Genet. Res., Camb. (1983), **41**, *pp.* 29–45
With 1 text-figure
Printed in Great Britain

Genetic analysis of transmission ratio distortion by *t*-haplotypes in the mouse

BY DOROTHEA BENNETT, ALTHEA K. ALTON AND KAREN ARTZT

Sloan-Kettering Institute for Cancer Research, New York, New York, USA

(*Received 22 February 1982 and in revised form 9 June 1982*)

SUMMARY

Lethal and semilethal *t*-haplotypes in the mouse are polymorphic in wild populations, where they are presumably maintained by transmission distortion from males, which transmit the aberrant chromosome to over 95% of progeny. We report here measurements of transmission ratio that show an unexpectedly strong effect of background genotype, specifically genes on the homologous chromosome, on the degree of transmission distortion of *t*-haplotypes. We also provide additional evidence that within *t*-haplotypes two separable elements co-operate to control transmission distortion, and that the system is thus closely analogous to segregation distortion in Drosophila, as has been previously proposed by Gluecksohn-Waelsch & Erickson (1970), Demin, Safronova & Lapkina (1978), and Styrna & Klein (1982).

INTRODUCTION

Among all the poorly understood effects of mouse *t*-haplotypes, perhaps the most studied but least well explained is the phenomenon called transmission ratio distortion. In its essence this is a very clear-cut effect that leads to the enhanced transmission of naturally occurring *t*-haplotypes from heterozygous males. It is well known that wild mouse populations are highly polymorphic for recessive lethal or semilethal *t*-haplotypes (Bennett, 1978*a*), and it appears that these polymorphisms are maintained by the selective advantage of non-Mendelian transmission through males (Lewontin & Dunn, 1960). The rate of transmission of these homozygously deleterious haplotypes by aboriginal males is astoundingly high; Dunn (1957) summarized the ratios of more than 50 wild-caught males carrying t^{w1}, t^{w5} and $t^{w\,semilethal}$ haplotypes as 96% in favour of the *t*-bearing chromosomes, with some individual ratios approaching 100%. Subsequent analysis of these haplotypes seemed to show that high transmission was an intrinsic property of the *t*-haplotype, and one that was not subject to modification by genetic background in general, or genes on the homologous 17th chromosome in particular. For example, when the haplotypes mentioned above were initially outcrossed to Dunn's laboratory stock carrying the dominant mutation *T* to produce tailless T/t animals, they maintained their high ratios, which continued to average about 96% (Dunn, 1957). However,

it was becoming evident that such extremely high transmission ratios were not necessarily typical of lethal *t*-haplotypes, since three others being described at about the same time showed only moderately high transmission distortion, with t^0 (Silagi, 1962) and t^{12} (Smith, 1956) averaging about 75 % and t^6 about 60–65 % (Lyon & Bechtol, 1977). In retrospect, since these three haplotypes had been maintained in laboratory stocks for some time when their transmission frequencies were measured, it seems that their relatively low ratios could have been taken as evidence that genetic background was in partial control of the ultimate transmission ratios of *t*-haplotypes. We present evidence here that this is in fact the case, and specifically that genes on the homologous chromosome play an important role in regulating the actual transmission of *t*-bearing chromosome in heterozygotes.

We also attempt to address another question, namely the number and interactions of the multiple factors in naturally occurring *t*-haplotypes that produce transmission ratio distortion. It has been known for a long time that multiple factors are responsible for this effect (Lyon & Meredith, 1964; Lyon & Mason, 1977). This information came from studies of exceptional recombinants from naturally occurring *t*-haplotypes where lethality or semilethality, and transmission ratio distortion are constant features. Although these wild *t*-chromosomes typically reduce recombination between themselves and homologous wild-type chromosomes to about $\frac{1}{100}$ the normal rate, studies of the occasional recombinants that do occur have been informative. Briefly, two reciprocal classes of chromosomes are produced by recombination occurring between the markers *T* and *tf*. The ones that obtain the centromeric end of the original *t*-haplotypes continue to interact with *T* to produce taillessness, and thus are defined as retaining the tail interaction factor t^T, but they have lost the lethal mutation (t^1) of the parent haplotype. The t^T-bearing recombinant chromosomes are uniformly transmitted in either normal or low (15–30 %) proportions. The reciprocal recombinant class retains the lethal mutation in the distal region of the *t*-haplotype, but no longer interacts with *T* and thus has lost t^T. The transmission ratios of these chromosomes are variable; only three have been reported on, and the ratios of these independently derived chromosomes range from about 50 % to 80 % (Lyon & Mason, 1977; E. Hsu, personal communication). Thus the high transmission ratio distortion of complete *t*-haplotypes clearly results from the interactions of at least two separable genetic factors that map near t^T and t^l respectively, and in fact more complex models involving more than three separable factors have been proposed (Lyon & Mason, 1977; Silver, 1981; Hammerberg, 1981) to account for the properties of complete *t*-haplotypes and both classes of recombinants derived from them. Styrna & Klein (1982) have recently presented data which support the strong analogy to the SD locus in Drosophila, suggested originally by Gluecksohn-Waelsch & Erickson (1970) and Demin *et al.* (1978).

We will show here new data derived from deletion mapping and from studies of recombinants obtained from animals doubly heterozygous for two different *t*-haplotypes. These data, considered together with our new information on genetic background effects, suggest that the existing models for the arrangement

and interaction of genetic factors in *t*-haplotypes responsible for transmission ratio distortion are unnecessarily complex. We will present here a simpler interpretation.

