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Deletion analysis of male sterility effects of *t*-haplotypes in the mouse

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Summary

We present data on the effects of three chromosome 17 deletions on transmission ratio distortion (TRD) and sterility of several *t*-haplotypes. All three deletions have similar effects on male TRD: that is, $T^{del}/t^{complete}$ genotypes all transmit their *t*-haplotype in very high proportion. However, each deletion has different effects on sterility of heterozygous males, with T^{Or}/t being fertile, T^{hp}/t less fertile, and T^{Orl}/t still less fertile. These data suggest that wild-type genes on chromosomes homologous to *t*-haplotypes can be important regulators of both TRD and fertility in males, and that the wild-type genes concerned with TRD and fertility are at least to some extent different. The data also provide a rough map of the positions of these genes.

1. Introduction

Disturbances of sperm function are amongst the outstanding phenotypes produced by mutant genes in the mouse *t*-complex. These disturbances range from vastly increased transmission of chromosomes carrying a mutant *t*-haplotype from $+/t$ males, to complete sterility in males carrying two different complementing mutant *t*-haplotypes and males homozygous for nonlethal *t*-haplotypes. The mechanistic or molecular bases for these phenomena are not understood, and virtually the only solid facts known about their etiology is that they depend on interactions between two or more mutant genes located in different regions of the *t*-complex. This information has come from observations on so-called partial *t*-haplotypes. Complete *t*-haplotypes are chromosomes that contain multiple inversions that effectively suppress genetic recombination between themselves and normal homologous chromosomes over a distance of about 15 centimorgans. However, occasional rare events of recombination in $t/+$ heterozygotes do occur, and generate chromosomes that contain varying lengths of '*t*-chromatin' either at their distal or proximal regions, or in some more complex cases, in the center. In general, the resulting partial *t*-haplotypes do not show the very high transmission through males that was typical of the parent mutant chromosome, nor are they always sterile as homozygotes. These data have led very logically to the idea that interactions of mutant genes produce these peculiar male phenotypes. The most complete and substantive models for explaining both transmission ratio distortion (TRD) and sterility have been provided by Mary Lyon (1984, 1986). She analyzed a very large number of combinations of partial *t*-haplotypes to reach the conclusion that at least three and possibly more mutant genes are involved in the observed phenomena, and that very likely the same genes are responsible for both TRD and sterility.

Evidence also exists that shows very clearly that the genetic background of a given *t*-haplotype can have very strong effects on the degree of transmission of that *t*-haplotype. This was first shown by Hammerberg (1981), and then by Bennett *et al.* (1983), where, for example, the transmission ratio of the t^0 haplotype was found to vary from 34% to 94% depending on genetic background. Further work (Gummere *et al.* 1986) showed that both general genetic background and also, more importantly, the homologous wild-type chromosome played a role in determining the ultimate transmission of any complete *t*-haplotype. In this paper, we have attempted to use two deletions and one deletion/duplication mutant to analyze the role of homologous chromosome genes in determining TRD and, more specifically, sterility. We review data on TRD, and analyze effects on fertility caused by these deletions. We conclude that deletions of wild-type genes on the chromosome homologous to the *t*-haplotypes can have profound effects on both TRD and sterility, but that different wild-type genes may be responsible, at least in some cases, for the two different types of effects.

