious problem in dealing with transmission ratios of *t* haplotypes is that there may be unaccountable variation among males of the same genotype. For this reason, the ranges of values for individual males are given in Table 2, rather than standard errors. There was in fact good consistency among males of the same genotype. All males of genotype Tt^{lowH}/t^{w32} gave less than 50% t^{lowH}, and all those in which t^{lowH} was heterozgyous with t^{w5}, t^{hr1}, or t^{hr15} gave more than 50% t^{lowH}.

Table 1. *Structure of partial haplotypes and their haplotype of origin*

Partial haplotype	Haplotype of origin	Allelic form of region: D1	*T* or *tct*	*Tcp-1*	R	D3	D2
t^{6}	t^{Lmb}	+	*t*	*a*	*t*	*t*	*t*
t^{h45}	t^{6}	+	*t*	*ab*	+	+	+
t^{h44}	t^{6}	+	*t*	*a*	+	+	+
t^{h51}	t^{w32}	*t*	*t*	*a*	+	+	+
t^{h49}	t^{w5}/t^{lowH}	*t*	*t*	*a*	*t*	+	+
t^{w18}	t^{w5}	*t*	*t*	*a*	*t*	*t*	+
t^{h18}, t^{h50}	t^{6}	+	+	*b*	+	+	*t*
t^{s6}	$t^{h17}/t^{w12}tf$	+	+	+	*t*	*t*	*t*
t^{Lub2}	t^{Lub1}	+	+*t*	—	*t*	*t*	*t*
t^{lowH}	t^{h2}/t^{h17}	+	+	*b*	*t*	+	+

+, wild type; *t*, *t*-complex form.
For *Tcp*−1, *a* is *t*-complex allele and *b* is wild type.
t^{h45} has a duplication of *Tcp-1*; t^{Lub2} has a duplication of *T*/*tct* and a deletion of *Tcp-1*.
D1, D2, D3 and R are abbreviations for *Tcd-1* to *Tcd-3* and *Tcr*.

Table 2. *Transmission ratios of males carrying* t$^{\text{lowH}}$ *in* trans *with a complete* t *haplotype*

Complete *t*	No. males	Offspring: *T*	*t*	t^{lowH} (%)	$\chi^2_{1:1}$
t^{0}	6	162	141	53·5 (42·9–61·8)	1·45
t^{6}	5	106	116	47·7 (41·2–60·8)	0·45
t^{w1} *	6	156	158	49·7 (40·4–55·3)	0·01
t^{w2}	3	50	47	51·5 (45·7–57·1)	0·19
t^{w73}	6	103	96	48·2 (45·2–66·7)	0·25
t^{w5} *	5	130	69	65·3 (56·4–84·2)	18·7
t^{hr1} *	5	177	107	62·3 (55·8–68·7)	17·3
t^{hr15}	5	168	86	66·1 (55·1–71·1)	26·5
t^{w32}	6	90	168	34·9 (28·9–46·3)	23·6

* Data of Lyon & Zenthon (1987).
Test males $Tt^{lowH}tf/t^{x}$+.
Range of values of % t^{lowH} for individual males given in parentheses.

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(ii) *Effect of* t^{h45} *on transmission ratio of distal partial haplotypes* t^{s6} *and* t^{Lub2}

In order to test for any effect of t^{h45} on transmission of distal partial haplotypes, lacking the distorter *Tcd-1*, males were bred which were doubly heterozgyous for t^{h45} and for the haplotype t^{s6}. Two groups of control males were also bred. The first carried the haplotype t^{h44} in place of t^{h45}, and the second were contemporaneous males heterozygous for t^{s6} and wild type, i.e. $Tt^{s6}/++$. All three groups of males gave transmission ratios of t^{s6} in the range of 50% (Table 3). There was no significant difference between the males carrying t^{h45} and those of genotype $Tt^{s6}/++$, nor between Tt^{s6}/t^{h44} and $Tt^{s6}/++$. In addition, the range of values of individual males within a group showed good consistency.