MATERIALS AND METHODS

Mice of all T/t-complex genotypes are maintained in our closed colony. The standard maintenance mode for recessive *t*-haplotypes is balanced lethal matings between *T tf*/t^x mice, where *tf* (tufted) is a marker about 10 units distal to *T* that serves to monitor recombination. This breeding system does not provide any

Table 1. *Adventures of transmission ratio on various genetic backgrounds*

Cross that produced test male	Test male genotype	No. of males tested	Total informative progeny	Progeny carrying *t*	Proportion of *t*
Ttf/t^0 × *Ttf*/t^0	*Ttf*/t^0	12	1278	434	0·34
Ttf/t^0 × CF_1	CF_1/t^0F_1	8	282	264	0·94
Ttf/t^0 × CF_1	*Ttf*/CF_1 F_1	4	813	396 (T)	0·49 (T)
CF_1/t^0F_1 × *Ttf*/CF_1 F_1	*Ttf*/t^0	3	88	38	0·43
See text	*T* + (ex CF_1)/t^0	1	155	66	0·43
Ttf/t^0 × BALB/c	BALB/t^0F_1	4	244	129	0·53
Ttf/t^0 × C3H (1977)	C3H/t^0F_1	2	218	187	0·86
Ttf/t^0 × BTBRTF/Nev. + *tf*	+ *tf*/t^0F_1	2	101	88	0·87
Wild population	+ /t^{w106}	1	40	40	1·0
Ttf/t^0 × + /t^{w106}	*Ttf*/t^{w106}	3	339	230	0·68

estimate of transmission ratio, which in general we have measured only sporadically, and usually as a by-product of outcrosses for other purposes. C3H.t congenics were constructed on C3H/DiSn by H. O. McDevitt at Stanford University from our foundation stocks bearing various *t*-haplotypes.

Transmission ratio testing was routinely done by crossing tailless males (T/t^x) to a harem of 4-6 CF_1 random-bred mice (Charles River), whose litters were classified at birth for Brachy (T/+) and normal-tailed (t/+) offspring. In some cases +/t^x males were tested by crosses to T/+ females, in which case the informative progeny classes are Brachy (T/+) and tailless (T/t).

Chromosomes derived from recombination between two different *t*-haplotypes were constructed and tested as outlined in Artzt, McCormick & Bennett (1982).

RESULTS

Effect of normal genetic backgrounds on the male transmission ratio of lethal haplotypes

1. *Detailed analysis of* t^0 (see Table 1).

Our first substantial data on the influence of genetic background on transmission ratio were obtained accidentally in 1977, when we outcrossed 12 *T tf*/t^0 males to

CF_1 females to produce $+/t^0$ heterozygotes for use in other experiments. In a sample size of almost 1300 progeny, the transmission ratio of t^0 was only 34%. This represented a substantial decrease in the expected ratio of 75–80% summarized in Bennett (1975). The question whether this decline was due to genetic background or a change intrinsic to the t^0-haplotype was clearly answered by progeny tests of the F_1 CF_1/t^0 males. Eight such males, with a total sample of 282 offspring, transmitted their t^0 chromosome to 264 progeny, or 94%. These data thus demonstrated a major effect of genetic background on the transmission of a 'high ratio' t-haplotype, and simultaneously raised a number of questions.

First of all we wondered whether the CF_1 chromosome 17 might simply segregate poorly with respect to any chromosome 17 in our stocks. This question was answered negatively by tests of Brachy CF_1/T tf segregants in which 4 males produced equal numbers of Brachy and normal-tailed offspring, thus showing that CF_1 did not induce distorted transmission in the absence of t^0.

We needed also to know whether the heightened transmission of t^0 from CF_1/t^0 F_1 males was due to some generalized genetic background effect, or whether it was caused specifically by a gene or genes on chromosome 17. We therefore crossed CF_1/t^0 males to CF_1/T *tf* F_1 mates to reconstitute mice of the *T tf*/t^0 tailless genotype, which would however contain the same proportion of CF_1-derived genes as the original CF_1/t^0 F_1 males. Three males of this kind were tested, and the transmission ratio of t^0 was 43%. The reversion to the low transmission ratio of our standard *T tf*/t^0 stock suggests strongly that the genetic content of the homologous chromosome 17 plays a significant role in the ultimate rate of transmission of t^0, and specifically that in a formal sense our *T tf* chromosome had accumulated minus modifiers, while the CF_1 chromosome 17 had plus modifiers.

We then tested the effect of other strain backgrounds on the transmission of t^0, and showed that BALB/c resulted in normal ratios, whereas both C3H and BTBRTF/Nev produced high ratios. The high ratio of the t^0 haplotype opposite the + *tf* chromosome from the BTBRTF/Nev stock was especially significant, since the same strain originally provided the *T tf* chromosome for the existing *T tf*/t^0 stock.

In a further test of the supposition that the *T tf* chromosome in the t^0 stock had accumulated one or more minus modifiers we crossed standard *T tf*/t^0 females to a wild-caught male heterozygous for the t^{w106} allele, whose transmission of t^{w106} was 100% in a progeny sample of 40. The resulting three *T tf*/t^{w106} male had an average transmission ratio of 68%, which thus again showed that the *T tf* chromosome had a pronounced effect in lowering transmission.

