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The influence of T^{Orl} upon male fertility in *t*-bearing mice

BY CRAIG HAMMERBERG

Department of Human Genetics, Box 015 University of Michigan Medical School, 1137 E. Catherine Street, Ann Arbor, Michigan 48109

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SUMMARY

The model of Lyon & Mason (1977) which defines three subregions of the *t* complex (*T*, tail-determining; *A*, abnormal transmission ratio; *L*, lethal) has been extended in an interpretation of male fertility data obtained from the combination of T^{Orl} with different *t* haplotypes. The results demonstrate: (1) the *T* region of most *t* haplotypes (t^{12}, t^{0}, t^{w18} and t^{w2}) possess gene(s) that interact with the *L* region to give quasi-sterility; (2) the *T* region of t^{6} lacks the allele(s) that result in quasi-sterility; and (3) the *T* region interacts with the *A* region to modify the transmission ratios of *t* haplotypes. The results were discussed in terms of an interacting genetic system controlling male fertility.

1. INTRODUCTION

One of the least understood, but probably most complex, properties of the *t* complex is its effect upon male reproduction (for review see Bennett, 1975; Klein & Hammerberg, 1977; Erickson, Hammerberg & Sanchez, 1980). The *t* complex can influence male reproduction in two seemingly opposing ways: (1) sterility occurs in compound heterozygotes (t^{x}/t^{y}) or homozygous semi-lethal *t* haplotypes; and (2) certain *t* haplotypes are transmitted by the male in greater or fewer numbers than the Mendelian ratios expected (transmission ratio distortion).

Lyon & Mason (1977) have demonstrated the presence of a sterility factor associated with the lethal factor (*L* region) of t^{6}. Dunn & Bennett (1969), using viable *t* haplotypes derived by recombination, found that the *T* (*T*, tail-determining) region of various *t* haplotypes interacts with the *t* complex or the *tf* end of a *t* complex to give quasi-sterile males. Lyon & Mason (1977) have also demonstrated that the transmission ratio distortion of t^{6} results from the interaction of three regions: *T*, *A* and *L* (Fig. 1). The *T* region is located next to the *Brachyury* (*T*) mutation and by itself has no effect upon transmission ratio distortion, but does interact with *T* to yield tailless mice (T/t^{x}). The *A* (abnormal transmission ratio) region lies in the middle of the *t* complex to the right of *Tcp-1* (Silver, Arzt & Bennett, 1979; Silver, White & Bennett, 1980). An *A* region of a *t* haplotype heterozygous with a normal seventeenth chromosome will have a low ratio. In the *L* (lethal) region, genetic factors have been found that interact with the *A* and *T* regions to give a moderate to high transmission ratio distortion. The *L* region is also believed to have the recessive lethal factors of most *t* haplotypes (Lyon, Jarvis & Sayers, 1979).

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Using T^{Orl}, a deletion of the T region that results in quasi-sterile males when combined with t haplotypes t^{12} and t^6 (Erickson *et al.* 1978), it was found that the t haplotype used by Lyon, t^6, is different from other t haplotypes in its proximal portion. The T^{Orl} deletion was also observed to influence the transmission ratios of various regions of t^6 which had been separated from each other by rare crossovers.

