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The effects of the *t*-complex upon male reproduction are due to complex interactions between its several regions

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SUMMARY

The three major regions (T, tail-determining; A, abnormal transmission ration; L, embryonic lethality) of the *t* complex on the murine seventeenth chromosome can interact to influence male reproduction (Lyon & Mason, 1977; Hammerberg, 1981). Evidence is presented here that suggests: (1) the presence of a polymorphic system within the T region that influences quasi-sterility in the male; (2) the existence of enhancing factors of transmission ratio distortion; and (3) that preferential interactions between *t*-haplotypes are found to occur both *cis* and *trans* which influence transmission ratio distortion.

1. INTRODUCTION

It has been known for some time that mutants in the *t*-complex can cause distortion of its transmission by the male (Chesley & Dunn, 1936) as well as male sterility or quasi-sterility (Dunn & Gluecksohn-Schoenheimer, 1943). More recently, it has seemed useful to divide the *t*-complex into three regions: T, A and L (Lyon & Mason, 1977). The regions of a *t*-haplotype can affect male fertility in the following manner:

A. *Transmission ratio distortion*

(i) The T, A and L regions (complete *t*-complex) in the *cis* configuration result in moderate to high transmission ratio distortion of the *t*-haplotype when in repulsion to a normal chromosome (Chesley & Dunn, 1936; Bennett & Dunn, 1971; Lyon & Mason, 1977).

(ii) The A region alone or combined *cis* or *trans* with a T region results in low transmission of the A region (Bennett & Dunn, 1971; Lyon & Mason, 1977).

(iii) The A region with or without a T or L region (*cis* or *trans*) in the homozygous state results in equal transmission of both chromosomes (Bennett & Dunn, 1971; Lyon & Mason, 1977).

(iv) An A region, with or without a *cis* T region derived from a t^6-haplotype,

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in *trans* with a complete t^6-haplotype gives equal transmission of both chromosomes (Lyon & Mason, 1977).

(v) An A region, with a *cis* T region derived from non-t^6-haplotypes, in *trans* with a complete non-t^6-haplotype often shows aberrant distortion of the complete non-t^6-haplotype (Bennett & Dunn, 1971).

Table 1. *List of* t-*haplotypes*

t-haplotype	*t*-haplotype derived from	Regions of *t*-complex T	A	L	Transmission ratio*
$t^{w100}tf$	t^{w18}†	t^{w5}	t^{w5}	+	Low
$t^{h2}tf$	t^6	t^6	t^6	+	Low
$t^{low}tf$	t^6	+	t^6	+	Low
t^6	t^6	t^6	t^6	t^6	Moderately high
t^0	t^0	t^0	t^0	t^0	Normal to moderate
t^{w5}	t^{w5}	t^{w5}	t^{w5}	t^{w5}	High

* Transmission ratio of the *t*-haplotype when *trans* to a wild-type chromosome.
† t^{w18} is a recombinant *t*-haplotype that is derived from a t^{w5}-like haplotype.

B. *Fertility*

(i) Males with T–A regions derived from a t^6-haplotype in *trans* with A–L or L regions are fertile (Lyon & Mason, 1977).

(ii) Males with T–A regions derived from a non-t^6-haplotype in *trans* with most complete *t*-haplotypes are quasi-sterile (Dunn & Bennett, 1969).

The discrepancy between the effects of the T–A regions of t^6 and non-t^6-haplotypes upon male fertility suggest that a polymorphism exists in the gene(s) in these regions. A deletion of the T region, T^{Orl}, was used to demonstrate that there are differences in the T region of t^6- and non-t^6-haplotypes (t^{12}, t^0, t^{w2} and t^{w18}) in the effects upon transmission ratios and male sterility (Erickson *et al.* 1978; Hammerberg, 1981). Using a *t*-recombinant, $t^{w100}tf$ (Table 1) derived from a non-t^6-haplotype, t^{w18} (Silver *et al.* 1980), it is demonstrated here that quasi-sterility induced by *t*-haplotypes in the male is dependent upon what *t*-haplotypes are placed together. In addition, it is shown that transmission ratio distortion can be altered by combining different regions of unrelated *t*-haplotypes.

2. MATERIALS AND METHODS

(i) *Mice*

The *t*-haplotypes: $t^{w100}tf$, $t^{h2}tf$, $t^{low}tf$, t^6, t^0 and t^{w5} (Table 1) were all maintained in the breeding colony of Dr. R. P. Erickson of the Department of Human Genetics at the University of Michigan Medical School.

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(ii) *Matings*

$t^{w100}tf/t^{w100}tf$ homozygotes were crossed to T/t^x (t^x:t^0, t^6 and t^{w5}) and tailless ($T/t^{w100}tf$) and normal-tail ($t^x/t^{w100}tf$) male progeny were selected for studies of fertility and transmission ratio distortion. $T/t^{w100}tf$ males were tested by mating them to normal-tail ($+/+$) females and newborn offspring were checked for tail-length at birth. Normal-tail ($t^x/t^{w100}tf$) littermates were mated to $+tf/+tf$

Table 2. *H-2 antisera and pattern of reactivity with* t^{w100}tf *and* t^{h2}tf

Antiserum		Donor strain	Region detected	Reactivity with* $t^{w100}tf$	Reactivity with* $t^{h2}tf$
D-2	(B10.A(5R) × LP.RIII)F_1	B10	D^b	−	−
Anti-D^d	(B10.AKM × A.SW)F_1	A.TH	D^d	+	−
D-17(2)	(D1.C × AKR.M)F_1	DBA/1	K^q	−	+
D-23(2)	(B10 × LP.RIII)F_1	B10.A(2R)	K^k	−	−
D-32	(B10.A(2R) × C3H.SW)F_1	C3H	D^k	±	+
Ia 11, 16	(A × B10)F_1	B10.D2	K^dI^d	+	±
Ia 9, 20	(A × B10.D2)F_1	B10.A(5R)	K^bI^b	−	−

* Range of cell lysis when reacted with antisera: −, 0–30% cell lysis; ±, 35–70% cell lysis; +, 100% cell lysis.

females to test for transmission ratio distortion. Their progeny were checked at four and/or eight weeks for the tufted phenotype. To determine fertility, a male was placed with two females for eight to twelve days, then rotated.

