

# Five of the nine genetically defined regions of mouse *t* haplotypes are involved in transmission ratio distortion

LEE M. SILVER AND DAVID REMIS

Department of Molecular Biology, Princeton University, Princeton, NJ 08544, U.S.A.

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## Summary

Mouse *t* haplotypes have been divided into nine subregions that are each defined by one or more molecular markers. In previous studies, three of these subregions were shown to contain 'distorter loci' that interact to effect the transmission-ratio distortion phenotype characteristic of all complete *t* haplotypes. To determine which of the remaining six subregions also play a role in this phenotype, we analysed the accumulated data on transmission ratio distortion from males that carried one of 26 different combinations of two partial *t* haplotypes. We have obtained evidence for the association of two additional subregions with distorter loci. First, we present further evidence for the existence of a previously postulated distorter locus, *Tcd-3*, and describe its mapping to the T66C subregion. Secondly, we describe the identification of a new distorter locus, *Tcd-4*, in association with the subregion defined by the structural gene for the TCP-1 protein. Further studies indicate that two doses of the *Tcd-4* locus are equivalent in effect to a single dose each of *Tcd-4* and a second distorter locus, *Tcd-1*. This result suggests that different distorter locus products could have a common mode of action.

## 1. Introduction

Mouse *t* haplotypes are a variant form of chromosome 17 present at high frequencies in wild populations of *Mus domesticus* and *Mus musculus* (see Silver, 1985 for a recent review). Evidence suggests that all *t* haplotypes are recent descendants of a single ancestral chromosome (Silver *et al.* 1987) that had acquired the ability to propagate itself at the expense of wild-type homologues from heterozygous  $+/t$  males, through a process known as transmission ratio distortion (TRD). Recent studies suggest that *t*-carrying spermatozoa are able to functionally inactivate their wild-type meiotic partners (Olds-Clarke & Peitz, 1986). The biochemical basis for this inactivation remains unknown.

Complete *t* haplotypes extend across a 12 cM stretch of chromosome 17 from a point near the centromere to a point between the major histocompatibility complex (MHC) and the *Pgk-2* locus. Crossing over between *t* haplotypes and wild-type chromosomes is greatly suppressed as a consequence of two non-overlapping inversions (Artzt, Shin & Bennett, 1982; Herrmann *et al.* 1986; Sarvetnick *et al.* 1986). Nevertheless, rare recombination events do occur, with the production of partial *t* haplotypes. The extent of *t*DNA present within each partial *t* haplotype can be determined by the presence or absence of *t*-specific

molecular markers that distinguish different subregions of the complete *t* haplotype (see Fig. 1; Silver *et al.* 1983*b*; Fox *et al.* 1985).

Analysis of males that carry different combinations of partial *t* haplotypes provided critical insight towards an understanding of the genetic basis for the TRD phenotype. The results of such studies allowed Lyon (1984) to postulate that TRD results from the action of a series of *t* complex distorter loci (*Tcd-1*, *Tcd-2* and *Tcd-3*) upon a single *t*-complex responder locus (*Tcr*). The effects of the distorter loci appear to be additive (the more *Tcd* loci present, the higher the ratio), and they can function in *cis* or *trans* to the *Tcr* locus. The chromosome that carries *Tcr* will be transmitted at a high ratio if enough *Tcd* loci are present. When *Tcr* is absent or homozygous, both homologues of chromosome 17 will be transmitted equally. Recent studies by Lyon (1986) suggest that the same *Tcd* loci responsible for transmission ratio distortion from heterozygous  $+/t$  males are also responsible for the sterility of males that carry two *t* haplotypes.

Together, the available partial *t* haplotypes, and available molecular markers, allow the division of a complete *t* haplotype into 9 subregions, of which 3 have been definitively associated with genes involved in TRD (*Tcr*, *Tcd-1* and *Tcd-2*; Fox *et al.* 1985). This report describes a genetic analysis of the remaining 6

subregions for their role, if any, in the TRD phenotype.