This work thus failed to confirm the finding of Silver & Remis (1987) in which males of genotype t^{h45}/t^{Lub2} showed enhanced transmission of t^{Lub2}. The possibility remained that the discrepancy was due to some difference between t^{Lub2} and t^{s6}. The work was therefore repeated using t^{Lub2}. Again three types of males were studied. Animals of genotype t^{h45}/t^{Lub2} were compared with controls having a wild-type chromosome 17 opposite t^{Lub2} ($Ttf/t^{Lub2}+$ or $+tf/t^{Lub2}+$) and with positive controls of genotype t^{h51}/t^{Lub2}. These latter were chosen so as to test the effect of the distorter *Tcd-1*, carried by t^{h51}, on the transmission of t^{Lub2}.

The transmission ratio of t^{Lub2} when opposite a wild-type chromosome 17 was surprisingly low. On the basis of molecular studies of its length t^{Lub2} is thought to carry *Tcd-2* and *Tcd-3* as well as Tcr^t (Fox *et al.* 1985; Sarvetnick *et al.* 1986) (Fig. 1), and thus would be expected to have a ratio like t^{s6}, or around 50%. In fact, the ratios of six $t^{Lub2}+/+tf$ or $t^{Lub2}+/Ttf$ males ranged from 10·5 to 34·6% (Table 3; individual values 10·5, 12·5, 16·7, 17·0, 23·7, 34·6%). One possible explanation of such a low transmission might be that the t^{Lub2} animals had undergone a rearrangement of some kind, since the molecular studies were undertaken, and leading to the loss of one or more distorters. This seems somewhat unlikely in that two distinct lines of t^{Lub2} were imported into this laboratory, and maintained separately, and both showed the low transmission. The second line carried t^{Lub2} on the Robertsonian translocation Rb(2.17)-11Rma (R11R) and four males from this line carrying R11R heterozgyously or homozygously and mated to females also carrying R11R and homozygous for tufted, *tf*, transmitted t^{Lub2} to 58 of 343 offspring or 16·9% (presence of t^{Lub2} assessed by classification for *tf*).

Like all other *t* haplotypes so far studied t^{Lub2} was indeed susceptible to the ratio-distorting effect of *Tcd-1* carried in t^{h51}. Males of genotype $t^{Lub2}+/t^{h51}tf$ transmitted t^{Lub2} to 64·4% of their 284 progeny (Table 3). However, in keeping with the low transmission of $t^{Lub2}+/+tf$ males, the transmission of $t^{Lub2}+/t^{h51}tf$ males was not as high as found previously with t^{s6}/t^{h51} males (Lyon, 1984), which gave 99% t^{s6} offspring. In addition, there was marked variability among the $t^{Lub2}+/t^{h51}tf$ animals, with the transmissions from individual males ranging from 22·4 to 89·6% (22·4, 52·2, 73·9, 78·6, 81·0, 89·6%). By contrast, t^{h45} had no effect on the transmission of t^{Lub2}, $t^{Lub2}+/t^{h45}tf$ males giving 14·5% of t^{Lub2} offspring, in good agreement with the 13·2 or 25·2% obtained from the control males. In addition, the six males of this genotype showed good consistency, with a range from 8·3 to 28·9% t^{Lub2} offspring.