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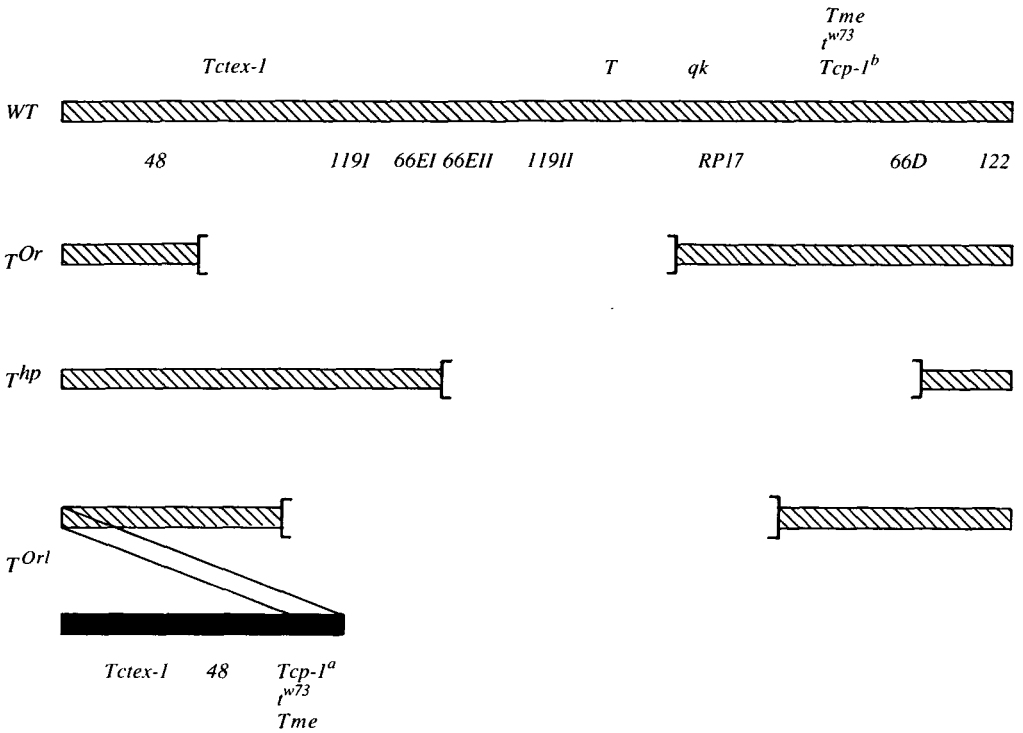

Fig. 1. Diagrammatic representation (not to absolute scale) of the T^{Or}, T^{hp}, and T^{Orl} deletions compared to a wild-type chromosome (WT). Positions of known genes (indicated by mutant symbols) and DNA markers are indicated respectively above and below the wild-type chromosome. DNA markers are abbreviated for clarity: '*48*' = *D17Leh48*, '*119I*' = *D17Leh119I*, '*66EI*' = *D17Leh66EI*, '*66EII*' = *D17Leh66EII*, '*119II*' = *D17Leh119II*, '*RP17*' = *D17RP17*, '*66D*' = *D17Leh66D*, and '*122*' = *D17Leh122*. T^{Orl} is a hybrid chromosome composed of both *t*-chromatin (black line) and wild-type, and carries wild-type and *t*-copies of several genes including *Tctex-1* and *Tcp-1*.

2. Materials and methods

The origins of the three deletions used in these experiments have been previously described: T^{Or} (Bennett *et al.* 1975), T^{hp} (Johnson, 1974), and T^{Orl} Moutier (1973). Their putative structure, as deduced from genetic and molecular studies is shown in Fig. 1 (Fox *et al.* 1985; Lader *et al.* 1989; C. A. Howard 1990, personal communication). As can be seen, T^{Orl} appears to represent both a deletion and a duplication, while the other two are most likely simple deletions of varying extent. These chromosomes were all maintained in our colony on the BTBRTF/Nev background.

Recessive *t*-haplotypes were maintained as $T\ tf/t+$ tailless stocks (*tf* is a recessive marker about 10 cM distal to *T*, and is used to monitor for rare recombinational events between the two chromosomes).

Animals heterozygous for deleted chromosomes and *t*-haplotypes were constructed by appropriate crosses. Since our aim was to test them for fertility, special care was taken to wean and mate only the most vigorous looking animals. Likewise, mates were chosen from exceptionally healthy random-bred (Carworth Farms) females. Test males were mated with harems of four to six females; their cages were monitored weekly for both pregnancy and health. Pregnant females were removed from the mating cages to give birth in isolation to prevent possible cannibalism by other animals in the cage, and animals looking in less than ideal condition were removed and replaced.