## 2. Materials and methods

All males analysed in this study carried two partial *t* haplotypes, one proximal (indicated generically as  $t^p$ ) and one distal (indicated generically as  $t^d$ ), in a trans-configuration on chromosome 17. To produce these genotypes, in all cases except those involving  $t^6$  and  $t^{w1ub2}$ , animals homozygous for a proximal partial *t* haplotype and a linked mutation at the *tufted* (*tf*) locus ( $t^p tf/t^p tf$ ) were mated with animals heterozygous for a distal *t* haplotype in cis-configuration with the dominant *T* locus mutation ( $Tt^d/+$ ). Recombination can occur between distal *t* haplotypes and the *T* locus; however, the *tf* locus is present within, and serves as an absolute marker for, the inversion characteristic of all distal *t* haplotypes. All proximal *t* haplotypes contain the *t-complex-T-interaction* (*tct*) locus, which causes taillessness in doubly heterozygous  $T/t^p$  animals.

The distal haplotypes  $t^{Tu2}$  and  $t^{s6}$  contain mutations at the *tufted* locus, and tailless tufted offspring from crosses with these haplotypes must have a genotype of  $Tt^d tf/t^p tf$ . The distal haplotypes  $t^{h17}$  and  $t^{h18}$  are maintained with a non-*t* homologue that carries a *tf* mutation ( $Tt^d +/+tf$ ), and tailless nontufted offspring from crosses with these haplotypes must have a genotype of  $Tt^d +/t^p tf$ . The transmission ratios of all  $Tt^d/t^p$  males were determined in crosses with wild-type females. (Two types of females were used, from the inbred strain 129/SvJ, and from an outbred background; no significant difference in transmission ratio was observed that could be attributed to the female genotype.) Offspring with short tails will have received the distal *t* haplotype ( $Tt^d/+$ ), and offspring with tails of normal length will have received the proximal *t* haplotype ( $t^p/+$ ).

The distal haplotypes  $t^6$  and  $t^{w1ub2}$  are maintained in trans-configurations with a chromosome that carries mutations at *T* and *tf* ( $Ttf/t^d +$ ), and mice with these genotypes were mated to animals homozygous for proximal haplotypes and the *tf* mutation. Non-tufted offspring with normal tails have a genotype of ( $t^p tf/t^d +$ ). The transmission ratios of ( $t^p tf/t^d +$ ) males were determined in crosses with females homozygous for *tf*, and wild-type at the *T* locus ( $+tf/+tf$ ). Offspring were scored at 28 days of age for the presence or absence of the tufted phenotype (Lyon, 1956). Tufted animals have received the proximal haplotype, and non-tufted animals have received the  $t^6$  haplotype.

The transmission ratios of ( $t^p/t^{w1ub2}$ ) males were determined in crosses with ( $T/+$ ) females. Doubly heterozygous ( $T/t^p$ ) offspring are tailless, whereas all other viable genotypes derived from this cross, including  $T/t^{w1ub2}$ , give rise to a tail of normal length, because the  $t^{w1ub2}$  haplotype suppresses the tail-

shortening effect of the *T* mutation (Winking & Silver, 1984). The transmission frequency of  $t^{w1ub2}$  from the doubly heterozygous males is calculated as:  $1 - [(2 \times \text{number of tailless offspring}) / (\text{total number of offspring})]$ .

All of the *t* haplotypes used in this study have been described in previous publications (see Silver *et al.* 1983*a, b*; Lyon *et al.* 1984; Styrna & Klein, 1981; Winking & Silver, 1984; and Fox *et al.* 1985).

## 3. Results and discussion

### (i) Strategy for the genetic analysis of transmission ratio distortion

In recent years, our laboratory and others have used recombinant DNA technology and two-dimensional gel electrophoresis to develop a series of *t*-specific molecular markers (Silver *et al.* 1979, 1983*b*; Fox, Silver & Martin, 1984; Röhme *et al.* 1984; Willison, Dudley & Potter, 1986; Mann, Silver & Elliott, 1986). We have used these markers to demonstrate that independently derived partial *t* haplotypes contain different lengths of *t* DNA (Silver *et al.* 1983*b*; Fox *et al.* 1985). The accumulated data allow us to divide a complete *t* haplotype into 9 subregions, each of which has been separated from adjacent regions through the generation of one or more partial *t* haplotypes (Fig. 1). Rare recombination between *t* haplotypes and wild-type chromosomes does not occur randomly throughout the *t* region, and as a consequence the relative size of each subregion can vary greatly. The most distal subregion, marked by the major histocompatibility complex (MHC), accounts for perhaps 50% of the complete *t* haplotype, whereas other subregions such as T66A may represent as little as 1% (J. Schimenti and L. M. Silver, unpublished observations).

