Narrowing the Critical Regions for Mouse *t* Complex Transmission Ratio Distortion Factors by Use of Deletions

Mary F. Lyon,* John C. Schimenti† and Edward P. Evans*

**MRC Mammalian Genetics Unit, Harwell, Didcot, Oxon OX11 0RD, United Kingdom and*
†The Jackson Laboratory, Bar Harbor, Maine 04609

Manuscript received November 19, 1999
Accepted for publication February 28, 2000

ABSTRACT

Previously a deletion in mouse chromosome 17, T^{22H}, was shown to behave like a *t* allele of the *t* complex distorter gene *Tcd1*, and this was attributed to deletion of this locus. Seven further deletions are studied here, with the aim of narrowing the critical region in which *Tcd1* must lie. One deletion, T^{30H}, together with three others, T^{31H}, T^{33H}, and T^{36H}, which extended more proximally, caused male sterility when heterozygous with a complete *t* haplotype and also enhanced transmission ratio of the partial *t* haplotype t^6, and this was attributed to deletion of the *Tcd1* locus. The deletions T^{29H}, T^{32H}, and T^{34H} that extended less proximally than T^{30H} permitted male fertility when opposite a complete *t* haplotype. These results enabled narrowing of the critical interval for *Tcd1* to between the markers *D17Mit164* and *D17Leh48*. In addition, T^{29H} and T^{32H} enhanced the transmission ratio of t^6, but significantly less so than T^{30H}. T^{34H} had no effect on transmission ratio. These results could be explained by a new distorter located between the breakpoints of T^{29H} and T^{34H} (between *T* and *D17Leh66E*). It is suggested that the original distorter *Tcd1* in fact consists of two loci: *Tcd1a*, lying between *D17Mit164* and *D17Leh48*, and *Tcd1b*, lying between *T* and *D17Leh66E*.

TWO of the peculiar features of the *t* complex on mouse chromosome 17 are the abnormally high transmission of the *t*-carrying chromosome to the offspring of male mice heterozygous for a complete *t* haplotype and the sterility of males homozygous for a nonlethal *t* haplotype or doubly heterozygous for two different lethal haplotypes. These phenomena have been attributed to the action of distorter genes on a responder gene in the complex. There are thought to be at least three distorters, *Tcd1*, *Tcd2*, and *Tcd3*, at different points in the complex, which act additively to produce a harmful effect on the wild-type allele of the responder (Lyon 1984, 1986, 1987). The *t* form of the responder, Tcr^t, is relatively resistant to this harmful action and hence, in heterozygotes, sperm carrying Tcr^t preferentially take part in fertilization, leading to high transmission of the *t* haplotype. When the distorters are homozygous their deleterious effects are so severe that the resistance of Tcr^t is overcome and the harmful effects on both Tcr^+ and Tcr^t mean that no sperm can fertilize and the male is sterile. Thus, the distorters are thought to be responsible for both transmission ratio distortion (TRD) and male sterility. The *t* complex occupies the proximal third of chromosome 17 (Lyon *et al.* 1986, 1988) and is maintained intact by four nonoverlapping inversions that prevent crossing over (Hammer *et al.* 1989) (Figure 1). The responder locus lies near the center of the complex and the distorter loci are spread through the region with *Tcd1* proximally, *Tcd2* in the distal inversion, and *Tcd3* just distal to the responder. The responder gene has recently been identified (Herrmann *et al.* 1999), but none of the distorters has so far been cloned, although various candidate genes have been suggested.

Previously, we studied a deletion, T^{22H}, which covers the locus of brachyury, *T*, and also the *Tcd1* locus (Lyon 1992). It behaved like the *t* allele of *Tcd1*, causing male sterility in heterozygotes with a complete *t* haplotype, t^{w32}, and enhanced TRD when heterozygous with a partial *t* haplotype carrying the responder. In the present article the effects on male sterility and TRD of seven further deletions covering the *T* locus have been studied. The aim was to narrow the critical region for the *Tcd1* locus and thus aid in identification of the underlying gene. The deletions arose in radiation mutagenesis experiments or spontaneously. Some of these deletions have already been used in positioning the head-tilt (*het*) locus that lies proximal to *T* (Bergstrom *et al.* 1998). The results complement those of Planchart *et al.* (2000). The region in which *Tcd1* must lie is considerably reduced, and the possibility that it consists of the two separable loci *Tcd1a* and *Tcd1b* is put forward.

Corresponding author: Mary F. Lyon, MRC Mammalian Genetics Unit, Harwell, Didcot, Oxon OX11 0RD, United Kingdom.
E-mail: m.lyon@har.mrc.ac.uk

MATERIALS AND METHODS

Origins: All except one of the deletions studied were induced by radiation treatment of males (Table 1), in some

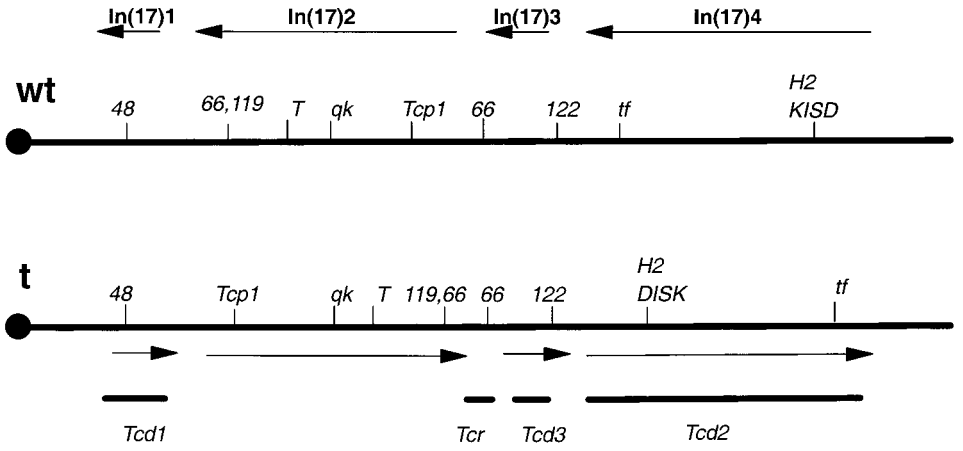

Figure 1.—Diagram of structure of the *t* complex. The four inversions are shown with the wild-type (wt) orientation above and *t* complex (t) below, and the centromere to the left. The relative positions of some known genes and DNA markers are indicated, as are the approximate positions of the *t* complex distorters *Tcd1* to *Tcd3* and responder *Tcr*. The numbered symbols are abbreviations for DNA markers, full symbols, *D17Leh48*, etc.

cases combined with chemical treatment. In the case of T^{29H} the treated male was from the BALB/c strain, and the single spontaneous deletion T^{34H} was found in a mixed mutant stock. In all other cases the treated male was of the F_1 hybrid stock C3H/HeH × 101/H (3H1).

