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Gene dosage effects on transmission ratio distortion and fertility in mice that carry *t* haplotypes

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Summary

Complete *t* haplotypes can be transmitted at distorted ratios from heterozygous $+/t$ male mice as a consequence of *t*-specific alleles at a series of *t complex distorter* loci (*Tcd-1*^{t} through *Tcd-4*^{t}) and a *t complex responder* locus. Partial *t* haplotypes that lack the *Tcd-2*^{t} allele cannot be transmitted at the very high ratios characteristic of complete *t* haplotypes. The breeding studies reported here tested the possibility that the absence of *Tcd-2*^{t} could be compensated for by the presence of double doses of other *Tcd*^{t} alleles. The results indicate that a double dose of *Tcd-4*^{t} alone will not work, but that a double dose of both *Tcd-1*^{t} and *Tcd-4*^{t} can promote a very high transmission ratio in the absence of *Tcd-2*^{t}. These results suggest that the extent to which transmission ratios are distorted is dependent upon the absolute level of expression of the individual *Tcd* genes. Further studies of genotypic effects on transmission ratio distortion, as well as fertility, lead to the suggestion of a fifth *t complex distorter* (*Tcd-5*) locus within *t* haplotypes.

1. Introduction

Mouse *t* haplotypes are variant forms of chromosome 17 that can be transmitted at non-Mendelian ratios from heterozygous $+/t$ males (see Silver, 1985 for a review). This transmission ratio distortion (TRD) results from the genetic interaction of a series of *t*-specific alleles at loci distributed along the 15 cm region present within a complete *t* haplotype (Lyon & Mason, 1977; Lyon, 1984). Two types of TRD loci have been identified. The *t complex responder* (*Tcr*) locus is defined by its role in determining which homologue of chromosome 17 will be transmitted at a high ratio; *Tcr* is the only mammalian locus known to have a haploid-specific effect on phenotype. The *t complex distorter* (*Tcd*) loci (*Tcd-1*, *Tcd-2*, *Tcd-3*, and *Tcd-4*) are defined by their ability to control the absolute level at which transmission ratios are distorted; *Tcd* loci can act in *cis* or *trans* configuration to *Tcr*. If a male carries one complete set of *Tcd*^{t} alleles and is heterozygous for *Tcr*^{t} and *Tcr*$^+$, the chromosome homologue with *Tcr*^{t} can be transmitted at frequencies of 95% or greater. If less than a full complement of *Tcd*^{t} alleles are present, this very high transmission of *Tcr*^{t} is not observed. The situation is somewhat more complicated because the genetic background of an animal (as well as epigenetic phenomena) can influence the level of TRD expressed with certain *t*-genotypes (Olds-Clarke & McCabe, 1982; Bennett *et al.* 1983; Gummere *et al.* 1986; Demin & Safronova, 1979). Nevertheless, with properly controlled breeding experiments, it has been possible to map and characterize the various TRD loci (Styrna & Klein, 1981; Lyon, 1984; Lyon, 1986; Silver & Remis, 1987; Lyon & Zenthon, 1987).

The relative 'strength' of the individually-defined *Tcd* loci can be gauged through a comparison of TRD values expressed by partial *t* haplotypes, or combinations thereof, that lack only a single one of the *Tcd*^{t} alleles. If only *Tcd-1*^{t} or *Tcd-3*^{t} is absent, transmission ratios are generally observed at a level intermediate between 50% and the very high values associated with complete *t* haplotypes (Lyon, 1984; Silver & Remis, 1987). However, if *Tcd-2*^{t} alone is absent, transmission ratios are reduced to approximately 50%. (Currently available partial *t* haplotypes do not allow the construction of genotypes that lack only *Tcd-4*^{t}.) These observations would suggest either that *Tcd-2*^{t} represents a gene with a more powerful effect on TRD than either *Tcd-1*^{t} or *Tcd-3*^{t} alone, or that *Tcd-2*^{t} actually represents two or more independent genes that have yet to be separated by recombination. The latter is a real possibility since the chromosomal region associated with *Tcd-2* is much larger than the regions associated with the other *Tcd* loci (Silver, 1985).