With the availability of a set of partial *t* haplotypes that define these 9 subregions (examples 1–15 in Fig. 1), it became possible to determine the contribution of each to the transmission ratio distortion phenotype characteristic of complete *t* haplotypes. Early results obtained by Lyon and her colleagues clearly demonstrated the existence of at least two distorter loci (*Tcd-1* and *Tcd-2*) and a single responder locus called *Tcr* (Lyon & Mason, 1977; Lyon, 1984). Molecular studies allowed the mapping of *Tcd-1* with the most proximal T48-defined subregion, *Tcd-2* with the large, distal, MHC-marked subregion, and *Tcr* with the T66B subregion (Fox *et al.* 1985). The *Tcd-2* 'locus' has a stronger effect on TRD than any other *Tcd* locus described in this report, and the large size of the *Tcd-2* subregion makes it likely that it actually contains multiple *Tcd* genes which have yet to be separated by recombination.

We set out to analyse the role, if any, of the remaining six *t* haplotype subregions. The strategy that we followed was to study males that carry two

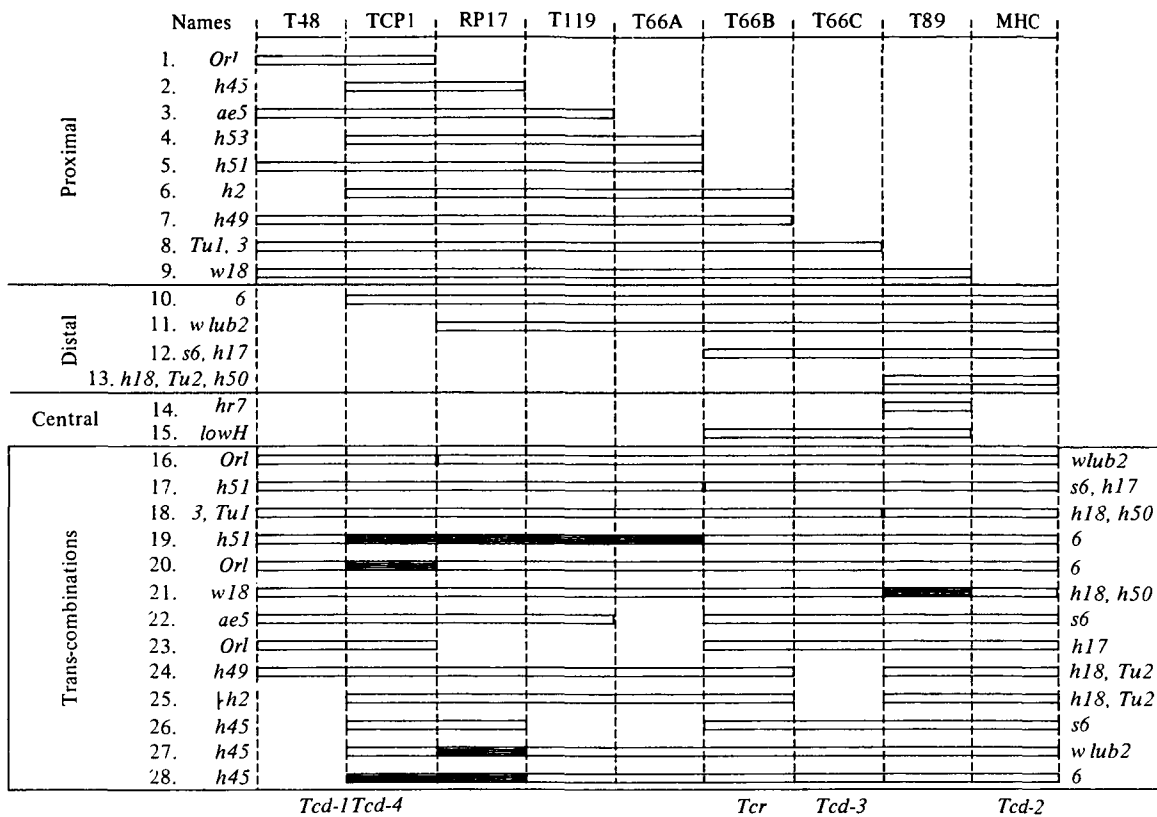


Fig. 1. Extent of *t*-DNA associated with partial *t* haplotypes and trans-combinations of partial *t* haplotypes. A complete *t* haplotype can be divided into nine subregions that are defined by one or more molecular markers (Silver *et al.* 1983*b*; Fox *et al.* 1985; Sarvetnick *et al.* 1986; Herrmann *et al.* 1986; Mann *et al.* 1986). We have chosen one marker to identify each subregion, and these are listed across the top of the figure, from left to right, according to their genetic order from the centromeric end to the telomeric end of a complete *t* haplotype. Fifteen classes of partial *t* haplotypes have been identified and grouped according to whether they contain the proximal portion, the distal portion, or only a

central portion of a complete *t* haplotype. Each partial haplotype is indicated only by its superscript name, and is numbered and schematically represented by an open box which extends over the subregions contained within it. Thirteen classes of trans-combinations of partial *t* haplotypes are represented schematically in the same way, with the proximal haplotype indicated on the left and the distal haplotype indicated on the right. Only those genotypes heterozygous at the *Tcr* locus are presented. These are numbered from 16 to 28. Subregions that are present in two doses are represented by filled-in boxes. The locations of all distorter loci identified to date are indicated at the bottom of the figure.