3. Results

Table 1 shows fertility data for males heterozygous for the three different deletions and a variety of *t*-haplotypes, together with previously published (Hammerberg, 1981; Bennett *et al.* 1983) transmission ratio data for comparison. The first thing to note is that all three deletions produce very high ratios of complete *t*-haplotypes, and that they are indistinguishable in this respect. The long proximal partial *t*-haplotype, t^{w18}, is variable in its response.

The fertility data are presented in three different ways. The first column shows the simplest estimate, that is, the number of fertile males versus the total number tested. Males carrying T^{Or} are all fertile, while a smaller proportion of those heterozygous for T^{hp} are also fertile, with those carrying t^{w18} very likely to be infertile. Males carrying T^{Orl} are as a group still less fertile, and none of the t^{w73} heterozygotes bred at all. These observations correspond very well with another commonly used measure of fertility, the 'fertility index', which represents the number of offspring born

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Table 1. *Relative fertility and transmission ratio of males heterozygous for* t-*haplotypes and three different proximal deletions*

Genotype	No. fertile/ no. tested	Male fertility index (offspring/MU*)	Average litter size (no. of litters)	Transmission ratio of *t* in % (*t*:*T*)	1977–78 'Standard *Tf*/*t* ratio (BTBRTF/Nev)
T^{Or}/t^{0}	4/4	6·1 (256/42)	8·5 (30)	95 % (243:13)	40 %
T^{Or}/t^{12}	2/2	9·9 (238/24)	10·8 (22)	99 % (236:2)	70 %
T^{Or}/t^{w1}	3/3	7·2 (280/39)	9·3 (30)	99 % (280:2)	75 %
T^{Or}/t^{w73}	5/5	10·1 (465/46)	9·9 (47)	97 % (450:15)	70 %
T^{Or}/t^{w5}	1/1	10·0 (140/14)	10.8 (13)	99 % (139:1)	70 %
T^{Or}/t^{w18}	5/5	8·2 (642/78)	11·1 (58)	74 % (447:165)	45 %
T^{hp}/t^{0}	3/3	4·3 (128/30)	9·8 (13)	85 % (109:19)	40 %
T^{hp}/t^{12}	1/2	2·6 (81/31)	9·0 (9)	95 % (77:4)	70 %
T^{hp}/t^{w1}	1/1	4·3 (126/29)	9·0 (14)	98 % (123:3)	75 %
T^{hp}/t^{w5}	5/6	4·8 (407/84)	9·3 (44)	97 % (395:12)	70 %
T^{hp}/t^{w18}	3/6	0·6 (33/51)	4·7 (7)	21 % (7:26)	45 %
T^{Orl}/t^{0}	2/4	1·4 (61/44)	7·6 (8)	98 % (60:1)	40 %
T^{Orl}/t^{6}†	3/3	5·6‡	—	98 % (352:7)	87 %
T^{Orl}/t^{12}	2/2	3·0 (75/25)	6·8 (11)	96 % (72:3)	70 %
T^{Orl}/t^{w1}	2/2	1·5 (66/44)	5·5 (12)	100 % (60:0)	75 %
T^{Orl}/t^{w5}	1/2	0·9 (27/30)	4·5 (6)	100 % (27:0)	70 %
T^{Orl}/t^{w73}	0/3	0·0 (0/30)	—	—	—
T^{Orl}/t^{w18}	1/2	1·7 (46/27)	5·8 (8)	87 % (40:6)	45 %

* Mating Unit = 1 month mated with 1 fertile female.
† Data from Hammerberg (1981).
‡ Offspring/female per week (359 total).