Lyon (1986) has presented compelling evidence that the same loci involved in the expression of TRD in $+/t$ heterozygotes may also be involved in sterility effects on males doubly heterozygous for two t haplotypes (t^x/t^y). Interestingly, the strongest effect on sterility is expressed by the *Tcd-2* locus, with weaker effects expressed by *Tcd-1*, *Tcd-3*, and *Tcd-4* respectively (see also Silver & Remis, 1984). Therefore, the *Tcd* loci appear to have the same relative strengths in the expression of both the TRD and sterility phenotypes.

Although candidate clones for a number of the loci involved in TRD have been obtained (Willison *et al.* 1986; Rappold *et al.* 1987; Schimenti *et al.* 1988), no information is available concerning the mechanisms by which these loci might act. In particular, it is not clear whether each of the Tcd^t loci provides an independent and essential factor necessary for complete expression of the TRD phenotype or whether alleles at different Tcd^t loci might be able to substitute for each other. To distinguish between these possibilities, breeding studies have been performed with mice that lack particular Tcd^t alleles, but have two copies of other Tcd^t alleles. The results of a previous study demonstrated that a double dose of $Tcd\text{-}4^t$ could compensate for the absence of $Tcd\text{-}1^t$ in the expression of a very high transmission ratio (Silver & Remis, 1987). This suggests that $Tcd\text{-}1^t$ and $Tcd\text{-}4^t$ might have a common mode of action within the context of the TRD phenotype. In the present report, these studies have been extended to determine whether the action of the most powerful *Tcd* locus – $Tcd\text{-}2^t$ – can be duplicated with extra doses of $Tcd\text{-}4^t$ and $Tcd\text{-}1^t$.

2. Materials and Methods

All mice were bred in our colony at Princeton University. Transmission ratios of Tt^{Or1}/t^{w18} and Tt^{Or1}/t^3 males were determined by breeding to wild-type ($+/+$) females and scoring tail lengths of progeny. [A short tail indicates the transmission of Tt^{Or1}, and a normal tail indicates the transmission of t^3 or t^{w18}.] Transmission ratios of t^{h45}/t^{w18} males were determined by breeding to T/t^{w18} females. This cross could result in embryos of four different genotypes – t^{w18}/t^{w18} embryos die *in utero*; t^{h45}/t^{w18} animals are born with a normal tail; and both T/t^{w18} and T/t^{h45} animals are born tailless. The subset of tailless animals that carry t^{h45} can be estimated as equivalent to the total number of animals with normal tails. Applying this estimate, it becomes possible to derive the following equation to obtain a value for the transmission of t^{w18} from t^{h45}/t^{w18} males: [(number of tailless offspring – number of offspring with normal tails)/(number of tailless offspring)]. Transmission ratios of $+/t^{w18}$ males were determined by breeding to $T/+$ females – a short tail indicates the transmission of the + chromosome, and taillessness indicates the transmission of the t^{w18} chromosome.

All experimental males were maintained with two healthy outbred females for a period of at least 4 months. Fertility was assessed by two parameters. First, if no offspring were produced during this period, males were deemed sterile. Second, if offspring were born to a particular male, a determination was made of the number of young born per female mate per month. The values obtained with all non-sterile males of the same genotype were averaged together for the final number shown in Fig. 1.

3. Results

(i) Tcd *genotypes of experimental animals*

Transmission ratio distortion and fertility were studied in experimental mice that carry one of three different t complex genotypes (Fig. 1). Tt^{Or1}/t^{w18} animals carry two doses of both $Tcd\text{-}1^t$ and $Tcd\text{-}4^t$, a single dose of $Tcd\text{-}3^t$ and *Tcr*, and no copies of $Tcd\text{-}2^t$. Tt^{Or1}/t^3 animals have an identical complement of TRD genes, as they are currently defined. Finally, t^{h45}/t^{w18} animals have a double dose of only $Tcd\text{-}4^t$, single doses of $Tcd\text{-}1^t$, $Tcd\text{-}3^t$, and *Tcr*, and no copies of $Tcd\text{-}2^t$.