partial *t* haplotypes, one proximal and one distal, in trans-configuration, such that one or a few *t*-subregions are either completely absent or present in two doses (indicated schematically as examples 16–28 in Fig. 1). Each male was mated to appropriate females for a determination of transmission ratio by progeny analysis, as described in the Materials and Methods. Other investigators have performed similar experiments (referenced in the legend to Fig. 2), and a summary of previous data together with data from this report are presented in Fig. 2. In total, 26 different combinations of partial *t* haplotypes have been analysed. All partial *t* haplotypes and genotypic combinations of partial *t* haplotypes used in this report (except those homozygous at the *Tcr* locus) will be referred to by their identifying number from Fig. 1.

(ii) Further evidence for the unique role of *Tcr* in transmission ratio distortion

The data in Fig. 2 are grouped according to the status

of the *Tcr* locus within each genotype. Results have been obtained from seven different combinations of partial *t* haplotypes in which the *Tcr* locus is either absent from both chromosome 17 homologues or present on both. In all seven cases the transmission ratio is not significantly different from 50% (by chi-square analysis) for each homologue. It is clear that heterozygosity for the *Tcr* locus is a first requirement for a significant distortion of transmission ratio, as postulated by Lyon (1984).

(iii) Further evidence for the existence of *Tcd-3* and localization to the *T66C* subregion

Lyon (1984) postulated the existence of a third distorter locus (*Tcd-3*) present within the  $t^{w18}$  haplotype and not within the  $t^{h49}$  haplotype based on the difference observed in transmission ratios of  $t^{w18}/t^{h18}$  and  $t^{w18}/t^{h50}$  males (genotype 21) compared to  $t^{h49}/t^{h18}$  males (genotype 24). Our data provide further support for the existence of *Tcd-3*. In three