Table 2. *Relative fertility of* +/t *and* +/+ *brothers of males listed in Table 1*

Genotype	No. fertile/ no. tested	Fertility index (offspring/MU)	Average litter size (no. of litters)
+(ex T^{Or})/t^{x}	1/2	8·9 (124/14)	11·3 (11)
+(ex T^{hp})/t^{x}	6/6	7·9 (773/98)	9·5 (81)
+(ex T^{Orl})/t^{x}	ND	—	—
+(ex T^{hp})/+	5/5	8·1 (593/73)	9·8 (63)

per month of pairing with a fertile female. As can be seen in Table 1, the fertility index of T^{Or} males is considerably higher than that of T^{hp} males, which in turn is much higher than that of T^{Orl} animals. A comparison of Table 1 with Tables 2 and 3 will show that on this criterion the fertility of T^{Or}/t males is equivalent to that of their $+/t$ littermates and their T^{Or}-bearing sisters. However, also on this criterion, the fertility of T^{hp} and T^{Orl} mice is very severely impaired, with T^{Orl} being by far the most infertile. This is perhaps best seen in the summary in Table 4, where it appears that the fertility index of T^{hp} is about half that of normal, whereas that of T^{Orl} is approximately 20 % of controls. (A single interesting exception to the infertility of T^{Orl} heterozygotes is seen in the genotype T^{Orl}/t^{6}, in which males are perfectly fertile.) Furthermore, the ranges for fertility index for males within each group are essentially non-overlapping, as can also be seen in Table 4. Surprisingly, however, yet another measure of male fertility does not conform entirely to these observations. When the average litter size was calculated (see Tables 1 and 4) it was evident that T^{Or} and T^{hp} males are indistinguishable from one another and from normal; they have the same average litter size, and essentially the same range of offspring per litter. T^{Orl} males show a deficit in fertility on this criterion also, but is not nearly as extreme as that suggested by fertility index comparisons, since their litter size is only about two-thirds that of normal. The reason for these differences is not at all understood, but their existence suggests that there must be parameters related to *t*-complex sterility effects that we do not yet know anything about.

We also studied the relative fertility of males carrying t^{low} heterozygous with either T^{hp} or T^{Orl}. The fertility index of four T^{Orl} males was 4·8 with a total offspring sample of 552, while three T^{hp} males gave an index of 9·6 from 306 offspring, thus again indicating

Table 3. *Relative fertility of deletion-bearing sisters of males listed in Table 1*

Genotype	No. fertile/ no. tested	Fertility index (offspring/MU)	Average litter size (no. of litters)
T^{Or}/t^{x}	9/11	10·2 (215/21)	10·2 (21)
T^{hp}/t^{x}*	N.D.	—	—
T^{Orl}/t^{x}	10/11	5·5 (121/22)	9·3 (13)

* T^{hp} is deleted for the *Tme* gene, which prevents the chromosome from being transmitted through females.

Table 4. *Summary of fertility characteristics of males heterozygous for lethal* t*-haplotypes and three different deletions*

Genotype	No. fertile/ no. tested	Fertility index (offspring/mating unit) average (range)	Average litter size (range)
All $T^{Or}/t^{x\,lethal}$	20/20	8·3 (6·1–10·1)	10·1 (8·5–11·1)
All* $T^{hp}/t^{x\,lethal}$	10/12	4·3 (2·6–4·8)	9·3 (9·0–9·8)
All† $T^{Orl}/t^{x\,lethal}$	8/15	1·6 (0·9–3·0)	6·1 (4·5–7·6)
Controls (from Table 2)	12/13	8·9 (7·9–8·9)	9·7

* Excluding T^{hp}/t^{w18} (unexplainedly semisterile), and T^{hp}/t^{w73} (lethal).
† Excluding T^{Orl}/t^{6} (perfectly fertile).

a fertility defect induced by T^{Orl}. In line with the data presented above, however, the average litter sizes were 9·2 and 11·8 respectively. In addition T^{Orl} apparently raised the ratio of t^{low}, from its usual 15% to 31%.