Breeding: Where possible the deletions were maintained by crossing to tufted animals from the inbred strain TFH/H, tufted *tf*, being a recessive mutant causing hair loss located a few centimorgans from the *T* locus, and the TFH/H strain being genetically *T tf*/+*tf* × +*tf*/+*tf*. However, in some cases these crosses bred poorly, and the deletions concerned were then maintained by crosses to 3H1.

The *t* haplotypes used were t^6, t^{h2}, t^{h51}, and t^{w32}, carrying the ratio and sterility factors shown in Table 2. They were maintained in separate closed stocks on nonstandard backgrounds.

Fertility tests: Males to be tested for fertility were placed with two +*tf*/+*tf* females from the TFH/H strain and left for 1 mo. If neither female became pregnant the male was judged to be sterile and the females were killed. If young were produced the mice were left to breed for at least 3 mo and the number of young per female per month was calculated as a measure of the male's fertility.

Transmission ratio: For tests of transmission ratio T^n/t males were crossed to +*tf*/+*tf* females and numbers of short-tailed (T^n/+) and normal-tailed (*t*/+) young were counted at birth. The control *t*/+ male sibs or half-sibs were crossed to *Ttf*/+*tf* females and numbers of short-tailed (*T*/+) and tailless (*T*/*t*) as well as normal-tailed (*t*/+ or +/+) young were counted.

Molecular mapping: For mapping the positions of the breakpoints of the deletions, some mice of genotype T^n/t^{w32} were studied to make use of polymorphisms associated with the *t* complex. In other cases the deletions were crossed with *Mus castaneus*. Restriction fragment length polymorphisms and simple sequence variants were used as in Bergstrom *et al.* (1998).

RESULTS

Characterization of the deletions: *(a) Extent of deletions:* All the deletions were originally detected through their short-tailed phenotype in heterozygotes. Allelism with *T* was shown by a tailless phenotype in offspring of crosses with the *t* haplotype t^{h2} and by linkage with tufted, *tf*. Evidence for the presence of deletions was provided by crosses to two nearby loci, quaking (*qk*), and head-tilt (*het*) (see Figure 3) and by cytogenetic tests. All except T^{30H} gave the quaking phenotype when crossed with *qk*/*qk*, indicating deletion of the *qk* locus, distal to *T* (Table 3). Three deletions, T^{31H}, T^{33H}, and T^{36H}, gave the head-tilt phenotype when crossed with *het*/*het* (Table 3), indicating deletion of the *het* locus, which lies proximal to *T* (Bergstrom *et al.* 1998). To test for deletion of the *T*-maternal effect (*Tme*) locus, deletions were tested for transmission from the female. Only T^{34H}/+ was not viably transmitted from females. Very few T^{34H}/+ offspring of T^{34H}/+ females survived birth, and in dissections of pregnant T^{34H}/+ females the short-

TABLE 1

Origins of chromosome 17 deletions

Brief symbol	Mutagenic treatment	Strain of origin	Full symbol	References
T^{29H}	5 Gy X rays	BALB/c	*Del(17)*T^{29H}*84H*	1
T^{30H}	3AB + 6Gy X rays	3H1	*Del(17)*T^{30H}*85H*	2
T^{31H}	TEM + 6Gy X rays	3H1	*Del(17)*T^{31H}*86H*	3
T^{32H}	TEM + 6Gy X rays	3H1	*Del(17)*T^{32H}*87H*	3
T^{33H}	TEM + 3AB + 6Gy X rays	3H1	*Del(17)*T^{33H}*88H*	2
T^{34H}	Spontaneous	Mutant stock	*Del(17)*T^{34H}*89H*	4
T^{36H}	3AB + 6Gy X rays	3H1	*Del(17)*T^{36H}*90H*	2

3AB, 3 aminobenzamide; TEM, triethylenemelamine; 3H1, (C3H/HeHx101/H)F1. References: 1, Cattanach *et al.* (1995); 2, B. M. Cattanach (personal communication); 3, Cattanach *et al.* (1989); 4, C. Beechey (personal communication).

TABLE 2

Transmission ratio and sterility factors carried by *t*-haplotypes used

Haplotype	Ratio factors			
t^{6}	+	R	D3	D2
t^{h2}	+	R	+	+
t^{h51}	D1	+	+	+
t^{w32}	D1	R	D3	D2

Symbols for the ratio and sterility factors are abbreviated. D1, D2, D3: *t*-alleles of distorters *Tcd1*^{t}, *Tcd2*^{t}, and *Tcd3*^{t}; R: *t*-allele of responder *Tcr*^{t}; +, normal allele of corresponding factor. Male sterility is thought to arise from homozygosity of D1, D2, and D3.

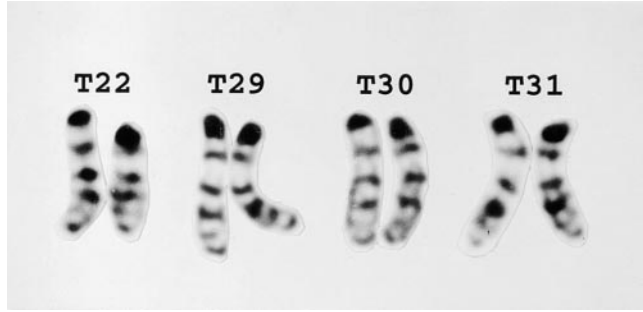

Figure 2.—Cytogenetic evidence of deletions. Some representative examples are shown, in each case with the deleted chromosome on the right. A shortening of the 17A3 band can be seen (uppermost light band).

tailed offspring showed the edema typical of fetuses with the *Tme* phenotype (data not shown).