(ii) *Transmission ratios*

Tt^{Or1}/t^{w18} males transmitted their *Tcr*-bearing chromosome (t^{w18}) to 96% (109/114) of their offspring. Transmission ratios determined for seven individual males with this genotype ranged from 93% to 100%. Sibling control animals with a genotype of $+/t^{w18}$ showed no significant transmission ratio distortion (58% of 50 offspring received the t^{w18} chromosome). The difference between our summed experimental (96%) and control (58%) values was determined by a chi-squared calculation to be highly significant ($P < 0{\cdot}0001$).

Tt^{Or1}/t^3 males transmitted their *Tcr*-bearing chromosome (t^3) to 80% (216/270) of their offspring. Transmission ratios determined for seven males with 11 or more offspring ranged from 63% to 87% (63%, 68%, 79%, 79%, 83%, 85%, 87%). Sibling control animals were not tested directly for TRD, however, a survey of past breeding records over an eight-year period in our colony of non-inbred t^3-bearing mice indicates no significant variation from a 50% transmission ratio by any individual male. In addition, Dunn & Gluecksohn-Waelsch (1951) have reported a transmission ratio of 48% in 745 offspring from T/t^3 males. The difference between our summed experimental value of 80% and the Mendelian ratio of 50% is highly significant ($P < 0{\cdot}0001$).

On average, t^{h45}/t^{w18} males transmitted their t^{w18} chromosome at less than Mendelian ratios (31%). The indirectly-calculated transmission ratios for 10

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males ranged from 0% to 64%. (A total of 124 tailless and 85 normal-tailed animals were born from the cross described in the Materials and Methods.) Clearly, a double dose of *Tcd-4*^{t} alone cannot compensate for the absence of *Tcd-2*^{t}.

(iii) *Fertility*

The fertility of Tt^{Or1}/t^{w18} males is significantly less than that of the two other experimental genotypes studied in this report. Four of the 11 animals tested appeared to be completely sterile, and the other seven produced an average of 1·4 offspring per female mate per month. Both Hammerberg (1981) and Bennett *et al.* (1983) have reported reduced fertility in Tt^{Or1}/t^{w18} males, and Lyon (1986) has obtained the same result with t^{h51}/t^{w18} males which appear to have a *Tcd* genotype identical to that of Tt^{Or1}/t^{w18}. In contrast, both Tt^{Or1}/t^3 and t^{h45}/t^{w18} males showed normal levels of fertility.

4. Discussion

(i) *Compensation relationships among* Tcd *loci*

TRD values for Tt^{Or1}/t^{w18} males have been reported in two previous publications. In one case, t^{w18} was transmitted at a ratio of 65% in a sample size of only 20 offspring (Hammerberg, 1981). In the second case, the observed transmission ratio was 87% in a sample size of 46 (Bennett *et al.* 1983). Although these values are not as high as reported here, in both cases, they were found to be greater than TRD values obtained with control $+/t^{w18}$ animals. [In the first case, the sample size was too small to provide significant information; in the second case, the calculated difference between experimental and control values as highly significant ($P < 0{\cdot}0001$).]

The accumulated data indicate that a double dose of both *Tcd-1*^{t} and *Tcd-4*^{t} alleles can compensate for the absence of *Tcd-2*^{t}, and within at least one genetic background (e.g. that present in the mice tested here), Tt^{Or1}/t^{w18} mice can express very high transmission ratios characteristic of heterozygotes for complete *t* haplotypes. Although a double dose of *Tcd-4*^{t} alone cannot compensate for the absence of *Tcd-2*^{t}, it can compensate for the absence of *Tcd-1*^{t} (Silver & Remis, 1987). These data lend support to the view of a *Tcd-2* locus that is approximately twice as strong as *Tcd-1* or *Tcd-4* alone in effecting the TRD phenotype.