Table 3. *Tests of effect of* t^{h45} *on transmission ratio of males also carrying* t^{s6} *or* t^{Lub2}

Male genotype	No. males	Offspring T	Offspring $+$	t^{s6} (%)
$Tt^{s6}/+$	5	90	129	41·1 (31·2–52·4)
Tt^{s6}/t^{h44}	5	137	123	52·7 (42·6–63·3)
Tt^{s6}/t^{h45}	3	87	101	46·3 (36·4–60·0)
		$+$	tf	t^{Lub2} (%)
$t^{Lub2}+/+tf$	3	36	107	25·2 (17·0–34·6)
$t^{Lub2}+/Ttf$	3	14	60	13·2 (10·5–16·7)
$t^{Lub2}+/t^{h51}tf$	6	183	101	64·4 (22·4–89·6)
$t^{Lub2}+/t^{h45}tf$	6	32	189	14·5 (8·3–28·9)

(iii) *Possible differences among* t *haplotypes in effects on male sterility*

Hammerberg (1982) reported that males doubly heterozgyous for the complete haplotype t^0 and for a proximal partial haplotype derived from t^{w5} were normally fertile. This result is in conflict with the results of Lyon (1986) that males heterozgyous for a complete and a proximal partial haplotype, namely t^{h49}/t^{w5} and t^{h51}/t^{w32}, are sterile, presumably due to homozygosity for the proximal distorter *Tcd-1*. This conflict raises the question whether the t^0 haplotype differs from others in the *Tcd-1* region. In order to investigate this, males doubly heterozgyous for t^0 and for t^{h49} (a proximal partial haplotype derived from t^{w5}) (Fig. 1, Table 1) were tested for fertility. All of five males tested were completely sterile. Thus, under the conditions of this work there is no evidence that the *Tcd-1* region of t^0 differs from that in other *t* haplotypes.

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4. Discussion

These results provide further evidence for differences among *t* haplotypes in the responder region. Lyon & Zenthon (1987) had found that complete *t* haplotypes could be divided into two types on the basis of their transmission when opposite a constant responder in t^{lowH}. The present work has revealed a third type. The majority of haplotypes tested were transmitted equally with t^{lowH}, namely t^0, t^6, t^{w1}, t^{w2} and t^{w73}. Further examples of equal transmission of two Tcr^t responders from double heterozygotes come from data of Silver & Remis (1987) who reported ratios not differing significantly from 50% from males of genotypes t^{h2}/t^{s6}, t^{h49}/t^{s6}, t^{h49}/t^{h17} and t^{h2}/t^{Lub2}. The haplotype t^{hr15} resembled t^{w5} and t^{hr1} (tested by Lyon and Zenthon) in that double heterozygotes with t^{lowH} gave over 60% offspring carrying t^{lowH}, and thus significantly less than 50% carrying t^{hr15}. The responder region of t^{hr15} is derived from t^{w5}. Thus, all three haplotypes with a t^{w5} responder region, namely t^{w5}, t^{hr1} and t^{hr15}, give a similar transmission of 60–65% t^{lowH}. This similarity is regarded as evidence of the repeatability of this phenomenon. The haplotype t^{w12} may be another example of this group, as Bennett *et al.* (1983) found that t^{lowH}/t^{w12} males produced a similar excess of t^{lowH} offspring (mean of 76%).

The single representative of the third group of haplotypes was t^{w32}, which gave significantly less than 50% offspring carrying t^{lowH}, and thus an excess of t^{w32}. This appears to provide further insight into the mechanism of unequal transmission of two responder regions. Unequal transmission of haplotypes from double heterozygotes could be due either to differences in the responders themselves, in their sensitivity to the action of the distorters, or the responders could be similar and the differential effect could be due to *cis*-acting effects of the distorters, in addition to the already established *trans*-acting effects (Lyon & Zenthon, 1987). Such a *cis*-acting effect could potentially be produced by the distorters continuing to have a harmful effect late in spermatogenesis after the intercellular bridges between spermatids had closed. However, if any such harmful effects occurred, a haplotype with more distorter genes would always have lower transmission than one with fewer or no distorters, such as t^{lowH}. The fact that t^{w32}, a complete haplotype, was transmitted at a significantly higher frequency than t^{lowH} suggests that this unequal transmission was not due to harmful effects of distorters, but instead due to differences in the responder itself. This is of interest in studying the molecular nature of the responder region, since it suggests that relevant differences should be found among *t*-haplotypes in the genes of the responder region. A candidate gene for the responder has already been found (Schimenti *et al.* 1988).