2. *Other naturally occurring lethal t-haplotypes*

The pronounced effect of genetic background on the transmission ratio of the t^0 haplotype prompted us to determine the ratios of other lethal t-haplotypes in our foundation stocks and compare them to those obtained on the CF_1 background. As Table 2 shows, only one of the complete lethal haplotypes tested in our standard tailless stocks was being transmitted at the very high ratios originally characteristic of them, and all of the others responded to the CF_1 background with pronounced

Table 2. *Transmission ratio of independently derived* t*-haplotypes on specific genetic backgrounds*

Alleles	Standard (Tf/t^n) 1977–8 ratio			Ratio on CF1			Ratio in C3H congenic stock		
	No. of males tested	Total informative offspring	Proportion *t*	No. of males tested	Total informative offspring	Proportion *t*	No. of males tested	Total informative offspring	Proportion *t*
(t^{w1} * / $t^{w12}tf$)	3	126	0·70	2	136	0·84	7	102	0·97
	21	432	0·93	—	—	—	3	138	0·99
t^{w5}	5	1032	0·68	2	190	0·98	5	41	1·0
(t^{w32} / t^{12})	12	2529	0·71	2	99	0·95	2	24	1·0
	—	—	—	—	—	—	15	452	0·97
t^{w73}	4	633	0·67	2	146	0·95	—	—	—
(t^{0} / t^{6})	12	1278	0·34	8	282	0·94	9	201	0·78
	—	—	—	—	—	—	6	237	0·89
t^{w18}	4	937	0·39	2	147	0·48	4	102	0·33

* Bracketed pairs are independently isolated haplotypes of the same complementation group.

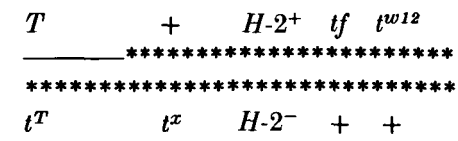
increases in ratio. Furthermore, the C3H congenic stocks all had very high ratios as well. Thus a substantial influence of genetic background on the transmission ratios of wild *t*-haplotypes seems to be a general phenomenon.

Effect of chromosome 17 deletions on transmission ratio

In a further attempt at localizing the gene(s) responsible for the *trans* modification of *t*-haplotype transmission ratios, we constructed heterozygotes of a representative panel of *t*-haplotypes with each of three apparent deletions around the region of the locus of *T*. T^{Orl} and T^{hp} are both thought to be deletions over loci of *T* and *qk* (a marker about 3 cM distal to *T*) because they have a short-tailed heterozygous phenotype and elicit pseudodominance of *qk* (Erickson, Lewis & Slusser, 1978; Bennett *et al.* 1975), although no clear cytological evidence of deletion has been seen (Lyon *et al.* 1979; B. Babiarz and M. Moses, personal communication). T^{OR} is a radiation-induced variant which appears to be a shorter deletion than the first two (Bennett *et al.* 1975). Table 3 shows that each of these three putative deletions in *trans* configuration has striking effects on the transmission ratio of lethal *t*-haplotypes, with the partial exception again of t^{w18}. Results comparable to these have already been published by Hammerberg (1981). However, the transmission of two viable, recombinant-derived haplotypes with low or normal ratios from + /*t* males was not affected. T^{Orl}, but not T^{hp}, also raised the ratio of t^{low} significantly. Two of the putative deletions, T^{hp} and T^{Orl}, also impaired fertility in T^x/t males, with T^{Orl} having by far the more severe effect. Interestingly, T^{Orl} also impaired fertility in t^{low} heterozygotes whose transmission ratio it also affected, whereas T^{hp} did not. Fertility data will be presented separately, but it should be noted in the present context that while all three deletions had essentially indistinguishable effects on transmission ratio, fertility was reduced only by the two most extensive ones.

Transmission ratios of chromosomes derived by recombination between two different lethal t*-haplotypes*

We have recently shown that recombination occurs freely along the length of two different *t*-haplotypes in female double heterozygotes, and thus generates chromosomes that carry two or more lethal factors, as well as chromosomes that carry none, and in which other *t*-related factors may also be presumed to differ (Artzt *et al.* 1982). The chromosomes of the original heterozygotes can be diagrammed as follows:



where the asterisks indicate the region of the *t*-haplotypes where recombination is suppressed when they are *trans* to normal chromosomes, and between which we

Table 3. *Male transmission ratio of* t-*haplotypes* trans *to three different deletions*

	T^{OR}/t^n			T^{hp}/t^n			T^{Orl}/t^n		
t^n	No. of males tested	Total offspring	Proportion t	No. of males tested	Total offspring	Proportion t	No. of males tested	Total offspring	Proportion t
t^0	4	256	0·95	3	128	0·85	2	61	0·98
t^{12}	2	238	0·99	1	81	0·95	2	75	0·96
t^{w1}	3	280	0·99	1	126	0·98	2	66	1·0
t^{w5}	1	140	0·99	5	407	0·97	1	27	1·0
t^{w18}	5	642	0·74	3	33	0·21	1	46	0·87
t^{w73}	5	465	0·97	0 (1)*	—	—	0†	—	—
t^{w82}†	2	137	0·53	ND	—	—	ND	—	—
t^{46}§	ND	—	—	1	104	0·27	ND	—	—
t^{low}‖	—	—	—	3	306	0·18	4	552	0·31

* T^{hp}/t^{w73} is lethal (Babiarz *et al.*, in the Press).

† 3/3 males tested were sterile.

‡ 'Standard' Tt/t^{w82} male ratio was 0·52 (165 t:155 T).

§ 'Standard' Tt/t^{46} male ratio was 0·18 (19 t:84 T).

‖ t^{low} is a factor isolated by recombination from t^{h17}; its only effects are a slight reduction of recombination in $t^{low}/+$ heterozygotes, and the reduction of its own transmission through males to 15% (Dunn & Bennett, 1971).

assume crossing over occurs in the doubly heterozygous configuration shown. Thus recombination generates both partial and complete *t*-haplotypes like the parent chromosomes, but with different combinations of genes. Table 4 and Fig. 1A summarize data on 75 independently derived chromosomes of both types, and show that recombinant chromosomes of the t^T *tf* type generally have very high rates of transmission through males, regardless of the haplotype or the position of the crossover(s) from which they were derived. The simplest interpretation of these

Table 4. *Summary of transmission ratios of derived* T/t-*haplotypes**

	Chromosome type				
Haplotype of origin	$T++$	$T\ t^x+$	t^T+t^{w12}	$t^T\ t^x\ t^{w12}$	$t^T\ t^x+$
t^{w32}					
Transmission† (no. of chromosomes)	0·54 (9)	0·44 (6)	0·96 (13)	0·99 (7)	—
Range‡	0·31–0·95	0·25–0·69	0·68–1·0	0·94–1·0	—
t^{w5}					
Transmission (no. of chromosomes)	0·54 (4)	0·51 (4)	0·96 (9)	0·96 (6)	0·98 (1)
Range	—	0·38–0·70	0·78–1·0	0·89–0·97	—
t^{w18}					
Transmission (no. of chromosomes)	—	0·22 (6)	0·94 (9)	—	—
Range	—	0·04–0·44	0·84–1·0	—	—

* Homologous chromosome was derived from *BTBRTF/Nev* see Table 1).