The cytogenetic tests provided further evidence of deletions. All the deletions showed visible shortening of the band 17A3, this being most marked in T^{33H} (Figure 2). With T^{30H}, the cytogenetics provided the only evidence at this stage of a deletion.

Since all these mutations involve cytogenetically visible deletions, they should be given the appropriate genetic symbols. These are *Del(17)*T^{29H}*84H*, etc. (Table 1). For convenience these symbols will be here abbreviated as T^{29H}, etc.

(b) Viability and fertility: It was necessary to know the viability and fertility of heterozygotes for the deletions with wild type before assessing effects on them of *t* complex factors. Tests revealed deleterious effects of the three deletions, T^{31H}, T^{33H}, and T^{36H}, which extended to cover *het* on both viability and fertility. Owing to poor breeding in crosses to the inbred strain TFH/H, it was necessary to maintain these deletions by crosses to the F_1 hybrid 3H1. All three then showed a statistically significant shortage of short-tailed offspring (Table 4). This was particularly marked in the case of T^{36H}, where only 27.1% of young born were short tailed. Although female $T^{36H}/+$ bred very poorly, the proportion of short-tailed young among their offspring was very similar to that from males, and thus T^{36H} did not show a *Tme* phenotype. Other possible causes of the deficiency of affected offspring include incomplete penetrance, reduced viability, or some form of distorted transmission. The evidence pointed to reduced viability. If incomplete penetrance were the case, then in crosses with *qk* or *het*, some normal-tailed quaking or head-tilt young might be found, and this was not so. In addition the shortage of affected young was still evident when T^{36H} was crossed to t^{h2}/t^{h2} or t^{h51}/t^{h51} when, owing to the obvious tailless phenotype, incomplete penetrance would not be expected. Further, among offspring of $T^{36H}+/t^{h2}$tf or $T^{36H}+/t^{h51}$tf, incomplete penetrance might lead to an excess of apparent crossovers of the type normal-tailed nontufted and again this was not so. Dissections of pregnant females failed to reveal the cause of the excess death of $T^{36}/+$ young. The deletions T^{31H} and T^{33H} also showed mild shortages of short-tailed offspring. Again there was no evidence of incomplete penetrance, and the shortages were ascribed to reduced viability. T^{29H} also showed mildly reduced viability.

The three deletions that covered the *het* locus also showed impaired fertility. With the four deletions that did not cover *het*, all $T^{n}/+$ males crossed to non-*T* fe-

TABLE 3

Results of crosses of $T^{n}+/++$ with $+qk/+qk$ or $+het/+het$ to tests for deletion of quaking (*qk*) and head-tilt (*het*)

Deletion	Phenotypes of offspring							
	$T^{n}+$	T^{n} *qk*	+ +	+ *qk*	$T^{n}+$	T^{n} *het*	+ +	+ *het*
T^{29H}	—	8	14	—	8	—	22	—
T^{30H}	18	—	22	—	11	—	13	—
T^{32H}	—	3	—	—	8	—	18	—
T^{34H}	6[a]	6	19	—	6	—	12	—
T^{31H}	—	15	12	—	—	3	2	—
T^{33H}	—	14	3	—	—	2	31	—
T^{36H}	—	3	17	—	—	7	16	—

T^{n} represents T^{29H}, T^{30H}, etc.
[a] In this case the cross was $T^{34H}/+ \times Tqk/+qk$.

TABLE 4

Offspring derived from crosses of $T^n/+$ males to normal females

Deletion	T^n+	T^ntf	$++$	$+tf$	% T^n	χ^2
		A. Crosses with $+tf/+tf$				
T^{29H}	71	2	—	97	42.9	3.39
T^{30H}	95	5	3	99	49.5	0.020
T^{32H}	134	3	4	144	48.1	0.42
T^{33H}	18	1	—	19	50.0	0.00
T^{34H}	96	1	3	100	48.5	0.18
		T^n	+	% T^n		χ^2
		B. Crosses with 3H1				
T^{29H}		210	298	41.3		15.24[a]
T^{31H}		213	295	41.9		13.20[a]
T^{33H}		184	257	41.7		12.08[a]
T^{36H}		201	543	27.1		157.21[a]

[a] χ^2 for 1:1 segregation, significant, $P < 0.001$.

males proved fertile (Table 5). For T^{31H}, T^{33H}, and T^{36H}, however, some $T^n/+$ males were sterile. The underlying basis of the impaired viability and fertility of these deletions is not known. It could be an effect of *t* complex factors but the deletions probably extend beyond the proximal limit of the *t* complex and hence other factors may be involved. In any case these impairments need to be taken into account in studies of the fertility and TRD of males also carrying *t* haplotypes.

Effect of deletions on *t*-complex male sterility: The fertility of males carrying the partial *t* haplotypes t^{h2} and t^{h51} opposite a deletion was compared with that of similar males carrying the complete *t* haplotype t^{w32} (Table 5). The t^{h2} and t^{h51} haplotypes were used as controls. Male sterility due to the *t* complex typically occurs when at least one *t* complex distorter gene is homozygous. t^{h2} carries no distorters and thus, even if the deletion behaved like the distorter *Tcd1*^{t}, males of genotype T^n/t^{h2} would be expected to be fertile. t^{h51} carries *Tcd1*^{t} but no other distorters. In earlier work, homozygosity for *Tcd1*^{t} alone did not result in male sterility, and thus T^n/t^{h51} would be expected to be fertile. By contrast, males of genotype t^{h51}/t^{w32} are typically sterile due to homozygosity of *Tcd1*^{t} combined with heterozygosity of *Tcd2*^{t} and *Tcd3*^{t}. Similarly, T^{22H}/t^{w32} males are sterile, presumably due to the deletion of the *Tcd1* locus in T^{22H}. Sterility of T^n/t^{w32} males carrying the new deletions would thus indicate deletion of the *Tcd1* locus.