The results concerning possible differences in distorters or presence of additional distorters are more puzzling. Firstly, some results appear not to be repeatable between one laboratory and another. Hammerberg (1982) found that male double heterozygotes of t^0 and a proximal partial haplotype derived from t^{w5} were fertile, whereas such males would be expected to be homozygous for the distorter *Tcd-1*, and hence to be sterile. In the present work similar males of genotype t^{h49}/t^0 were fully sterile, as expected, and differing from Hammerberg's results. Earlier, Dunn & Bennett (1969) had also found males of similar genotype to be sterile or near sterile. Thus, the evidence of Dunn and Bennett and the present work indicates that t^0 resembles other *t* haplotypes in carrying a distorter or sterility gene in its proximal region. It is possible that Hammerberg's results stem from a difference in genetic background. Both transmission ratio (Bennett *et al.* 1983; Gummere *et al.* 1986) and male sterility (Lyon, 1987) can be affected by genetic background. In particular, Bennett *et al.* showed that t^0 was susceptible to genetic background effects leading to reduction of its transmission ratio to less than 50%. The genetic background in Hammerberg's work was mixed, and the transmission ratio of t^0 was only 58%. Thus, it is possible that the background was such as to mitigate the effects of the distorters, not only on transmission ratio, but also on male sterility.

A second discrepancy in results from different laboratories concerns the effect of t^{h45} on transmission ratio. Silver & Remis (1987) found that t^{h45} increased transmission of t^{Lub2} from 38 to 87%. In the present work t^{h45} had no significant effect on the transmission of t^{Lub2} or of a second distal partial haplotype t^{s6}. The interpretation is complicated by the unexpected finding of a low transmission of t^{Lub2} from heterozygotes with wild type. Molecular evidence on the structure of t^{Lub2} (Fox *et al.* 1985; Sarvetnick *et al.* 1986) suggests that it should carry Tcr^t, *Tcd-3*^{t} and *Tcd-2*^{t} and it would thus be expected to have a transmission ratio around or mildly above 50%, like that of t^{s6}. Instead only about 20% (13·2 and 25·2% in two groups of males) of offspring carried t^{Lub2}. This apparent difference between t^{Lub2} and t^{s6} could be a genuine one, or could be due to various extraneous factors such as genetic background. One possibility is that the t^{Lub2} haplotype had undergone some genetic change since the molecular studies were carried out, and no longer carried *Tcd-3* or *Tcd-2*. This seems an unlikely explanation since two separate lines of t^{Lub2}, with or without the Robertsonian translocation Rb(2.17)11Rma, showed similar transmission, and it would be surprising if both had undergone similar genetic change. Another possibility is that the apparent low transmission of t^{Lub2} was in fact due to low viability of t^{Lub2} offspring. With most *t* haplotypes this could be checked by breeding from heterozygous females, but t^{Lub2} cannot be transmitted from females (Winking & Silver, 1984). However, there was no evidence of reduced litter-size or increased post-natal

losses such as would be expected from reduced viability, and surviving t^{Lub2} animals were vigorous. Hence there seems no reason to suspect poor viability of t^{Lub2}. Yet a third possible explanation is that the genetic background of the t^{Lub2} stock was causing the low transmission. This possibility must indeed be entertained because the t^{Lub2} haplotype had been recently introduced into this laboratory and was on an unknown genetic background. Most *t* haplotypes used in this work had been crossed for various numbers of generations to the inbred strain TFH/H. The t^{Lub2} stock had similarly been crossed to TFH/H, but only for 2–4 generations. Bennett *et al.* (1983) showed that the transmission of various distal partial haplotypes, derived from t^{w5}, t^{w12} or t^{w32}, and thought to have a set of distorter and responder genes like t^{Lub2} (i.e. lacking *Tcd-1*) had a mean slightly above 50%, but varied from 22 to 99% in individual males. Thus, the low transmission of t^{Lub2} may have been due to a different genetic background; if so, this will become clear after further crosses to TFH/H.