† Transmission ratios were normalized to 100 per sample/per independently derived haplotype because males with extremely aberrant ratios were often more abundantly tested.

‡ Ranges are given only for progeny samples of 25 or more from individual males.

data is that the t^T *tf* recombinant chromosomes carry two interacting factors responsible for elevated ratio, one of which maps near t^T and one of which appears to be associated with each of the three lethal factors, t^{w5}, t^{w12} and t^{w32}, since all chromosomes that carry t^T and at least one of these lethal factors have extremely high transmission ratios.

On the other hand, the transmission ratios of *T*-bearing recombinants are highly variable and clearly differ according to the haplotype from which they were derived. Such chromosomes derived from t^{w5}, t^{w12} and t^{w32} usually result in slightly high but very variable transmission ratios, which are not different whether or not the chromosome contains a *t*-lethal factor. This suggests that the distal ratio-distorting factor is separable from the lethal mutations, and that in the absence of the distorting factor associated with t^T it produces an unstable ratio that is subject to modification by environment and/or genetic background. The chromosome carrying *T tf* t^{w12}, we have examined in detail, is a good example; 38 *T tf* $t^{w12}/+\ +\ +$ males transmitted the mutant chromosome in an average ratio of

75%, with a range of 22–99% in progeny samples of over 50 (data not shown). Fig. 1 B shows a case in point, with transmission ratio data from 27 males, all sons, grandsons, great grandsons, or great-great-grandsons of a single *T tf* t^{w12}/+ male on the genetic background of the CF_1 strain. In this case the *T tf* t^{w12} chromosomes tested were monitored for and known to contain all three markers; nevertheless

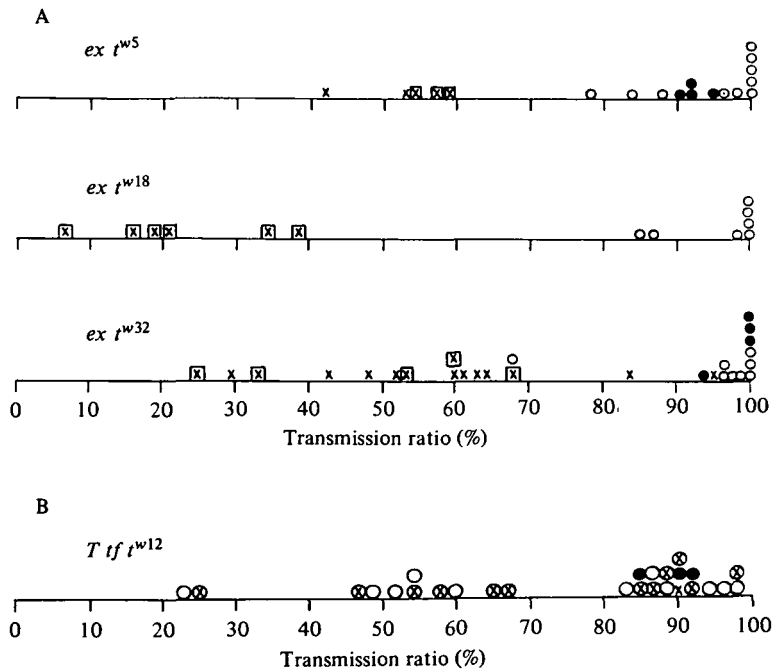


Fig. 1. (A) Distribution of transmission ratios of male *T*- and t^T-bearing recombinants from *T tf* t^{w12}/t^x ($t^x = t^{w5}, t^{w18}, t^{w32}$) on genetic background of BTBRTF/Nev. Progeny sample size from individual *T*−/+ males, whose ratios are relatively variable, was at least 25, and averaged over 50; sample sizes from t^T-/*T* males whose ratios are uniformly quite high was at least 10, but also averaged over 50. ×, *T*+++; ⊠, *T* x^x++; ○, t^T+*tf* t^{w12}; ●, t^T t^x *tf* t^{w12}. (B) Distribution of transmission ratios of *T tf* t^{w12}/CF_1 males, all sons (×), grandsons (⊗), great-grandsons (○), or great-great-grandsons (●) descended from one male. The number of test progeny was at least 50, and usually over 100.

the transmission ratios of these males not only varied dramatically but fell into discontinuous categories that themselves had a 9 : 6 : 1 ratio, suggesting the possibility that two unlinked genes are segregating in CF_1 and controlling the ratio of individual males. Distal end recombinants arising from exceptional recombination in single heterozygotes behave similarly; our own unpublished data for t^{h17} (a chromosome much studied by Lyon & Mason, 1977) show an average transmission from 28 males of 57%, but a range of 15–83%.

The tendency to high ratio and its instability is evidently a real phenomenon, and not due to sampling errors since, for example, a random sample of similar

progeny numbers from 7 $T/+$ males on comparable genetic backgrounds gave segregation ratios of 0·40, 0·45, 0·49, 0·50, 0·52, 0·52 and 0·55.

The t^{w18} haplotype generates recombinant T-bearing chromosomes that are clearly different as a group from any others, since their transmission ratios, although also variable, are generally very low.