As expected, all males of the control genotypes T^n/t^{h2} were fertile, consistent with the absence of any distorter genes in t^{h2}. With t^{h51}, however, the results were somewhat unexpected. All males carrying the deletions not covering *het* were fertile. However, several males carrying the deletions T^{31H} and T^{33H} were sterile, and the remainder sired a low number of young per female per month. Although all of four $T^{36H}+/t^{h51}tf$ males were fertile, again the number of young sired was abnormally low. Here the sterility of some heterozygotes with wild type has to be taken into account. Other evidence given below suggests that T^{31H}, T^{33H}, and T^{36H} all delete *Tcd1*. Thus, the heterozygotes with t^{h51} would in effect be homozygous for *Tcd1*^{t}, and a relatively mild impairment due to this may be acting additively with factors causing sterility of some heterozygotes with wild type.

When tested against t^{w32}, the deletions T^{29H}, T^{32H}, and T^{34H} gave fully fertile males (except for one $T^{29H}+/t^{w32}+$ male). By contrast, all of six T^{30H}/t^{w32} males were completely sterile. This is similar to the results previously obtained with T^{22H} and is consistent with the T^{30H} deletion covering the *Tcd1* locus. The T^{30H}/t^{w32} males would thus have no normal copies of *Tcd1* and would also carry *t* alleles of the remaining distorters.

Among the deletions covering the *het* locus, all of three T^{31H}/t^{w32} males were sterile. Out of eight T^{33H}/t^{w32} males, seven were fertile, but all were poorly fertile, with an average of only 1.1 young per female per month, well below the normal value. With T^{36H}, the heterozygotes with t^{w32} were poorly viable and no males survived to adulthood. If the poor fertility of T^{33H}/t^{w32} males is considered as a variant of the sterility due to *t* complex

TABLE 5

Results of tests of fertility of males of various genotypes

	$T^n/+$		T^n/t^{h2}		T^n/t^{h51}			T^n/t^{w32}		
Males	No. tested	No. fertile	No. tested	No. fertile	No. tested	No. fertile	Y/MU	No. tested	No. fertile	Y/MU
$T^{29H}+$	10	10	5	5	7	7	4.8	3	2	6.8
$T^{30H}+$	7	7	6	6	6	6	3.4	6	0	—
$T^{31H}+$	14	9	3	3	6	1	0.9	3	0	—
$T^{32H}+$	11	11	5	5	4	4	2.0	5	5	6.3
$T^{33H}+$	15	9	3	3	8	3	1.9	8	7	1.1
$T^{34H}+$	6	6	6	6	3	3	4.3	4	4	5.7
$T^{36H}+$	21	19	4	4	4	4	1.6	—	—	—

Y/MU, young per female per month, for fertile males only.

TABLE 6

Transmission ratios of males heterozygous for T^n and for t^{h2}, t^{h51}, or t^{w32}

	T^n/t^{h2}			T^n/t^{h51}			T^n/t^{w32}		
Deletion	T^n	t^{h2}	$\%t^{h2}$	T^n	t^{h51}	$\%t^{h51}$	T^n	t^{w32}	$\%t^{w32}$
T^{29H}	188	70	27.1	61	77	55.8	—	81	100.0
T^{30H}	178	92	34.1	55	58	51.3	—	—	—
T^{31H}	65	33	33.7	4	5	55.5	—	—	
T^{32H}	106	57	35.0	47	25	34.7	2	194	99.0
T^{33H}	99	43	30.3	15	26	63.4	1	51	98.1
T^{34H}	96	42	30.4	53	64	54.7	21	157	88.2
T^{36H}	72	63	46.7	24	51	68.0	—	—	

factors, these results with T^{31H} and T^{33H} are consistent with deletion of *Tcd1*. As these deletions cover the *het* locus they must extend more proximally than T^{30H}, and hence their deletion of *Tcd1* is as expected.

Effects of deletions on transmission ratio distortion: Data on TRD were obtained with the *t* haplotypes t^6, t^{h2}, t^{h51}, and t^{w32}, but the main test was with t^6. t^{h51} served as a negative control, since it does not carry the responder Tcr^t. For TRD to occur the responder must be heterozygous. Therefore, no TRD is expected among the offspring of T^n/t^{h51} males. The results were as expected except for $T^{32H}/t^{h51}tf$, which gave a shortage of t^{h51} offspring (Table 6). The explanation of this is not clear, but since the responder Tcr^t is not present in these males there is no reason to suppose that this discrepancy is due to *t* complex factors. With T^{36H}/t^{h51} there was a shortage of $T^{36H}/+$ offspring but this is as expected from the poor viability of $T^{36H}/+$. The t^{w32} haplotype was a positive control. Since it is a complete haplotype, T^n/t^{w32} males would be expected to give strong TRD in favor of t^{w32} with all T^n that permitted fertility of such heterozygotes, but the TRD might be so high that any enhancement by the deletions could not be detected. This was indeed found. An unexpected result was that of heterogeneity among the deletions, with T^{34H} giving a lower TRD of t^{w32} than the other three. Of the four deletions involved, T^{33H} is thought to delete the *Tcd1* locus (see above) whereas the other three apparently do not. Thus, these three, T^{29H}, T^{32H}, and T^{34H}, might have been expected to give similar TRD of t^{w32}. However, the heterogeneity χ^2 among this group is 27.82, with 2 d.f. and $P < 0.0001$.

The t^{h2} and t^6 haplotypes were the test haplotypes. t^{h2} carries the responder but no distorters, and in heterozygotes with wild type it is transmitted in a low ratio. The deletion T^{22H}, in which the distorter *Tcd1* is deleted, raised the ratio of t^{h2} in T^{22H}/t^{h2} heterozygotes (Lyon 1992). In the present work, all deletions gave very similar ratios, with the exception of T^{36H}. The proportion of t^{h2} offspring of T^{36H}/t^{h2} males was 46.7%. However, the poor viability of $T^{36H}/+$ young mentioned above must be taken into account, since it alone could give an apparent increase in the ratio of t^{h2} young born. If the viability of $T^{36H}/+$ relative to $+/+$ or $+/t$ is v, and the true transmission ratio of the *t* haplotype concerned is *r*, then among the offspring of T^{36H}/t males the ratio of *t* carrying to total offspring scored at birth is given by

$$\frac{t}{N} = \frac{r}{r + v(1 - r)},$$

where *t* and *N* indicate the numbers of *t* and total offspring, respectively.