4. Discussion

The data presented here show quite unequivocally that all three deletions tested result in very high transmission ratios of complete lethal *t*-haplotypes in heterozygous males. This suggests that the absence of a wild-type gene or genes common to the deleted region of all of them permits the *t*-haplotypes to be transmitted in very high proportion. If so, these genes must map to the region surrounding the locus of *T* and the D17Leh119II marker. Many observations show that the tranmission ratio of complete lethal *t*-haplotypes can vary dramatically depending on their particular homologous chromosome. This suggests further that the putatively deleted genes may occur in wild-type chromosomes in a variety of allelic forms capable of differently modifying the transmission ratio of *t*-haplotypes. Similar suppressors and enhancers have been described as operating in the *Segregation Distortion* system in *Drosophila* (Sandler & Golic, 1985).

With respect to male fertility, all three deletions were found to have different effects. This suggests primarily that either different wild-type genes or different combinations of such genes play a role in transmission distortion and in sterility. T^{Or}/t males are completely fertile, while T^{hp}/t animals show a comparatively much reduced fertility index. This suggests that a gene or genes deleted in T^{hp} in the region of non-overlap with T^{Or}, that is, in the region from *qk* to D17LehT66D, may be necessary for full fertility in T^{hp}/t males. It is also worth noting that T^{Or}/t mice are effectively hemizygous for the mutant form of *Tctex-1*, a gene which when homozygous *and* in combination with at least one other more distal *t*-complex mutant gene produces a sterile or semisterile phenotype (Lader *et al.* 1989). In mice that carry two mutant copies of *Tctex-1*, its messenger RNA levels are eight-fold that seen in normal mice, a factor which we suspect is connected with the infertility seen in mice that carry two copies of *Tctex-1* and at least one distal *t*-haplotype. However, mice which carry one wild-type copy of *Tctex-1* and one mutant copy produce approximately four-fold levels of the relevant messages, and are nevertheless fertile even when they contain the distal mutant genes mentioned above. Thus, the imputation of sterility due to increased message levels in *Tctex-1* mutant homozygotes requires the rather uncomfortable argument that an eight-fold excess results in sterility, while a four-fold excess has no effect. An equally attractive explanation could be that the presence of one wild-type copy of *Tctex-1* is sufficient for fertility. The finding that T^{Or}/t

males, whose *Tctex-1* genotype is *null/mutant*, are fertile obviates the argument that a wild-type gene is necessary for fertility.

The most severely impaired group of males tested were the genotype T^{Orl}/t; with the exception of t^6 heterozygotes all these males were semisterile. These observations are entirely explainable, since the T^{Orl} chromosome contains a portion of *t*-chromatin at its proximal end, and thus these heterozygotes (other than those containing t^6) are genotypically like the sterile $t^{prox}/t^{complete}$ heterozygotes carrying chromosomes other than t^6 and its derivatives that have by now been well-studied (Bennett & Dunn, 1969; Lyon, 1986). Heterozygotes of this deletion with t^6 are normally fertile; this is in line with initial observations of Lyon & Mason (1977) who showed that proximal partial haplotypes derived from t^6 did not cause sterility when heterozygous with other complete *t*-haplotypes. It was later shown that t^6 differs from all other known 'complete' *t*-haplotypes by having wild-type chromatin at its most proximal end, and is in fact a very long partial distal haplotype (Fox *et al.* 1985). In addition, Lader *et al.* (1989) showed that t^6 contains the wild-type allele of *Tctex-1*. Thus, since T^{Orl}/t^6 heterozygotes have only one mutant copy of *Tctex-1* and they are completely fertile, they provide additional evidence that two mutant copies of the overexpressed gene *Tctex-1* are necessary for sterility.

Lyon *et al.* (1989) have recently published a brief report of studies of another deletion, T^{22H}, located also in the proximal end of chromosome 17, and known to be deleted for the DNA marker D17Leh48. They obtained similar results with respect to transmission ratio, and showed that heterozygotes with t^{w32}, but not t^6, were sterile.

These studies of the effects of deletion mutants on transmission distortion and sterility in homologous *t*-haplotypes have shown quite clearly that genes in the wild-type chromosome in $t/+$ heterozygotes can have profound effects on these phenotypes. A comparison amongst these deletions offers the opportunity to define and localize the relevant genes for further study.

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