Table 5. *Transmission ratios and recombination* in males with* t^{w86} *and* t^{h17} *in* cis *and in* trans

Male genotype	Offspring by $\frac{+tf}{+tf}$							
	At birth			At weaning				
	T	t	Apparent proportion t^{h17}	$T\,tf$	$T+$!	$t^{w86}+$	$t^{w86}tf$!	Proportion t^{h17}
$\frac{T+t^{h17}}{t^{w86}tf+}$	163	48	0·77		—			
$\frac{t^{w86}+t^{h17}}{T\,tf+}$	73	203	0·74	1) Real numbers 15	47 !	151	10 !	
				2) Corrected to total at birth (18)	(55 !)	(190)	(13 !)	0·89

* In + heterozygotes, t^{w86} and $T\,t^{h17}$ are transmitted from males at 50% (69:70) and 70% (Silver & Artzt, 1981) respectively. $T\,t^{h17}/t^{w86}$ females transmit both chromosomes equally, as expected, and show 8·5% (3/38) recombination between T and tf. Genetic background was in all cases derived from BTBRTF/Nev (see Table 1).

Transmission ratios in cis *and* trans *heterozygotes for proximal* (t^{w86}) *and distal* (t^{h17}) *partial* t-*haplotypes*

Table 5 shows data from tests of two partial t-haplotypes, in *cis* and *trans*, and shows that observations at birth (using tail phenotype as a criterion for genotype) would suggest that t^{h17} is transmitted from such males at approximately the same frequency as it is from $T\,t^{h17}/+$ + males. However, in one set of experiments we raised progeny to weaning, when they could also be scored for phenotype with respect to tf, and thus obtained information on recombination between T and tf in the male parents. Table 5 shows unexpected results; more than 75% of the T-bearing gametes transmitted were recombinants and therefore carried t^{h17}. Since the rate of recombination as measured in females is just over 8%, this clearly means that the transmission of the T-bearing gametes that receive t^{h17} by recombination is greatly enhanced *versus* the non-recombinant $T\,tf$ gametes. Table 5 shows that when the true rate of transmission of t^{h17} is measured, by taking recombinants into account, it is as high as 89%. Since we did not measure recombination in $T\,t^{h17}/+$ + males, we do not know their actual rate of transmission of t^{h17}.

https://doi.org/10.1017/S0016672300021042 Published online by Cambridge University Press

Transmission ratio in males carrying 't^{low}' *factors*

On three independent occasions the t^6 haplotype has generated, by recombination, peculiar partial *t*-haplotypes that have no obvious effect except to reduce their own transmission through heterozygous males, and slightly curtail recombination with normal homologous chromosomes (Dunn & Bennett, 1968, 1971; Lyon & Mason, 1977; Lyon *et al.* 1979). Table 6 shows results of tests of each of these *trans*

Table 6. *Transmission ratios of males with three independently isolated* 't^{low}' *factors* trans *to normal or* t^{w12} *chromosomes*

	Male genotype and offspring					
	T 't^{low}'/++			$T\ t^{low}/t^{w12}$		
Specific 't^{low}'	++	t 't^{low}'	Proportion T	t^{w12}	T 't^{low}'	Proportion T
t^{low}	169	37	0·18	4	11	0·73
	111*	29	0·21	6	15	0·71
	111*	29	0·21	0	6	1·00
Totals and average proportion T	391	95	0·20	10	32	0·76
t^{low2H}	132	19	0·13	34	88	0·72
	29	6	0·17	31	18	0·37
	31	9	0·23			
Totals and average proportion T	192	34	0·15	65	106	0·62
t^{low3H}	35	4	0·10	28	94	0·77
	35	3	0·08	49	32	0·40
	96	11	0·09	56	47	0·46
	87	13	0·13	41	32	0·44
Totals and average proportion T	253	32	0·11	174	205	0·54

* These two lines are not inadvertent repeats; two different males by chance had identical numbers and proportions of progeny!

to a wild-type chromosome or to the t^{w12} haplotype, which confirm previous data that the transmission ratio of each of them (t^{low}, t^{low2H}, t^{low3H}) is very much reduced in T 't^{low}'/++ males. From t^{w12} heterozygotes, on the other hand, each of them is transmitted at much higher ratios. However, in these cases pronounced inter-male variability is again evident. For t^{low2H}, for example, two males, who happened to be litter mates, gave ratios of 0·72 and 0·37 respectively; the χ^2 for this difference is 17·4; $P > 0{\cdot}001$). Likewise, for t^{low3H} the two largest samples showed ratios of 0·77 and 0·46, which were again statistically significantly different ($\chi^2 = 24{\cdot}8$, $P > 0{\cdot}001$). Thus it is clear that the 't^{low}' factors and the complete lethal *t*-haplotype interact in some way, but that the actual extent of the interaction is elusive. Hidden recombination is not a factor here (as it was for t^{w86} and t^{h17}) since

https://doi.org/10.1017/S0016672300021042 Published online by Cambridge University Press

recombination is suppressed in $T\ t^{low}/t^{w12}$ heterozygotes to a similar degree as in $T+/t^{w12}$ heterozygotes (data not shown).

However, we did find and analyse one recombinant chromosome that occurred in a $+t^{low}\ tf/t^{T}\ t^{w12}$ female; it carried *tf* and the lethal factor t^{w12}, but had lost t^{T}. It seems likely that the recombinant chromosome also had acquired t^{low}, since its male transmission ratio was very different from that of any other distal end recombinant ever attained from a naturally occurring lethal *t*-haplotype. Six males tested transmitted the chromosome to 19 % of 425 offspring, with a range of 12–27 %. This ratio is indistinguishable from that caused by t^{low} alone, but clearly different from that seen when t^{low} is *trans* to the complete $t^{T}\ t^{w12}$ haplotype.