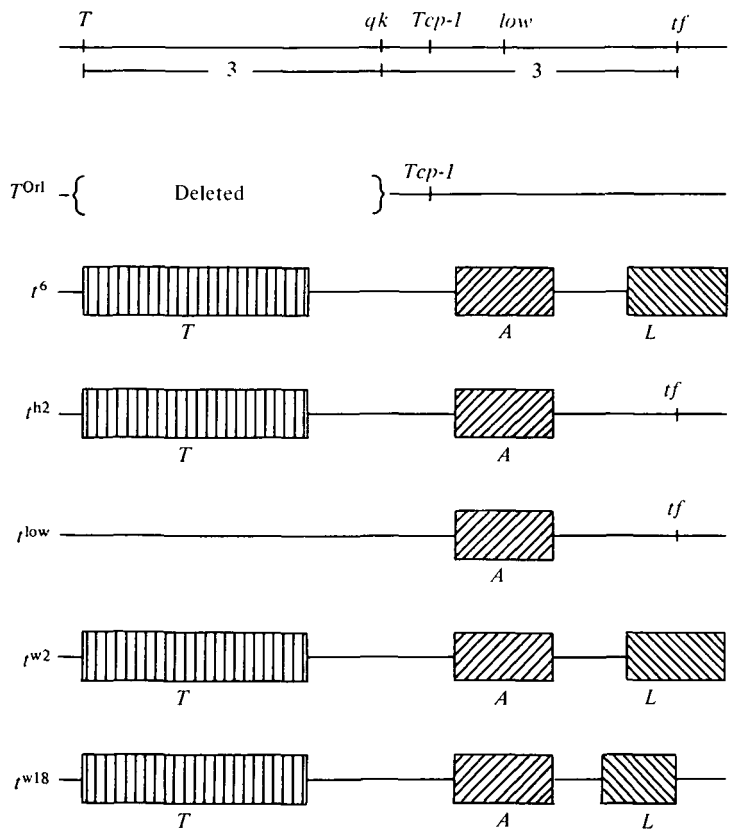

Fig. 1. Schematic representation of the various t haplotypes and their regions. Upper line represents the seventeenth chromosome and various genetic markers: *T* (Brachyury), *gk* (quaking), *Tcp-1* (*t*-complex protein-1), *low* (low transmission ratio), and *tf* (tufted).

2. MATERIALS AND METHODS

(i) *Mice*

B6.T^{Orl} was maintained in the breeding colony of Dr R. P. Erickson. Breeding stocks of T tf/f^6 and $T(t^{h18})/t^{h2}$ were obtained from Dr M. Lyon. T gk tf/t^{w2} and $t^{low}tf/t^{low}tf$ were obtained from Dr D. Bennett. C3H.T tf/t^{w18} were provided by Dr H. O. McDevitt. The recombinant t haplotype, t^{h2}, is derived from t^6 and possesses the T and A regions of t^6. t^{low} is derived from the t^6 recombinant t^{h17} (Bennett *et al.* 1979) and maintains the mid-portion of t^6, or the A region.

(ii) *Matings*

T^{Orl} mice used for these studies were obtained by outcrossing B6.T^{Orl} to CF-1 random bred mice. Males used for fertility and transmission ratio distortion testing were derived from crosses to $T^{Orl}/+$. T/t^{x} were mated to $T^{Orl}/+$ and T^{Orl}/t^{x} (tailless) and $+/t^{x}$ (normal-tail) litter-mates were tested. $T^{Orl}/t^{h2}tf$ (tailless) and $+/t^{h2}tf$ (normal-tail) litter-mates were derived from crosses of $t^{h2}tf/t^{h2}tf$ to $T^{Orl}/+$. $T^{Orl}/+$ mated to $t^{low}tf/t^{low}tf$ resulted in $T^{Orl}/t^{low}tf$ (short-tail) and $t^{low}tf/++$ (normal-tail) offspring. Tailless and short-tail males were tested by mating them to normal-tail females. Normal-tail litter-mates were mated to $T/+$ females. Newborns were checked for tail-length and discarded. Because $++/t^{low}tf$ males lack a T (tail-determining) region, they were crossed to $+tf/+tf$ females to test for transmission ratio distortion. Their offspring were checked at four and eight weeks for the tufted phenotype. A male was placed with two females of proven fertility for eight to twelve days, then rotated.

Table 1. *Effect of* T^{Orl} *upon fertility of various t haplotypes*

Male genotype	No. tested	No. female weeks per male	Newborns per female per week
T^{Orl}/t^{6}	3	56	5·64
$+/t^{6}$	3	54	5·71
T^{Orl}/t^{h2}	3	50	7·06
$+/t^{h2}$	3	26	6·03
T^{Orl}/t^{low}	3	49	6·48
$+/t^{low}$	3	16	5·03
T^{Orl}/t^{w18}	3	34	0·58
$+/t^{w18}$	3	27	4·18
T^{Orl}/t^{w2}	2	29	0
$+/t^{w2}$	2	13	5·66

3. RESULTS

(i) *Effect of* T^{Orl} *upon male fertility*

Male fertility was determined according to the number of newborns per female per week (Table 1). T^{Orl} in combination with t^{w2} resulted in complete sterility. T^{Orl}/t^{w18} males have greatly reduced fertility and can be considered quasi-sterile. However, there is no difference between T^{Orl}/t^{6} and its litter-mate controls, $+/t^{6}$. This is a marked contrast to the data found for t^{0} (Erickson *et al.* 1978), a member of the same complementation group as t^{6}. T^{Orl}, in combination with two recombinants of t^{6}, t^{h2} and t^{low}, did not effect their fertility.