		<i>Tcr</i> absent				<i>Tcr</i> present				
		<i>h45</i>	<i>Orl</i>	<i>ae5</i>	<i>h51</i>	<i>h2</i>	<i>h49</i>	$b = Tu1/a = 3$	<i>w18</i>	
		2	1	3	5	6	7	8	9	
		50%	52% (88) <sup>1</sup>	47% (485) <sup>2</sup> 40% (664) <sup>1</sup>	57% (127) <sup>4</sup>	47% (86) <sup>4</sup>	20% (113) <sup>2</sup>	40% (206) <sup>4</sup>	46% (745) a <sup>1</sup> 45% (194) b <sup>7</sup>	49% (55) <sup>1</sup>
<i>Tcr</i> absent	A = <i>h18</i>	13 53% (141) B <sup>1</sup>	47% (129) A <sup>4</sup>			54% (102) A <sup>4</sup>	25 67% (207) A <sup>1</sup>	24 83% (191) A <sup>4</sup>	18 96% (189) Aa <sup>1</sup>	21 99% (227) A <sup>4</sup>
	B = <i>Tu2</i>	53% (175) A <sup>1</sup>				81% (36) B <sup>1</sup>	84% (81) A <sup>1</sup>	94% (269) Bb <sup>7</sup>	99% (210) C <sup>4</sup>	
	C = <i>h50</i>	41% (216) B <sup>7</sup>					82% (60) B <sup>1</sup>			
<i>Tcr</i> present	A = <i>s6</i>	12 54% (158) B <sup>1</sup>	26 80% (66) A <sup>1</sup>	23 95% (91) B <sup>4</sup>	22 90% (181) A <sup>1</sup>	17 87% (128) B <sup>1</sup>	51% (154) A <sub>1</sub>	56% (72) B <sup>1</sup>		
	B = <i>h17</i>	67% (333) A <sup>4</sup> 69% (512) A <sup>1</sup>				99% (201) A <sup>4</sup>	48% (58) A <sup>1</sup>			
	<i>wlub2</i>	11 38% (115) <sup>8</sup>	27 87% (191) <sup>1</sup>	16 95% (78) <sup>6</sup>			57% (42) <sup>1</sup>			
	6	10 54% (136) <sup>4</sup> 70% (20) <sup>2</sup> 86% (261) <sup>1</sup> * 73% (88) <sup>1</sup> 93% (165) <sup>1</sup> *	28 99% (197) <sup>1</sup>	20 98% (359) <sup>2</sup>		19 99% (267) <sup>4</sup>		49% (160) <sup>4</sup>		

Fig. 2. Transmission ratios expressed by partial *t* haplotypes. Each column is headed by a proximal *t* haplotype, and each row is headed by a distal *t* haplotype. Each box represents the intersection of two forms of chromosome 17 and contains transmission ratio data from males that carry both chromosomes in their genotype. The first row of boxes contains data from males with only a proximal *t* haplotype and a wild-type chromosome, and the first column of boxes contains data from males with only a distal *t* haplotype and a wild-type chromosome. In both cases the transmission ratios of the chromosome that carries the partial haplotype are indicated. All other boxes contain data from males with a combination of a proximal and a distal haplotype. All partial haplotype combinations were in a trans-configuration, except for  $t^{h45}t^{h18}$  and  $t^{h51}t^{h18}$ , which were analysed in cis-configuration. In cases where *Tcr* is heterozygous, the transmission ratio of the *Tcr*-containing chromosome is indicated. In all remaining cases, the transmission ratio of the distal haplotype is indicated. The number within the top of many boxes corresponds to the numbered representation of the genotype in Fig. 1. (Only

trans-combinations that are heterozygous at the *Tcr* locus are represented in Fig. 1.) Each data line within each box contains up to four components. First is the transmission ratio obtained in a particular experiment. Second, in parentheses, is the total sample size analysed. Third, in some boxes only, are one or more letters that indicate the particular haplotypes used in the experiment when a class of partial haplotypes is represented by multiple, independent isolates. (The upper-case letter distinguishes the distal haplotype used, and the lower-case letter distinguishes the proximal haplotype used.) Finally, the publication describing the experiment is indicated as a superscripted number that corresponds to one of the following references: (1) this report; (2) Hammerberg (1981); (3) Dunn & Gluecksohn-Waelsch (1951); (4) Lyon (1984); (5) Lyon & Mason (1977); (6) Silver, Lukralle & Garrels (1983a); (7) Styrna & Klein (1981); (8) Winking & Silver (1984). The two data lines in the +/*t*<sup>6</sup> box that are followed by an asterisk (\*) represent ratios obtained from sets of males derived from special outcrosses described in the text.

independent experiments, males with genotype 24 expressed ratios of 82%, 83% and 84% compared to ratios of 99% in two separate experiments carried out by Lyon on males with genotype 21 (Fig. 2). When the data from each genotype are compared in both directions by a chi-square contingency test, strong support ( $P < 0.0001$  in all cases) is provided for the existence of the *Tcd-3* locus in the region of the  $t^{w18}$  haplotype (9) not shared by  $t^{h49}$  (7).