DISCUSSION

The high rate at which *t*-haplotypes are transmitted from wild male heterozygotes is undoubtedly an important factor in maintaining polymorphic levels of these lethal chromosomes in wild populations. The degree of transmission ratio distortion in such wild males is extreme since, as was pointed out earlier, they almost invariably transmit their lethal gamete at a frequency of 95 % or more. Our present finding that high transmission ratios decay on prolonged breeding without selection in the laboratory therefore suggests that some selective mechanism for maintaining high *t*-haplotype transmission is operating in natural populations. It is clear, furthermore, that the main target of selection is not the *t*-haplotype itself but rather the genetic background, specifically genes on the normal chromosome 17. This is most sharply shown by two points. First, transferring *t*-haplotypes from our laboratory stocks, in which their transmission was low, to several different genetic backgrounds results in an immediate restoration of high transmission; this is good evidence that no change intrinsic to the *t*-haplotypes had occurred. Second, three different apparent deletions at the proximal end of chromosome 17, in the vicinity of the locus of *T*, all result in extremely high transmission frequencies of naturally occurring *t*-haplotypes. These observations, coupled with the evidence that implicates the *T tf* chromosome from our standard laboratory stocks as being responsible for lowering transmission of *t*-haplotypes, suggests strongly the presence in the vicinity of the locus of *T* of one or more modifier genes that are very important in governing the ultimate transmission characteristics of *t*-haplotypes. Moreover, these modifier(s) seem to have a distinct tendency to undergo changes with time in the absence of selection that result in a lowering of *t*-haplotype transmission. For example, the transmission ratios of 5 of the 6 naturally occurring *t*-haplotypes shown in Table 2 are much lower in their standard stocks than they were when those stocks were first initiated. Yet each of those stocks has been bred quite separately from one another, and thus the modifier(s) responsible for lowered transmission ratio must have accumulated independently. It has been suggested (Silver (1981), who incorrectly cited a personal communication (Bennett, 1978*b*) as the source of the notion), that inbreeding *per se* is responsible for declining transmission ratio; this is patently incorrect, as inbred C3H congenic

lines show extremely high rates of *t*-haplotype transmission. The mode of action of the modifier gene(s) must be, in at least a formal sense, inhibitory, since their removal by deletion results in very high ratios. This is a point to consider in the context of experiments reported by Shur & Bennett (1979), who found increased galactosyltransferase activity in sperm populations from high-ratio males, and proposed that *t*-haplotypes were associated with relatively ineffective inhibitors of galactosyltransferase activity.

Regardless of the interesting evolutionary relationship between *t*-haplotypes and their modifiers in natural populations, this evidence that the same *t*-haplotypes can vary so greatly in transmission ratio clearly has negative implications for models previously constructed to explain the aberrant transmission of *t*-haplotypes. Until now it has widely been assumed that the various naturally occurring *t*-haplotypes had intrinsically different transmission ratios which, although always higher than normal, ranged from 'moderately high' to 'very high' (Bennett, 1975; Lyon & Mason, 1977). However, we have shown here that although these haplotypes have the potential to be transmitted from males in very high proportions, their actual transmission is very dependent on the homologous chromosome. This calls into question a number of issues, among them the reality of the transmission ratio measurements that have been used in making models. For example, several reports (Demin & Safronova, 1978; Demin *et al.* 1978; McGrath & Hillman, 1980) have suggested a role of female genotype in determining male transmission ratio. In the light of the data we have presented, the putative female effects could perhaps as well be explained by statistical sampling errors that would result from testing small numbers of males with variable transmission ratios.

The most extensive attempts at dissecting the factors within *t*-haplotypes responsible for altered transmission ratio have been those of Lyon (Lyon & Mason, 1977) who focused on the t^6-haplotype, which has conventionally been classified as having a 'moderately high' ratio of about 60–65 %. They examined exceptional recombinants from t^6, and found that proximal *t*-haplotypes (which retained t^T but not the lethal factor) had either low or normal transmission ratios, and that one of the two distal haplotypes they described had a transmission ratio that was normal (t^{h18}), and the other a moderately high and unstable ratio (t^{h17}). Furthermore, on three separate occasions there have appeared, apparently by recombination, isolated ratio factors that had no effect other than to lower the rate at which they were transmitted; Lyon & Mason have referred to these as t^{low}. To explain these data, they postulated that the t^6-haplotypes contained an 'A' factor (A for abnormal ratio) which in combination with t^T or by itself produced low ratios, but which when *cis* to the t^6 lethal factor produced the moderately high but unstable ratio they considered typical of t^{h17}. However, in order to explain their observations that the complete t^6 haplotype (t^T *A* t^l on their model) had a higher transmission ratio than t^{h17}, they had also to assume that the t^T factor (or some factor close to it) also influenced transmission ratio. They went on to try to test this hypothesis more generally, and concluded that 'the A-factor is essential for the occurrence of an abnormal ratio, and in haplotypes, or combination of haplotypes, which lack

this factor the ratios are always normal'. However, in a sense this represented circular reasoning, since haplotypes were classified as having or not having the putative A-factor on the basis of their transmission ratio, without any independent verification of that classification. This is especially true since Lyon & Mason report many specific instances where their results did not coincide with the predictions of their hypothesis, and thus required one or more additional assumptions. It seems likely that the difficulties Lyon & Mason encountered in formulating and evaluating their model were caused by the capriciousness of transmission ratio that we have demonstrated here. Although we have not proposed specific models, we now believe that we have also fallen into the trap of paying too much attention to detail (see Bennett & Dunn, 1971). Here we would like to propose a different model, which we have arrived at by ignoring the 'noise' in the system, and abstracting from our data and those of Lyon & Mason what we believe to be the important relevant points.