(ii) *Effect of* T^{Orl} *upon transmission ratio distortion*

T^{Orl} did not have an abnormal transmission ratio when placed across from a normal chromosome (Table 2). However, in combination with t^{6} extreme distortion, which is unusual for the t^{6} haplotype, resulted. An unusually high transmission ratio distortion occurred for litter-mate controls ($+/t^{6}$), suggesting that the

Table 2. *Effect of T^{Orl} upon transmission ratio distortion*

Mating ♂		♀	No. males tested	Offspring tail-length NT	ST	OT	%t (mean ± S.D.)*	χ^2 †
$T^{Orl}/+$	×	$+/+$	8	259	226		47(44 ± 7·63)	2·25
T^{Orl}/t^{w18}	×	$+/+$	3	13	7		65(76·33 ± 20·65)	2·32
$+/t^{w18}$	×	$T/+$	3		34	31	48(35·67 ± 33·71)	
T^{Orl}/t^{6}	×	$+/+$	3	352	7		98(98·3 ± 0·58)	43·30
$+/t^{6}$	×	$T/+$	3		36	225	86(86·33 ± 5·51)	32·65
T/t^{6}	×	$T^{Orl}/+$	1	14	6		70	
T^{Orl}/t^{h2}	×	$+/+$	3	136	235		37(36·33 ± 8·74)	292·94
$+/t^{h2}$	×	$T/+$	3		69	8	10(10·00 ± 4·36)	
T^{Orl}/t^{low}	×	$+/+$	3	115	275		29(29·67 ± 1·53)	164·56
$++/t^{low}\ tf$	×	$+tf/+tf$	3	16 (tf/tf)	142 ($+/tf$)		10(10·83 ± 1·53)	

* Frequency of a t haplotype transmitted by males. The mean of the individual males ± standard deviation is given in the parentheses.

† $T^{Orl}/+$ was compared to expected Mendelian values and $+/t^{6}$ was compared to T/t^{6}. All other chi-square values were calculated using the frequency of the litter-mate controls as the expected frequency.

genetic background of T^{Orl} has an influence upon the transmission ratio distortion of t^6. However, the transmission ratio distortion of T^{Orl}/t^6 is significantly different from that of $+/t^6$ and is consistently around 98%.

The effect upon transmission ratio distortion is even more significant when T^{Orl} is placed with t^6 recombinants. T^{Orl} in combination with t^{h2} (T and A region) increases the frequency of t^{h2} transmission almost fourfold. T^{Orl} has a similar effect upon the frequency of t^{low} transmission. However, T^{Orl} does not appear to have an influence upon the transmission ratio of t^{w18}.

DISCUSSION

These results support the model presented by Lyon & Mason (1977) which postulates three interacting regions within the t complex responsible for transmission ratio distortion. In addition, they demonstrate that t^6, unlike other haplotypes (t^0, t^{12}, t^{w18} and t^{w2}), has a different T region.

T^{Orl} deletes the T region from *Brachyury* to *quaking* (Fig. 1). It does not extend beyond the gene coding for the protein p63/6·9 (*Tcp-1*), which lies to the right of *qk* (Silver *et al.* 1980), as it is probably a duplication for this gene (Silver, personal communication). t^{low}, which defines the A region of the t complex, places the A region to the right of *Tcp-1* because the *L-A* region recombinant of t^6, t^{17}, from which t^{low} is derived (Bennett *et al.* 1979) picked up the wild-type allelle of *Tcp-1* (Silver *et al.* 1979). Thus, T^{Orl} only covers the T region of the t complex and does not extend into the A region. Although borderline data for positive transmission ratio distortion were previously found with T^{Orl} (Erickson *et al.* 1978), the current data, alone or when pooled with the previous data, show that transmission ratios of T^{Orl} are not abnormal. These data support the notion that T^{Orl} does not extend to the A region or carry pieces of t chromatin picked up by unequal crossing-over that influence transmission ratios of the seventeenth chromosome. In addition, the T^{Orl} chromosome does not bear parts of the L region, as it is fully viable in combination with all t haplotypes (t^{w18}, t^0, t^{12}, t^{w2} and t^6) it has been tested with.