From the data on viability of T^{36H} given earlier, 27.1% of $T^{36H}/+$ offspring were found when, with full viability, 50% would be expected. Therefore, the viability is given by

$$\frac{T}{N} = \frac{v}{1 + v}$$

and

$$v = 0.37.$$

Therefore, if *t* and *N* are 63 and 135, then

$$\frac{63}{135} = \frac{r}{r + 0.37(1 - r)}$$

$$r = 0.25 \text{ or } 25\%.$$

Thus, when allowance is made for the reduced viability of $T^{36H}/+$ young the transmission ratio of T^{36H}/t^{h2} males is very similar to that of the other deletions (Table 6). The data in Table 4 showed that three other deletions, T^{29H}, T^{31H}, and T^{33H}, also had slightly reduced viability. Correction of the transmission ratios of these for viability produced only small changes: 20.7, 26.8, and 23.8%, respectively. This means that the data provide no evidence that any of this group of deletions alters the TRD of t^{h2}. In view of the clear enhancement of TRD found earlier with T^{22H} the reason for the present negative finding is not clear.

The main test of ratio distortion was made with t^6, since its mildly raised ratio in T/t^6 or $+/t^6$ males provides scope for detection of changes. Because of the known dependence of TRD on genetic background, test males

TABLE 7

Transmission ratios of t^6 from T^n/t^6 males and control $+/t^6$ males

Deletion	No. males	T^n/t^6			No. males	$+/t^6$			χ^2	P
		T^n	t^6	$\%t^6$		$T+$	Tt^6	$\%Tt^6$		
A. Non-outcross males										
T^{29H}	5	34	191	84.9	5	78	95	54.9	42.0	<0.0001
T^{30H}	6	8	183	95.8	4	65	56	46.3	98.6	<0.0001
T^{32H}	1	10	49	83.1	1	14	22	61.1	4.60	0.032
T^{34H}	5	89	96	51.9	5	89	84	48.6	0.276	0.5994
B. Outcross males										
T^{32H}	3	1	67	98.5	2	22	46	67.6	—	<0.0001[a]
T^{31H}	5	2	69	97.2	4	4	142	97.3	—	1.00[a]
T^{33H}	8	4	407	99.0	3	8	66	89.2	—	<0.0001[a]
T^{36H}	3	5	142	96.6	5	28	95	77.2	—	<0.0001[a]

Statistical tests are for differences between T^n/t^6 males and their control $+/t^6$ sibs.
[a] Probability determined by Fisher's exact test.

of genotype T^n/t^6 were compared with their sibs or half-sibs of genotype $+/t^6$. Where possible, the test males and sibs were bred without outcrossing the stocks. However, in some cases the poor breeding behavior of the deletions necessitated the use of animals derived from outcrosses, and the data from outcross and nonoutcross animals are shown separately.

(a) Deletions not covering the het locus: For these deletions (T^{29H}, T^{30H}, T^{32H}, and T^{34H}) almost all the data were from non-outcross animals. The four sets of data from the control $t^6+/+tf$ sibs showed good agreement (heterogeneity $\chi^2 = 1.28$, d.f. = 3, $P = 0.734$), with transmission ratios ranging from 46.3 to 61.1% (Table 7). This indicates that the general genetic backgrounds of the stocks were reasonably similar. By contrast, among the sets of data from T^n+/t^6+ males there was strong heterogeneity ($\chi^2 = 107.42$, d.f. = 3, $P < 0.0001$), T^{30H} giving a markedly high value of 95.8% and T^{34H} a lower value of 51.9%. When T^{30H} was removed from the test, heterogeneity still remained ($\chi^2 = 58.7$, d.f. = 2, $P < 0.0001$), but when T^{30H} and T^{34H} were both removed, the two remaining deletions T^{29H} and T^{32H} showed good agreement ($\chi^2 = 0.122$, d.f. = 1, $P = 0.728$). Of the four deletions only T^{34H} showed no significant difference from its control. T^{29H} and T^{30H} both showed a highly significant difference. T^{32H}, with fewer data, gave a marginally significant χ^2, but some data were also available from outcross animals, and again a significantly raised transmission of t^6 from the test males was seen (Fisher's exact test $P < 0.0001$). Correction of the T^{29H} data for reduced viability of T^{29H} produced only a small change in ratio from 84.9 to 79.7% and did not affect the conclusion.

Thus, it appeared that T^{29H}, T^{30H}, and T^{32H} all led to an increased TRD of t^6, with T^{30H} having a significantly stronger effect than the other two.

(b) Deletions covering the het locus: For the deletions T^{31H}, T^{33H}, and T^{36H} only data from outcross animals were available. The outcrosses led to an increase in the TRD of the control $t^6/+$ males, making detection of any enhancing effect of the deletions more difficult (Table 7). There was statistically significant heterogeneity among the transmission ratios of the three sets of control $t^6/+$ males ($\chi^2 = 26.05$, d.f. = 2, $P < 0.0001$), but none among the data from the test T^n/t^6 males ($\chi^2 = 4.25$, d.f. = 2, $P = 0.120$). All three groups of test males gave a very high transmission ratio, ranging from 96.6 to 99.0%. With T^{33H} and T^{36H} there was a significant difference between test and control males, but with T^{31H} there was no such difference. The TRD of control $t^6/+$ males for T^{31H} was very high, at 97.3%. For this reason any enhancing effect of T^{31H} would be very difficult to detect, and hence the interpretation of the negative result with T^{31H} is not clear.

As in the tests with t^{h2}, it is necessary to correct for the reduced viability of $T^{36H}/+$. As before the viability, v, of $T^{36H}/+$ is taken as 0.37. Using the same formula as before

$$\frac{t}{N} = \frac{r}{r + v(1 - r)}$$

$$r = 0.914 \text{ or } 91.4\%.$$

Thus, after allowing for the reduced viability, the TRD of T^{36H}/t^6 is still considerably higher than that of the $t^6/+$ sibs. Correction for the mildly reduced viability of T^{31H} and T^{33H} resulted in only small changes, to 96.1 and 98.7%, and did not affect the conclusions.