(1) All naturally occurring *t*-haplotypes are potentially capable of being consistently transmitted at very high ratios (over 90%) from an appropriate genetic background (see Table 2).

(2) Exceptional recombination in a wild *t*-haplotype yields the following.

(*a*) Proximal end (t^T-containing) recombinant chromosomes that fall into two sharply different classes with respect to transmission ratio, either consistently low (under about 25%) or normal.

(*b*) Distal end (t^{lethal}-containing) recombinant chromosomes that as a group are highly variable, and show pronounced inter-male variation. The transmission ratio characteristics of these chromosomes are entirely comparable to those of distal end recombinants obtained from double *t*-heterozygotes, although the latter recombinants may or may not carry a lethal factor. Thus there must be some factor in the distal end of *t*-haplotypes that by itself confers a tendency to higher than normal ratios, although there is much individual variation around the mean.

(*c*) Lethal *t*-haplotypes of the t^9 complementation group have been generated independently five times by the naturally occurring haplotypes t^{12}, t^{w5} and t^{w1}, and where appropriate markers were available these events were accompanied by recombination. Recent evidence (Artzt *et al.* 1982) has shown that the t^9-haplotype, t^{w18}, has a much shorter distal segment than do the naturally occurring lethal *t*-haplotypes. Since the intact t^{w18}-haplotype has a variable but generally *low* ratio (see Table 4), it seems likely that it is missing the distal factor responsible for high and variable ratios, which must therefore map distal to the t^{w18} lethal factor.

(3) When the proximal and distal ends of a naturally occurring *t*-haplotype are arranged in *trans*, males transmit one chromosome in ratios that can be as high as those produced by intact natural haplotypes. Our data show that the distal marker is preferentially transmitted, but Styrna & Klein (1981) as well as Lyon & Jarvis (1980) have evidence that the chromosome containing the proximal end *t*-haplotype is preferentially transmitted. This is true whether or not the t^T end has a normal or high ratio. This suggests that only t^T and a distal factor (t^{distal}) intrinsically govern distorted transmission, and further that the interaction is

premeiotic, since in our experiments t^{distal} is transmitted in high ratio even when it has been separated from t^T by recombination (Table 5). Thus it seems that the factor called t^{low} by Lyon & Mason (1977), which is clearly present in all naturally occurring *t*-haplotypes (since all are capable of generating proximal end recombinants with low ratios) plays no evident role in its *cis* configuration within complete *t*-haplotypes, but that when it is separated out either alone (as t^{low}), or with either t^T or *t*-distal segments, it acts independently to lower the ratio of its chromosome. This brings up the interesting speculation that t^{low} may function to eliminate recombinant partial *t*-haplotypes promptly from wild populations, and thus serve to maintain the integrity of complete *t*-haplotypes with which they might otherwise undergo recombination.

We are unable to present a substantive model for the mechanism of transmission distortion by mouse *t*-haplotypes. It is worth noting, however, that a closely analogous situation, termed 'segregation distortion' is well known and studied in Drosophila. There are many parallels with transmission distortion, which include a requirement for at least two genes (segregation distortion S, and responder R), lethality, male sterility, and recombination suppression, and high frequencies and polymorphisms in wild populations.

Styrna & Klein (1982) have already also presented evidence for a two-factor control of transmission ratio of *t*-haplotypes, and pointed out the analogy to the Drosophila SD system, a possibility that had been raised before by Gluecksohn-Waelsch & Erickson (1970) and Demin *et al.* (1978). In Drosophila it is clear that in heterozygotes chromosomes carrying both *S* and *R* 'poison' their meiotic partners, since microscopic evidence shows that about half of their immature sperm cells degenerate (Tokuyasu, Peacock & Hardy, 1977; Kettaneh & Hartl, 1980). The mechanism by which this occurs is interesting and complex; it appears that during meiosis the *SR* chromosome somehow induces the wild-type chromosome to produce actively some product or products that lead to a dysfunction later in the haploid stages of spermiogenesis, which is in some way connected with a failure of transition from somatic to sperm-specific histones (Kettaneh & Hartl, 1976). The mouse and Drosophila meiotic drive systems are so similar in all other respects that the possibility of 'meiotic poisoning' by *t*-haplotypes seems a strong one that should be explored in more detail. The only evidence at present for morphological abnormalities of spermatozoa in *t*-haplotypes has been presented by Yanagisawa (1965) and Olds (1971), who found defective axonemal elements in a proportion of sperm, although the fraction of visibly abnormal spermatozoa was not high enough to account for the observed transmission ratio. Nevertheless these morphological differences may be reflected in function, since Tessler, Carey & Olds-Clarke (1981) report that sperm from $t^{w32}/+$ males show reduced velocity and abnormal swimming patterns. The same group reported effects of genetic background on sperm phenotype and function *in vitro* (Olds-Clarke & McCabe, 1981).

These experiments were supported by NIH grants HD-10668, CA-21651 and CA-08748, and Department of Energy contract DE-AC02-77EV04159. We thank J. Cookingham and C. Calo for help in the experiments, and A. Kennard for the manuscript.

REFERENCES

ARTZT, K., MCCORMICK, P. & BENNETT, D. (1982). Gene mapping within the T/t-complex of the mouse. I. *t*-Lethal genes are nonallelic. *Cell* **28**, 463–470.

BENNETT, D. (1975). The T-locus of the mouse: a review. *Cell* **6**, 441–454.

BENNETT, D. (1978*a*). Population genetics of T/t-complex mutations. NIH Workshop on *Origins of Inbred Mice* (Morse, H. C. ed.), pp. 615–632. New York: Academic Press.

BENNETT, D. (1978*b*). *Mouse News Letter* **59**, 60–61.