T^{Orl} has a striking effect upon the transmission ratio distortion of t^6 and its recombinants. Limited data with other t haplotypes (t^0 and t^{12}) indicate that T^{Orl} also increases their transmission ratio distortion (Erickson, personal communication). The increase in transmission ratios suggest that T^{Orl} deletes genes which, when on a normal chromosome, can modify the transmission ratio distortion of a t complex. In the case of t^6, these genes act as suppressors of high transmission ratio distortion. The increase in transmission ratios of t^{h2} (T and A region) and t^{low} (A region) suggests that the T region gene(s) modify the abnormal effect of the A region. The L region then interacts with the modified or unmodified A region. Lyon & Mason (1977) showed that t haplotypes consisting of the T and A regions combined with the t^6 haplotype (T, A and L regions) resulting in equal segregation of both t haplotypes. Equally, when an A region (t^{low}) is combined with t^1 (T, A and L region), equal segregation of both haplotypes is seen (Bennett & Dunn, 1971). Thus, the A region plays a central role in obtaining transmission

ratio distortion. The T region acts as a modifier of the A region or of the interacting A and L regions.

The existence of a polygenic modifier system in the T region would explain the ability of T^{Orl} to affect the transmission ratios of an A region combined with a T region derived from t^6 (t^{h2}) or a wild-type chromosome (t^{low}). It is possible that similarity at some genes exists between t^6 and the normal t-complex. These genes influence the effects of the A region upon transmission ratios, while a different set of genes from the T region of t^6 are involved in the modified A region–L region interaction.

The T region of t^6 also differs from other t haplotypes in its interaction with the L region as measured by its effect upon male fertility. It has been demonstrated that recombinant t^6 haplotypes (T and/or A regions) and t^6 (Lyon & Meredith, 1964) or t^{w5} (Lyon & Mason, 1977) as compound heterozygotes are fertile. Dunn & Bennett (1969) using a t haplotype, t^0, of the same complementation group as t^6, found that males with a t^0 recombinant chromosome paired with t^0 are quasi-sterile. T^{Orl}/t^0 also was found to be quasi-sterile (Erickson *et al.* 1978). However, we have now demonstrated that T^{Orl}/t^6 is fertile, and thus must differ from t^0 in the region deleted by T^{Orl}.

Because homozygous recombinant t haplotypes are fertile (Dunn & Bennett, 1969; Lyon & Meredith, 1964; Lyon & Mason, 1977) and $t^x/+$ males are fertile, quasi-sterility only results when the homozygous or hemizygous T region interacts with the $L(A)$ region(s). The exception, t^6, lacks the quasi-sterility gene(s) of most other t haplotypes.

The absence of an effect of T^{Orl} upon the transmission ratio of t^{w18} also suggests that the L region can be altered. The t^{w18} haplotype was derived by recombination from a t^{w5} haplotype (Bennett & Dunn, 1960). Normally such a recombinational event results in a viable t haplotype. However, t^{w18} remains a lethal t haplotype, but affects a different stage of development than does t^{w5}. In addition, t^{w18} permits normal recombination between T and tf (Bennett, 1975). The T region and A region [low transmission ratios are seen in viable t haplotypes derived from t^{w18} Hammerberg, unpublished data)] remain from t^{w5}. It would appear that the L region of t^{w18} has been altered. The transmission ratio distortion gene(s) of the L region involved in the interaction with the A region may also be affected, explaining the near-normal transmission ratios seen for t^{w18}. The failure of T^{Orl} to affect the t^{w18} transmission rates may be due to an alteration of transmission ratio gene(s) in the L region.

The existence of a polygenic system controlling male fertility was proposed by Hammerberg & Klein (1975) as an explanation for the evolution of the t complex. The evidence presented in this communication would support such a complex of genes involved in male reproduction. The differences between t^6 and t^0, members of the same complementation group, at their T region and *H-2* complex, demonstrate that t haplotypes should not be classified only by their embryonic lethal effects: the t complex is truly a complex of interacting related genes.

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