The region of difference between  $t^{w18}$  and  $t^{h49}$  has been split into two subregions defined by the markers T66C and T89. It is possible to sort out the roles played by these subregions through an analysis of males that carry either  $t^3$  or  $t^{Tu1}$  (8) in place of  $t^{w18}$  (9) in the genotypes described above. The no. 8 haplotypes are associated with only one of the two subregions (T66C) that distinguishes  $t^{w18}$  from  $t^{h49}$ . In two independent experiments, males with genotype 18 expressed ratios of 94% and 96% (Fig. 2). Both of these ratios are significantly different ( $P < 0.0001$  in all cases) from those obtained with genotypes 24 (82–84%). This result implies that the T66C subregion contains a strong distorter locus, defined as *Tcd-3*.

(iv) Evidence for the existence of a fourth distorter locus, *Tcd-4*

One of the nine *t* haplotype subregions, to which a role has not yet been assigned, is marked by a gene (*Tcp-1*) that codes for a protein expressed preferentially in the testes of mature adults (Silver *et al.* 1979; Willison *et al.* 1986). It seemed reasonable to hypothesize that such a gene could play a role in the TRD phenotype. To test this hypothesis, we analysed transmission ratios from animals that carried the partial haplotypes  $t^{h45}$  (2) or  $t^{w18}t^{wub2}$  (11) separately or together (27). The  $t^{w18}t^{wub2}$  haplotype is missing only two of the nine subregions, marked by T48/*Tcd-1* and *Tcp-1*. The  $t^{h45}$  haplotype is associated with only two of the nine subregions, marked by *Tcp-1* and *RP17*. Separately, each haplotype is transmitted at an accumulated ratio of 52% or less. (Of 13 individual +/ $t^{w18}t^{wub2}$  males analysed in our colony, none expressed a transmission ratio of greater than 57%.) However, when both are present in the same genotype, the  $t^{w18}t^{wub2}$  haplotype is transmitted at a significantly higher ratio of 87% ( $P < 0.0001$  in comparison with each partial *t*



haplotype alone). This result demonstrates the existence of a fourth distorter locus (*Tcd-4*) in association with the  $t^{h45}$  haplotype.

(v) *Localization of Tcd-4 and the identification of three subregions that do not play a major role in TRD*

A further localization of *Tcd-4* is possible through a comparison of genotypes 16 and 23 that differ by the presence or absence of three subregions – RP17, T119 and T66A. No significant difference is observed in the high transmission ratios (95% versus 95%) expressed by both genotypes. Hence there is no evidence for the existence of loci that strongly affect TRD in any of these three subregions. Since  $t^{h45}$  contains only the two subregions marked by *Tcp-1* and RP17, the *Tcd-4* locus must be associated with *Tcp-1*.

(vi) *Evidence for a role of Tcd-4 in the sterility phenotype*

In her analysis of the genetic basis for sterility in males homozygous for *t*-chromatin, Lyon (1986) demonstrated that each of the three subregions previously postulated to carry a *Tcd* locus (*Tcd-1*, *Tcd-2*, *Tcd-3*) also played a role in the sterility phenotype. Therefore, it was important to determine whether the *Tcd-4* locus might also be associated with the expression of this second phenotype. Evidence for this possibility comes from an analysis of data obtained by Lyon (1986), on the relative fertility of males that are identical in genotype at all *Tcd* loci except *Tcd-4*. The  $t^6/t^{w18}$  genotype is homozygous for *Tcd-4*, whereas  $t^{s6}/t^{w18}$  is heterozygous with only a single copy of *Tcd-4* (see Fig. 1 for a schematic diagram of each partial *t* haplotype); both genotypes are also homozygous for *Tcd-3*, and heterozygous for *Tcd-1* and *Tcd-2*. The frequency of sterile males was significantly greater ( $P < 0.05$ ) with the  $t^6/t^{w18}$  genotype compared to the  $t^{s6}/t^{w18}$  genotype (chi-square analysis performed on data from table 2 of Lyon, 1986). Although the sample sizes were small (7 and 17), the results are consistent with the hypothesis that all four *Tcd* loci function both in the distortion of transmission ratios from heterozygous males, and the sterility of homozygous males.

(vii) *Effect of gene dosage at the Tcd-4 locus*

The accumulated data indicate that the various *Tcd* loci can act additively to increase the transmission ratio of a chromosome 17 homologue that carries the *Tcr* locus. When all four *Tcd* loci are present, ratios of greater than 95% can be expressed. If either *Tcd-3* or *Tcd-1* alone is absent within a trans-configured partial *t* genotype (24, 26, 27), ratios between 80 and 87% are observed. These results suggest that the products of different *Tcd* genes might function in a similar manner, and that the actual levels of *Tcd* expression might play a determining role in the particular transmission ratio expressed by a male.