Molecular mapping of deletion breakpoints: Some idea of the extent of the deletions had already been obtained by crosses to *qk* and *het* and by cytogenetic tests. To obtain more precise estimates of the positions of the deletion breakpoints mice heterozygous for the deletions and a *t* haplotype or with *M. castaneus* were

TABLE 8

Results of testing deletions for various genetic markers

Deletion	*245*	*19*	*164*	*Tul*	*Aus9*	*196*	*T48*	*119*	*66E*	*T*	*48*	*57*	*156*	*qk*	*114*	*Tme*
T^{22H}	+	—	—	—						—			+			
T^{29H}								+	—	—				—		+
T^{30H}		+	+	—	—		—			—		+		+	+	+
T^{32H}							+	—	—	—				—		+
T^{33H}							—			—				—		+
T^{34H}								+	+	—				—		—

−, marker deleted; +, marker present. Symbols of the markers are abbreviated: *T48*, *66E*, and *119* by removal of *D17Leh*; *Tul* and *Aus9* by removal of *D17*; and others (except *T*, *qk*, and *Tme*) by removal of *D17Mit*.

analyzed for informative polymorphisms. Some of the results have already been published (Bergstrom *et al.* 1998). Further work showed that in T^{30H} the markers *D17Mit196* and *D17Tu1* were deleted (Table 8 and Figure 3), whereas in T^{29H} and T^{32H} they were not. In addition, in T^{32H} the locus of *T48* was not deleted. Because T^{30H} shows the phenotype of a deletion of *Tcd1*, whereas T^{29H} and T^{32H} do not, these mapping results imply that the *Tcd1* locus is situated between the markers *D17Leh48* and *D17Mit164*.

The proximal breakpoint of T^{34H} lay more distally, since this deletion did not extend to the marker nearest to the brachyury locus, *D17Leh66E*. T^{29H} and T^{30H} both deleted the *D17Leh66E* locus. Phenotypically T^{34H} differed from T^{29H} and T^{32H} in that the latter two enhanced the TRD of t^6 and T^{34H} did not. It is possible that this difference is attributable to deletion in T^{29H} and T^{32H} of a distorter locus lying between *T* and *D17Leh66E*.

Among the distal breakpoints, that of T^{30H} was the most proximal and that of T^{34H} the most distal (Table 3 and Figure 3). Thus, there is no evidence that the phenotypic differences among the deletions could be attributed to the positions of their distal breakpoints or to an unspecific effect of their general length.

DISCUSSION

The deletions studied here extend knowledge of the genetic basis of TRD due to the *t* complex and of the identity and location of distorter and male-sterility factors in the proximal region of mouse chromosome 17. Previously (Lyon 1992) we had shown that the deletion T^{22H} behaved as though it deleted the locus of *Tcd1* and that the effect of this deletion was like that of a *t* allele at this locus. The aim of the present work was to find which of the new group of deletions behaved like T^{22H}, and thus to narrow the critical region for *Tcd1*.

Concerning male sterility the effect of the T^{30H} dele-

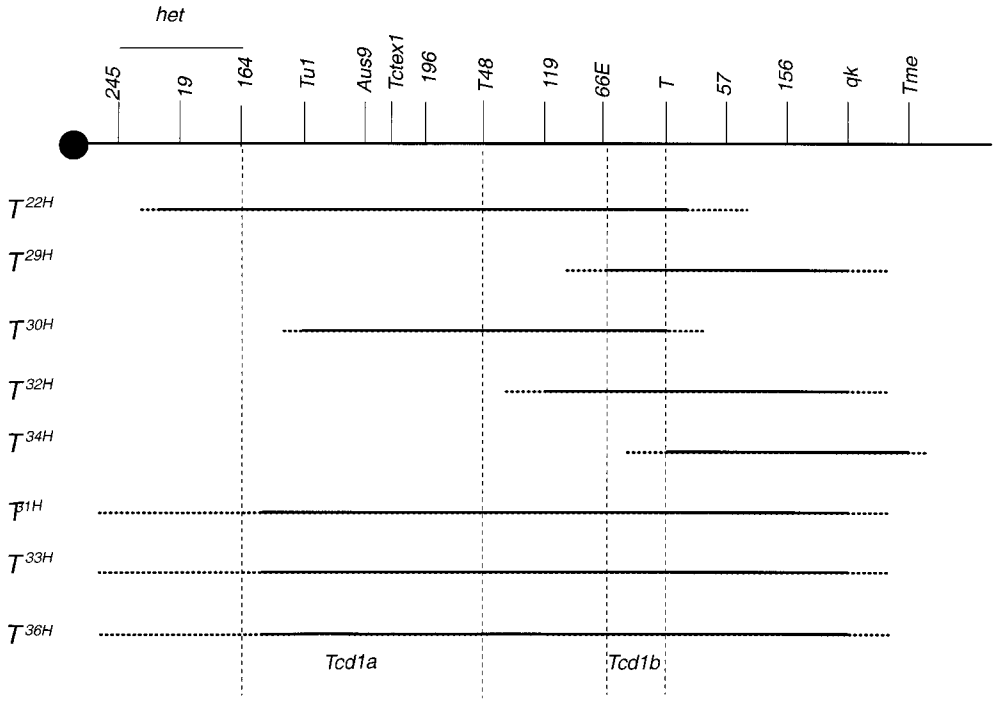

Figure 3.—Diagrammatic representation of the extent of the various deletions. Solid lines indicate regions deleted, with dotted extensions showing uncertainty. Symbols of some markers are abbreviated: *T48*, *66E*, and *119* by removal of *D17Leh*; *Tu1* and *Aus9* by removal of *D17*; and others (except *het*, *T*, *qk*, and *Tme*) by removal of *D17Mit*. Distances are not to scale. The critical regions for location of *Tcd1a* and *Tcd1b* are shown.