One interpretation of the accumulated results is that at least three of the four *Tcd*^{t} loci characterized to date have a common mode of action in the expression of the TRD phenotype. (Analogous information on *Tcd-3* cannot be obtained with the currently available partial *t* haplotypes.) Alternatively, it is possible that each *Tcd* locus has a distinct mode of action, and that the underlying cause of TRD is different in animals with different *t*-genotypes. This latter interpretation is supported by comparative physiological studies on sperm from $+/t$ mice (Olds-Clarke, 1989; Brown *et al.* 1989). In either case, the results strongly suggest that the extent to which transmission ratios are distorted is dependent upon the absolute level of expression of the individual *Tcd* genes.

(ii) *The effect of the* t^{h45} *haplotype on TRD*

Unexpectedly, we observed that t^{h45}/t^{w18} males transmitted the t^{w18} haplotype at a very low ratio of 31%. In fact, Moser & Gluecksohn-Waelsch (1967) also obtained a value of 31% for the transmission of a t^{w18}-like haplotype from $+/t$ males. Therefore, one interpretation of the result reported here is that the t^{h45} haplotype is acting identically to the wild-type chromosome in this particular genotype. However, we have not observed a ratio this low with $+/t^{w18}$ mice in our colony. An alternative explanation is based on the results of molecular studies which imply that the t^{h45} chromosome carries a duplication of the *Tcd-4* locus, with one *Tcd-4*^{t} allele and one *Tcd-4*$^+$ allele (Herrmann *et al.* 1986). It is possible that this duplication actually reduces the transmission of t^{w18} relative to that observed with $+/t^{W18}$ *animals.*

(iii) *Evidence for the existence of a fifth* Tcd *locus*

Highly significant differences in the expression of both the TRD and fertility phenotypes were observed in a comparison of Tt^{Or1}/t^3 males with Tt^{Or1}/t^{w18} males, even though t^{w18} and t^3 are thought to carry an equivalent set of TRD loci (Fig. 1). Possible explanations for these differences include: (1) genetic background effects; (2) haplotype-specific differences in the *Tcr*^{t} region, of the type described by Lyon (1988); and (3) an additional distorter/sterility (*Tcd-5*) locus in the region of *t*-DNA present in t^{w18} and not in t^3 (Fig. 1).

Two independent lines of evidence argue in favour of this third hypothesis. First, all haplotypes of the t^{w18} class appear to act differently from haplotypes of the t^3 class within the context of simple $+/t$ heterozygotes. To date, five independent examples of t^{w18}-like chromosomes – t^4, t^9, t^{w18}, t^{w52} and t^{ks1} – have been characterized in various experiments (Moser & Gluecksohn-Waelsch, 1967; Bennett *et al.* 1976; Bennett & Dunn, 1960). Three of these chromosomes – t^4, t^{w18}, and t^{ks1} – have been characterized in molecular studies and appear indistinguishable from each other (Bucan *et al.* 1987). Four (all except t^{ks1}) have been characterized in breeding studies which have demonstrated a high degree of variability in transmission ratios expressed by $+/t$ mice from different colonies as well as individual males within a colony, with values as low as 31%, in a sample size of 1239 offspring, and as high as 63%, in a sample size of 580