We set out to determine if two doses of one *Tcd* gene (*Tcd-4*) could substitute for the complete absence of another *Tcd* gene (*Tcd-1*) in the expression of a very high transmission ratio. To generate the required genotypes for analysis, we crossed  $Tf/t^6+$  males with  $Tf/t^{h45}tf$  females. The  $t^{h45}/t^6$  males that are generated (genotype 28) have single doses of *Tcd-2* and *Tcd-3*, a double dose of *Tcd-4* and no dose of *Tcd-1* (Fig. 1). The transmission ratio obtained with this genotype is 99%. This is significantly greater ( $P < 0.0001$ ) than the ratios usually obtained with males that have single doses of *Tcd-2*, *Tcd-3* and *Tcd-4*, in genotypes represented by the  $t^6$  haplotype alone (10, 54–86%), the  $t^{h45}/t^{w18}t^{ub2}$  combination (27, 87%), and the  $t^{h45}/t^{s6}$  combination (26, 80%). These observations suggest that the extent of transmission ratio distortion can be affected by the dosage of a particular *Tcd* gene.

With the cross used to generate  $t^{h45}/t^6$  males, we also obtained control siblings that carried only  $t^6$  or  $t^{h45}$  in trans-configuration with the dominant *T* mutation. The two different control genotypes could be distinguished through expression of the tufted phenotype, and the transmission ratios of each could be determined through progeny analysis. As expected, males that carried only the  $t^{h45}$  haplotype expressed ratios that were not significantly different from 50%. However, control males with only  $t^6$  expressed a ratio of 93%, which is significantly higher ( $P < 0.0001$ ) than the usual  $t^6$  ratio observed both in our colony and by other investigators (Fig. 2). A similar increase in the transmission ratio of  $t^6$ , from 70 to 86% ( $P < 0.0001$ ), was also observed by Hammerberg (1981) after outcrossing to a stock with the  $Tt^{or1}$  haplotype. In both cases, the mothers of the high transmission  $+/t^6$  males carried an additional distorter locus or dose that was not segregated to their sons. However, we cannot provide a satisfactory mechanism to explain these unusual results.

Nevertheless, the results just discussed require a re-analysis of the significance of the transmission ratio obtained with  $t^{h45}/t^6$  males (99%) in comparison with that obtained with sibling  $+/t^6$  males (93%), to control for potential genetic background effects. The difference between the two ratios is still highly significant ( $P < 0.001$ ). Therefore, we can state with a high degree of confidence that the dosage of *Tcd-4* can affect the extent of transmission ratio distortion expressed by males that do not carry the *Tcd-1* locus.

(viii) *The T89 subregion*

The available partial *t* haplotypes do not allow us to construct a genotype that contains the large *Tcd-2* subregion, but is missing T89. Therefore, it is not possible to determine the single-dose effect of this subregion on TRD. Nevertheless, a partial *t* haplotype consisting only of the T89 subregion,  $t^{hr7}$  (14), has been derived by Lyon, and it is possible to compare genotypes that differ only in their dosage of the T89 subregion. Recently we developed a congenic line that

carries the  $t^{hr7}$  haplotype on the 129/SvJ background after 10 sequential generations of backcrossing.

Although  $t^0$  is a complete  $t$  haplotype, it expresses a highly reproducible ratio of only 74% within the context of the 129/SvJ background, presumably as a consequence of a variant allele at one of the genes involved in TRD (L. M. Silver, unpublished observations). To determine if an extra dose of the T89 subregion would increase the transmission ratio of the  $t^0$  haplotype, we crossed 129/SvJ- $Tt^{hr7}$  N10 animals with 129/SvJ- $t^0$  (N20 or greater) animals to obtain the congenic double heterozygote 129/SvJ- $Tt^{hr7}/t^0$ . Our analysis of 54 progeny from these males yields a transmission ratio of 70%, which is not significantly different from that obtained with  $t^0$  alone. Therefore, no evidence exists for an effect of the T89 subregion on TRD. However, at the present time, with the available partial haplotypes, a role for this subregion cannot be definitely ruled out.

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