tion was very clear. Heterozygotes of T^{30H} with wild type or with the two partial haplotypes t^{h2} and t^{h51} showed normal fertility, but heterozygotes with the complete *t* haplotype t^{w32} were totally sterile. In this, T^{30H} resembles T^{22H}. The deletions T^{31H} and T^{33H} also impaired male fertility but the effects were less clear. In the case of T^{33H}, T^{33H}/t^{w32} males showed much impaired fertility, rather than total sterility. With T^{31H}, the sterility of T^{31H}/t^{w32} males was total, but some $T^{31H}/+$ males were also sterile, complicating the interpretation. With T^{36H}, no T^{36H}/t^{w32} males could be tested. T^{31H}, T^{33H}, and T^{36H} were all shown to delete the locus of *het*, which lies proximal to *T* (Bergstrom *et al.* 1998), and hence to extend more proximally than the rest (Figure 3). Thus, these results are consistent with T^{30H}, T^{31H}, T^{33H}, and T^{36H} all deleting the locus of a *t* complex sterility factor, presumably *Tcd1*, and, as with T^{22H}, absence of this locus having an effect like a *t* allele.

The current interpretation of the effects of the *t* complex on TRD and male sterility is that the sterility is due to homozygosity of distorter genes that, when heterozygous, result in TRD. The deletions that affected male sterility would therefore be expected also to affect TRD. As expected from its effect on male sterility, T^{30H} showed a strong enhancement of TRD of t^6. T^{33H} and T^{36H} also showed ratio distorting effects, as expected. With T^{31H} no significant effect on TRD was detected. Nevertheless, the transmission of t^6 from the test males was very high and the failure to detect any enhancement of ratio could be due to the unusually high TRD of the control males in this test. Thus, the results concerning TRD are in accord with the effects on male sterility.

These results narrow the critical region in which the *Tcd1* locus lies. Previously, the results with T^{22H} had placed this locus between a point proximal to the *D17Tu1* locus and the locus of brachyury (Howard *et al.* 1990). Among the present group of deletions, the breakpoint in T^{30H} is distal to *D17Mit164* and in T^{32H} is proximal to *D17Leh119*, placing the *Tcd1* locus between these two markers (Figure 3). The data of Planchart *et al.* (2000) on embryonic stem cell-derived deletions narrow the interval further to between *D17Aus9* and *Tctex1*. Thus, the interval in which this locus can lie is now quite small, and this knowledge will be valuable in cloning the gene. The critical region now appears to exclude the locus of *Tctex1*, previously a possible candidate gene (Lader *et al.* 1989; Howard *et al.* 1990; O'Neill and Artzt 1995).

An unexpected result was an effect of the deletions T^{29H} and T^{32H} in enhancing TRD, but not inducing male sterility. In each case the effect was highly significant, but also significantly lower than that seen with T^{30H}. This raises the possibility of deletion of another distorter locus in T^{29H} and T^{32H} but not in T^{34H}. T^{34H} showed no effect on TRD although it extends distally farther than all the other deletions studied here in that it deletes the *Tme* locus. Thus, this second effect on TRD is not likely to be due to a locus distal to *T* or to an unspecific effect of the length of the deletions. However, proximally T^{34H} extends for the least distance. It fails to delete the locus of *D17Leh66E*, whereas T^{29H} and T^{32H} both delete this locus. Thus, it is possible that there is a distorter locus situated between *T* and *D17Leh66E* (not deleted in T^{34H}; Figure 3). Then T^{34H} would have no distorter loci deleted, T^{29H} and T^{32H} would have one, and the remaining deletions, T^{30H}, T^{31H}, T^{33H}, T^{36H}, and T^{22H}, studied previously, would have two. It is of interest that T^{34H} also gave a lower transmission of the complete haplotype t^{w32} than did T^{29H} and T^{32H}.

If there is indeed a distorter locus between *T* and *D17Leh66E*, then the hairpin-tail deletion, T^{hp}, should also show an enhancing effect on TRD since, like T^{29H}, it deletes *D17Leh66E* (Bergstrom *et al.* 1998). It is not possible to test males of genotype T^{hp}/t^6, since T^{hp} carries the t^6 recessive lethal factor. However, in earlier work T^{hp} enhanced the transmission of t^{h2} from T^{hp}/t^{h2} males (Lyon 1992).

Deletion of this apparent new distorter did not result in sterility of males also carrying the complete haplotype t^{w32}. This raises the question whether the current interpretation that homozygosity of distorters leads to sterility is correct, or whether there are distorters that do not affect fertility. It seems not possible to say. The effect of the various distorters is cumulative, both on TRD and on sterility. It is known that homozygosity for the partial haplotype t^{h51}, previously thought to carry *Tcd1* and now also appearing to carry the new distorter, does not lead to sterility. Perhaps the sterility seen in T^{30H}/t^{w32} (and also in t^{h51}/t^{w32}) requires homozygosity or deletion of both *Tcd1* and the new distorter (as well as the presence of $Tcd2^t$ and $Tcd3^t$). This distorter may impair sperm function but not sufficiently to cause sterility when it is the only homozygous locus. On the other hand, it is also possible that there are two types of distorters, some affecting fertility and others not. Among this group of deletions there is no evidence for a sterility locus not affecting ratio distortion.

The suggestion of this new distorter implies that there are more distorters than the three (*Tcd1*, *Tcd2*, and *Tcd3*) originally postulated. There have already been such suggestions from other work. Silver and Remis (1987) postulated the existence of *Tcd4*, located distal to *T*, in the region of *Tcp1*, and Silver (1989) suggested a *Tcd5* locus lying distal to *Tcd3*. From their positions neither of these could be identical with the new distorter described here. Gummere *et al.* (1986) studied the effect of genetic background on TRD and found influences of the general genetic background and of the homologous chromosome 17. An effect of chromosome 17 could be due to polymorphisms among wild-type alleles of distorter genes, but Gummere *et al.*'s work did not identify the location of the genes concerned. A problem with the present work is that effects of genetic background cannot be excluded. T^{29H}, T^{30H}, T^{32H}, and T^{34H} were all

maintained by crossing to the inbred strain TFH/H and had at least two to three backcrosses to this strain when the crosses to t^6 were made. Hence, their general genetic backgrounds were reasonably similar, and this is confirmed by the homogeneity of the four sets of control males from these crosses. However, these deletions arose by mutation in different strains. T^{29H} arose in BALB/c and T^{34H} in a mutant stock, whereas T^{30H} and T^{32H} arose in the F_1 strain 3H1 (C3H/HeH × 101/H). Thus, it is possible that there could be polymorphism in undeleted regions of chromosome 17 that are responsible for the effects. The identification of the new distorter is therefore tentative. However, support for its existence is provided by the work of Bennett and Artzt (1990) who found that the three deletions T^{Or}, T^{hp}, and T^{Orl}, all with proximal breakpoints between the markers *D17Leh48* and *D17Leh66E*, also enhanced TRD but did not cause male sterility when opposite a complete *t* haplotype. This means that all five deletions (these three and T^{29H} and T^{32H}) that delete the region between *T* and *D17Leh66E* have a similar effect on TRD.