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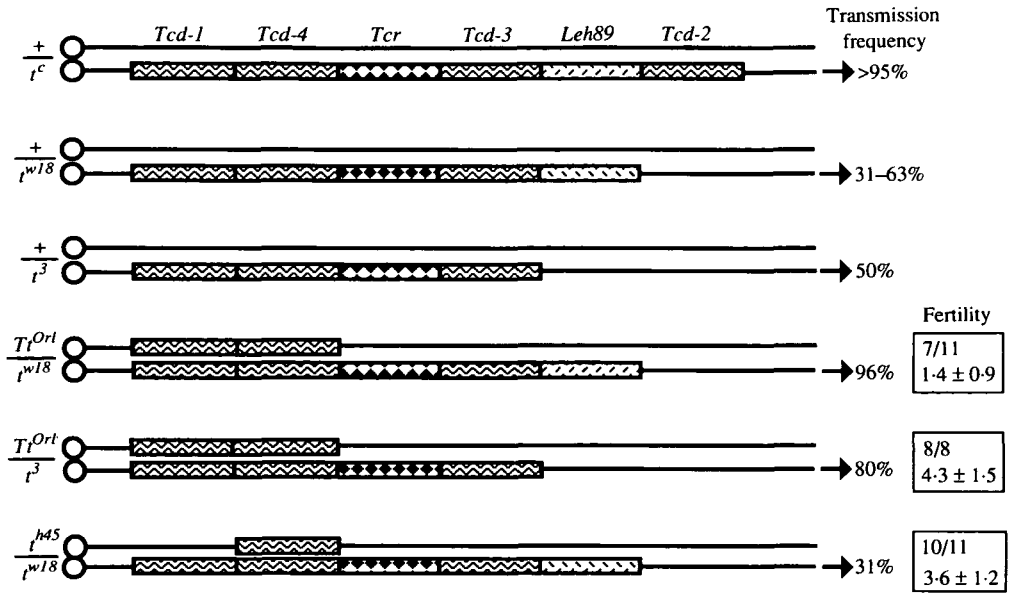

Fig. 1. Dosage effects of *Tcd*^{t} loci on transmission ratio distortion and fertility. The extent of *t*-DNA associated with each homologue in six diffferent genotypes is shown (Silver & Remis, 1987). The presence of a particular region of *t*-DNA is indicated by a patterned box – one pattern is used for *Tcd* loci, a second pattern is used for *Tcr*, and a third pattern is used for the *D17Leh89* region with the proposed *Tcd-5* locus. The actual size of genomic regions associated with each locus varies greatly, and is not indicated in this figure. The transmission frequency of the *Tcr*-bearing chromosome is indicated at the right of each genotype. In addition, for the three compound genotypes, a box is presented with fertility figures: the top fraction represents (number of fertile males)/(total males tested); the lower number represents the average offspring born per female mate per month for non-sterile animals, followed by the standard deviation in this number.

offspring (Moser & Gluecksohn-Waelsch, 1967; Bennett *et al.* 1976). In contrast, significant departures from Mendelian ratios have not been observed with heterozygotes for either of the two *t* haplotypes known to be members of the t^3 class (Dunn & Gluecksohn-Waelsch, 1951; Styrna & Klein, 1981; unpublished data).

Second, in studies from four different laboratories, reduced fertility was observed with mice that carry t^{w18} in a genotype with either Tt^{Or1} or t^{h51} (both of which carry *Tcd-1*^{t} and *Tcd-4*^{t}; Hammerberg, 1981; Bennett *et al.* 1983; Lyon, 1986; this report). In contrast, normal levels of fertility are observed with Tt^{Or1}/t^3 males (Fig. 1). Furthermore, in our colony, 91% (42/46) of males homozygous for the t^3 haplotype were found to be fertile, and these males generally sired frequent, large litters (unpublished data). Therefore, homozygosity for *Tcd-1*^{t}, *Tcd-3*^{t} and *Tcd-4*^{t} is not sufficient significantly to reduce fertility levels.

The simplest explanation for the accumulated observations is that (1) a fifth *t complex distorter* locus (*Tcd-5*) exists in the region defined by the *D17Leh89* marker (Fig. 1); (2) haplotypes of the t^{w18} class are more susceptible than t^3 to genetic background effects on TRD as a consequence of a *t*-allele at this locus; (3) the *Tcd-5*^{t} allele is absolutely required for the expression of a very high transmission ratio (over 90%), and (4) the *Tcd-5*^{t} allele acts within animals homozygous for *Tcd-1*^{t} and *Tcd-4*^{t} to reduce fertility. However, further experiments will be necessary to substantiate the existence of *Tcd-5*. If the data presented here are confirmed, six independent genes will have been defined that play a role in *t* haplotype effects on sperm function.