There remains the possibility that the transmission of t^{Lub2} is truly lower than that of t^{s6} and similar haplotypes. This possibility is strengthened by the fact that the original description of t^{Lub2} by Winking & Silver (1984) gave its transmission ratio as 38% among a total of 115 offspring, a value significantly below 50% ($\chi^2 = 6{\cdot}34$, $P < 0{\cdot}01$). Although t^{Lub2} resembles t^{s6} in the distorters *Tcd-1*, *Tcd-2* and *Tcd-3* and the responder, it differs in the proximal region. In t^{Lub2} there is a duplication of the region including the tail interaction factor, and a deletion including the *Tcp-1* gene (Sarvetnick *et al.* 1986) (Fig. 1). Perhaps the duplication of the tail interaction factor might tend to lower transmission. Lyon & Meredith (1964) found the transmission of the haplotype t^{h7}, also suspected of having a duplication of the tail interaction factor, to be lower than that of its parent haplotype t^{6}. Furthermore, a haplotype derived from t^{h7} apparently by reversion of the duplication, namely t^{h16}, also showed a reversion in transmission ratio to that of t^{6}. Conversely, Bennett *et al.* (1983) found that T^{hp} and T^{0r}, which both involve a deletion in the same region, tended to enhance transmission of *t* haplotypes. Thus, it is possible that duplications in the region of *T* or *tct* may lower transmission, and deletions may enhance it. Further work is needed to determine whether the transmission of t^{Lub2} is indeed lower than that of t^{s6} and similar haplotypes, and if so whether the effect is due to the duplication of the tail factor.

In any case the discrepancy between the results of Silver & Remis (1987) and the present work concerning the effect of t^{h45} remains. In our crosses t^{h45} had no effect on the transmission of either t^{Lub2} or t^{s6}. However, t^{Lub2} did respond by increased transmission to the haplotype t^{h51} which carries *Tcd-1* and was used as a positive control. The reason for the discrepancy is not clear, but again genetic background may be involved. Silver and Remis did not use any exactly comparable controls. Hence their experimental group, t^{Lub2}/t^{h45}, may have differed in background from the animals used as controls. The individual t^{Lub2}/t^{h51} males in the present work varied markedly in transmission ratio from 22·4 to 89·6%, and this suggests that t^{Lub2} is indeed subject to background effects, and that genes producing these effects were segregating in our own stocks.

This work has thus provided further evidence for differences in the responder region of *t* haplotypes but no clear evidence for differences in distorters. However, differences in distorter or sterility genes may well emerge in future studies. It is already known that males homozygous for t^{w2} show abnormalities in spermiogenesis not seen in other haplotypes (Dooher & Bennett, 1977), suggesting that t^{w2} carries an additional sterility gene which may or may not also affect ratio distortion. It is reasonable to expect that *t* haplotypes have undergone change during evolution, by mutation and selection of alleles favourable to maintenance of the haplotypes in wild populations (Lewontin & Dunn, 1960; Lewontin, 1962, 1968). It is of interest that the t^{w73} haplotype, which occurs only in the subspecies *Mus m. musculus* (Klein *et al.* 1984), in our tests on the responder region fell in with the majority of haplotypes from *M. m. domesticus*. However, this does not exclude the possibility of other differences between t^{w73} and the *domesticus* haplotypes. It is not clear from this work which type of responder is selectively most advantageous. The *t* form of the responder is transmitted in excess when distorter genes are present, but at markedly less than 50% frequency in the absence of distorters (Lyon, 1984; Lyon & Zenthon, 1987). This suggests that the same property that confers resistance to the distorters also impairs sperm function relative to normal sperm. Thus, it may be that natural selection on the responder involves a balance between increasing resistance to the distorters and impairment of sperm function.

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