Another difficulty in the identification of distorters is the crossover suppression due to the four inversions in the *t* complex. The three original distorters were identified by study of partial *t* haplotypes arising by crossing over. However, this crossing over leaves chromosomal segments still intact. The *Tcd1* locus was identified by absence of its *t* allele from the t^6 haplotype. This haplotype has wild-type chromatin in the proximal region of chromosome 17 extending distally as far as *D17Aus3II* (Howard *et al.* 1990). Since the exact position of the new distorter is not known it is not clear whether t^6 has a *t* or wild-type allele of this distorter. The results appear to suggest that it has a wild-type allele. If so, the effect of the present results is to indicate that *Tcd1* can be split into two loci, one deleted in T^{29H} and T^{32H} and both deleted in T^{22H} and T^{30H}. Provisionally, the more proximal one may be designated *Tcd1a* and the other *Tcd1b*.

The recent identification of the responder as a mutant form of a sperm motility kinase, Smok (Herrmann *et al.* 1999), makes it possible to speculate on the function of the distorters. Herrmann *et al.* (1999) suggest that they may constitute components of a signal cascade regulating Smok functions, which in turn may affect sperm flagellar function. This knowledge, combined with the narrowing of the critical region for *Tcd1* provided by the present work, should bring closer the cloning of the distorters and hence a full understanding of TRD by the *t* complex.

We are grateful to Bruce Cattanach for the gift of T^{29H}–T^{33H} and T^{36H} and to Colin Beechey for the gift of T^{34H}. We thank Mark Harrison for animal care. M.F.L. was partly supported by EU contract no. CHRX-CT93-0181. The animal stocks at Harwell were maintained under the guidance issued by the Medical Research Council in "Responsibility in the Use of Animals for Medical Research" (July 1993) and Home Office Project Licence no. 30/00875.

LITERATURE CITED

Bennett, D., and K. Artzt, 1990 Deletion analysis of male sterility effects of *t* haplotypes in the mouse. Genet. Res. **56:** 179–183.

Bergstrom, R. A., Y. You, L. C. Erway, M. F. Lyon and J. C. Schimenti, 1998 Deletion mapping of the head tilt (*het*) gene in mice: a vestibular mutation causing specific absence of otoliths. Genetics **150:** 815–822.

Cattanach, B. M., J. Peters and C. Rasberry, 1989 Induction of specific locus mutations in mouse spermatogonial stem cells by combined chemical X-ray treatments. Mutat. Res. **212:** 91–101.

Cattanach, B. M., G. Patrick, D. Papworth, D. T. Goodhead, T. Hacker *et al.*, 1995 Investigation of lung tumour induction in BALB/cJ mice following paternal X-irradiation. Int. J. Radiat. Biol. **67:** 607–615.

Gummere, G. R., P. J. McCormick and D. Bennett, 1986 The influence of genetic background and the homologous chromosome 17 on *t* haplotype transmission ratio distortion in mice. Genetics **114:** 235–245.

Hammer, M. F., J. Schimenti and L. M. Silver, 1989 Evolution of mouse chromosome 17 and the origins of inversions associated with *t* haplotypes. Proc. Natl. Acad. Sci. USA **86:** 3261–3265.

Herrmann, B. G., B. Koschorz, K. Wertz, K. J. McLaughlin and A. Kispert, 1999 A protein kinase encoded by the *t complex responder* gene causes non-mendelian inheritance. Nature **402:** 141–146.

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

Lader, E., H.-S. Ha, M. O'Neill, K. Artzt and D. Bennett, 1989 *tctex-1*: a candidate gene family for a mouse *t* complex sterility locus. Cell **58:** 969–979.

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

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

Lyon, M. F., 1987 Distorter genes of the mouse *t* complex impair male fertility when heterozygous. Genet. Res. **49:** 57–60.

Lyon, M. F., 1992 Deletion of mouse *t* complex distorter-1 produces an effect like that of the *t* form of the distorter. Genet. Res. **59:** 27–33.

Lyon, M. F., J. Zenthon, E. P. Evans, M. D. Burtenshaw, K. Dudley *et al.*, 1986 Location of the *t* complex on mouse chromosome 17 by in situ hybridization with *Tcp-1*. Immunogenetics **24:** 125–127.

Lyon, M. F., J. Zenthon, E. P. Evans, M. D. Burtenshaw and K. R. Willison, 1988 Extent of the mouse *t* complex and its inversions shown by in situ hybridization. Immunogenetics **27:** 375–382.

O'Neill, M. J., and K. Artzt, 1995 Identification of a germ-cell-specific transcriptional repressor in the promoter of *Tctex-1*. Development **121:** 561–568.

Planchart, A., Y. You and J. C. Schimenti, 2000 Physical mapping of male fertility and meiotic drive quantitative trait loci in the mouse *t* complex using chromosome deficiencies. Genetics **155:** 803–812.

Silver, L. M., 1989 Gene dosage effects on transmission ratio distortion and fertility in mice that carry *t* haplotypes. Genet. Res. **54:** 221–225.

Silver, L. M., and D. Remis, 1987 Five of the nine genetically defined regions of mouse *t* haplotypes are involved in transmission ratio distortion. Genet. Res. **49:** 51–56.

Communicating editor: N. A. Jenkins