BENNETT, D. & DUNN, L. C. (1971). Transmission ratio distorting genes on chromosome IX and their interactions. *Proceedings of Symposium on Immunogenetics of the H-2 System* (ed. A. Lengerova and M. Vojtiskova), pp. 90–103. Basel: Karger.

BENNETT, D., DUNN, L. C., SPIEGELMAN, M., ARTZT, K., COOKINGHAM, J. & SCHERMERHORN, E. (1975). Observations on a set of radiation-induced dominant T-like mutations in the mouse. *Genet. Res., Camb.* **26**, 95–108.

DEMIN, J. S. & SAFRONOVA, L. D. (1978). Effect of female genotype on non-Mendelian segregation in offspring of male carriers of t-haplotypes in the house mouse (*Mus musculus* L.). *Proc. USSR Acad. Sci.* **243**, 1306–1308.

DEMIN, J. S., SAFRONOVA, L. D. & LAPKINA, J. A. (1978). Modifying effect of female genotype on the frequency of t-haplotypes transmission from T/t^6 males to offspring of mice. *Cytology and Genetics* **12**, 36–39.

DUNN, L. C. (1957). Studies of the genetic variability in populations of wild house mice. II. Analysis of eight additional alleles at locus T. *Genetics* **42**, 299–311.

DUNN, L. C. & BENNETT, D. (1968). A new case of transmission ratio distortion in the house mouse. *Proc. natn. Acad. Sci. USA* **61**, 570–573.

DUNN, L. C. & BENNETT, D. (1971). Further studies of a mutant (*Low*) which distorts transmission ratios in the house mouse. *Genetics* **67**, 543–558.

ERICKSON, R. P., LEWIS, S. E. & SLUSSER, K. S. (1978). Deletion mapping of the *t* complex of chromosome 17 of the mouse. *Nature* 274, 163–164.

GLUECKSOHN-WAELSCH, S. & ERICKSON, R. P. (1970). The *T*-locus of the mouse: implications for mechanisms of development. *Current Topics in Developmental Biology* **5**, 281–312.

HAMMERBERG, C. (1981). The influence of T^{Orl} upon male fertility in *t*-bearing mice. *Genet. Res., Camb.* **37**, 71–77.

KETTANEH, N. P. & HARTL, D. L. (1976). Histone transition during spermiogenesis is absent in segregation distorter males of *Drosophila melanogaster*. *Science* **193**, 1020–1021.

KETTANEH, N. P. & HARTL, D. L. (1980). Ultrastructural analysis of spermiogenesis in segregation distorter males of *Drosophila melanogaster*. *Genetics* **96**, 665–683.

LEWONTIN, R. C. & DUNN, L. C. (1960). The evolutionary dynamics of a polymorphism in the house mouse. *Genetics* **45**, 705–722.

LYON, M. F. & BECHTOL, K. (1977). Derivation of mutant *t*-haplotypes of the mouse by presumed duplication or deletion. *Genet. Res., Camb.* **30**, 63–76.

LYON, M. F., EVANS, E. P., JARVIS, S. E., SAYER, I. (1979). *t*-Haplotypes of the mouse may involve a change in intercalary DNA. *Nature* **279**, 38–42.

LYON, M. F. & JARVIS, S. E. (1980). *Mouse News Letter* **62**, 49.

LYON, M. F. & MASON, I. (1977). Information on the nature of *t*-haplotypes from the interaction of mutant haplotypes in male fertility and segregation distortion. *Genet. Res., Camb.* **29**, 255–266.

LYON, M. F. & MEREDITH, R. (1964). The nature of t-alleles in the mouse. I, II, III. *Heredity* **19**, 301–312, 313–325, 327–330.

MCGRATH, J. & HILLMAN, N. (1980). The *in vitro* transmission frequency of the t^6 allele. *Nature* **283**, 479–481.

OLDS, P. J. (1971). Effect of the T-locus on sperm ultrastructure in the house mouse. *J. Anat.* **109**, 31–37.

OLDS-CLARKE, P. & MCCABE, S. (1982). Genetic background affects expression of *t*-haplotypes in mouse sperm. *Genet. Res., Camb.* **40**, 249–254.

SHUR, B. D. & BENNETT, D. (1979). A specific defect in galactosyltransferase on sperm bearing mutant alleles of the *T/t* locus. *Devl. Biol.* **71**, 243–259.

SILAGI, S. (1962). A genetical and embryological study of partial complementation between lethal alleles at the *T*-locus of the house mouse. *Devl. Biol.* **5**, 35–67.

SILVER, L. M. (1981). A structural gene (*Tcp-1*) within the mouse *t* complex is separable from effects on tail length and lethality, but may be associated with effects on spermatogenesis. *Genet. Res., Camb.* **38**, 123.

SMITH, L. J. (1956). A morphological and histochemical investigation of a preimplantation lethal (t^{12}) in the house mouse. *J. Exp. Zool.* **132**, 51–84.

STYRNA, J. & KLEIN, J. (1981). Evidence for two regions in the mouse *t* complex controlling transmission ratios. *Genet. Res., Camb.* **38**, 315–325.

TESSLER, S., CAREY, J. E. & OLDS-CLARKE, P. (1981). Mouse sperm motility affected by factors in the T/t-complex. *J. Exp. Zool.* **217**, 277–285.

TOKUYASU, K. T., PEACOCK, W. J. & HARDY, R. W. (1977). Dynamics of spermiogenesis in *Drosophila melanogaster*. VII. Effects of *segregation distortion* (*SD*) chromosome. *J. Ultrastruct. Res.* **58**, 96–107.

YANAGISAWA, K. (1965). Studies on the mechanism of abnormal transmission ratios at the *T*-locus in the house mouse. IV. Some morphological studies on the mature sperm in males heterozygous for *t*-alleles. *Jap. J. Genet.* **40**, 97–104.