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References

Bennett, D., Dunn, L. C. & Artzt, K. (1976). Genetic changes in mutants at the T/*t*-locus in the mouse. *Genetics* **83**, 361–372.

Bennett, D., Alton, A. & Artzt, K. (1983). Genetic analysis of transmission ratio distortion by *t*-haplotypes in the mouse. *Genetic Research* **41**, 29–45.

Bennett, D. & Dunn, D. C. (1960). A lethal mutant (t^{w18}) in the house mouse showing partial duplications. *Journal of Experimental Zoology* **143**, 203–219.

Brown, J., Cebra-Thomas, J. A., Bleil, J. D., Wassarman, P. M. & Silver, L. M. (1989). Mouse *t* haplotypes induce a premature acrosome reaction which could play a role in transmission ratio distortion. *Development*, in press.

Bucan, M., Herrmann, B. G., Frischauf, A.-M., Bautch, V. L., Bode, V., Silver, L. M., Martin, G. R. & Lehrach, H. (1987). Deletion and duplication of DNA sequences is associated with the embryonic lethal phenotype of the t^9 complementation group of the mouse *t* complex. *Genes and Development* **1**, 376–385.

Demin, Y. S. & Safronova, L. D. (1979). Effect of the female genotype on non-Mendelian segregation in progeny of male carriers of *t*-haplotypes in the house mouse (*Mus musculus*). *Doklady Biol. Sci.* **243**, 582–584.

Dunn, L. C. & Gluecksohn-Waelsch, S. (1951). On the

origin and genetic behavior of a new mutation (t^3) at a mutable locus in the mouse. *Genetics* **36**, 4–12.

Gummere, G., Mccormick, P. & Bennett, D. (1986). The influence of genetic background and the homologous chromosomes 17 on *t* haplotype transmission ratio distortion in mice. *Genetics* **114**, 235–245.

Hammerberg, C. (1981). The influence of Tt^{Or1} upon male fertility in *t*-bearing mice. *Genetical Research* **37**, 71–77.

Herrmann, B., Bucan, M., Mains, P. E., Frischauf, A.-M., Silver, L. M. & Lehrach, H. (1986). Genetic analysis of the proximal portion of mouse *t* complex: evidence for a second inversion within *t* haplotypes. *Cell* **44**, 469–476.

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. & Zenthon, J. (1987). Differences in or near the responder region of complete and partial mouse *t*-haplotypes. *Genetical Research* **50**, 29–34.

Lyon, M. F. & Mason, I. (1977). Information on the nature of *t*-haplotypes from the interaction of mutant haplotypes in male fertility and segregation ratio. *Genetical Research* **29**, 255–266.

Moser, G. C. & Gluecksohn-Waelsch, S. (1967). Developmental genetics of a recessive allele at the complex *T*-locus in the mouse. *Developmental Biology* **16**, 564–576.

Olds-Clarke, P. (1989). Sperm from $t^{w32}/+$ mice: capacitation is normal, but hyperactivation is premature and non-hyperactivated sperm are slow. *Developmental Biology* **131**, 475–482.

Olds-Clarke, P. & Mccabe, S. (1982). Genetic background affects expression of *t* haloptype in mouse sperm. *Genetical Research* **40**, 249–254.

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.

Schimenti, J., Cebra-Thomas, J. A., Decker, C., Islam, S., Pilder, S. H. & Silver, L. M. (1988). A candidate gene family for the mouse *t complex responder* locus responsible for haploid effects on sperm function. *Cell* **55**, 71–78.

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

Silver, L. & Remis, D. (1987). Five of the nine genetically defined regions of mouse *t* haplotypes are involved in transmission ratio distortion. *Genetical Research* **49**, 51–56.

Styrna, J. & Klein, J. (1981). Evidence for two regions in the mouse *t* complex controlling transmission ratios. *Genetical Research* **38**, 315–325.

Willison, K. R., Dudley, K. & Potter, J. (1986). Molecular cloning and sequence analysis of a haploid expressed gene encoding *t* complex polypeptide 1. *Cell* **44**, 727–738.