$t^{w100}tf/t^{w100}tf$ homozygotes were also mated to *$T(t^{h18})+/t^{h2}tf$* and their normal-tail tufted ($t^{w100}tf/t^{h2}tf$) male offspring were tested for transmission ratio distortion. Because both of these *t*-haplotypes carry the tufted allele and have the tail-enhancing factor, it was necessary to use their H-2 antigens as genetic markers for measuring their transmission ratios. *$t^{w100}tf$ H-2^d/$t^{h2}tf$ H-$2^{q/k}$* males were mated to *$++$H-2^b/$++$H-2^b* or *$++$H-2^b/$++$H-2^k* females and their offspring typed at four weeks of age for their H-2 antigens (see below).

$t^{w100}tf/t^{low}tf$ males were obtained by mating *$t^{w100}tf/t^{w100}tf$* to *$t^{low}tf/t^{low}tf$*. The transmission ratio of *$t^{w100}tf$* was determined by mating them to *$T/+$* females and checking their newborn for tail-length.

(iii) *H-2 antisera*

The H-2 antisera used in these studies were obtained from the National Institutes of Health (Table 2).

(iv) *Microcytotoxicity assay*

2 μl of antiserum in 5 doubling dilutions were mixed in a Terasaki plate with 2 μl of lymphocytes (2×10^6 cells/ml), taken from the mesenteric lymph nodes, for 20 min at room temperature. After washing with a drop of medium (Hanks, with 5% heat inactivated newborn calf serum) for 10 min, 2 μl of complement (Baby Rabbit serum, Pel-Freez) was added and incubated for 30 min at room temperature.

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Plates were read with a Zeiss-inverted phase microscope after fixing the cells with 5% formaldehyde in phosphate-buffered saline.

3. RESULTS

(i) *The effect of* $t^{w100}tf$ *upon male fertility*

Depending upon the *t*-haplotype which is placed in *trans* with $t^{w100}tf$ different effects are seen upon male fertility. Males of the $t^{w100}tf/t^6$ + genotype have normal fertility, whereas $t^{w100}tf/t^{w5}$ + males are quasi-sterile (Table 3). This behaviour of $t^{w100}tf$ resembles that of t^6-recombinants of the T–A regions in one respect in that

Table 3. *Effect of* $t^{w100}tf$ *upon male fertility of various* t*-haplotypes*

Male genotype	No. tested	No. female weeks permale	Newborns per female per week
$t^{w100}tf/T$*	3	45	5·3
$t^{w100}tf/T$†	3	27	3·12
$t^{w100}tf/t^6$	3	39	3·3
$t^{w100}tf/t^0$	2	17	4·64
$t^{w100}tf/t^{w5}$	6	59	0·42

* Littermates to $t^{w100}tf/t^6$ derived from the cross of $t^{w100}tf/t^{w100}tf$ to Ttf/t^6.
† Littermates to $t^{w100}tf/t^0$ derived from the cross of $t^{w100}tf/t^{w100}tf$ to T/t^0.

in combination with t^6, normal male fertility is obtained. Such t^6-recombinants would also have normal fertility when placed in *trans* to t^{w5} (Lyon & Mason, 1977). However, the quasi-sterility of $t^{w100}tf/t^{w5}$ + is what would be expected for most non-t^6 recombinants of the T–A regions in *trans* with a complete non-t^6-complex (Dunn & Bennett, 1969). It has also been shown (Dunn & Bennett, 1969) that near-sterility occurs in the t^{w29}/t^0 combination, where t^{w29} is a t^{w18} recombinant with normal transmission ratios. However, the fertility of $t^{w100}tf/t^0$ + males is normal (Table 3).

(ii) *Transmission ratio distortion effects of* $t^{w100}tf$

The transmission of $t^{w100}tf$ can be altered by the particular *t*-haplotype it is combined with. Transmission of $t^{w100}tf$ by $t^{w100}tf/T$ + males is lower than normal Mendelian values (Table 4, line 1). When $t^{w100}tf$ is combined with t^6, it behaves like a low distorting t^6-recombinant in that both *t*-haplotypes are transmitted close to equality (Table 4, line 3). The combination $t^{w100}tf/t^0$ also has transmission ratios close to Mendelian values (Table 4, line 4).

The transmission ratios of $t^{w100}tf/t^{w5}$ are close to what would be expected if the relative ratio formula of Bennett & Dunn (1971) is applied. According to this formula, the transmission ratios of a *t*-haplotype will vary relative to the ratio of the seventeenth chromosome *trans* to it. Assuming a transmission ratio of 0·90 for t^{w5} (Bennett, 1975), the expected ratio for t^{w5} in *trans* with $t^{w100}tf$ would be

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Table 4. *Crosses demonstrating the influence of various* t*-haplotypes upon* t^{w100}tf

Mating ♂	Mating ♀	No. males tested	Phenotype of Offspring: Tail-length NT	Tail-length ST	Tail-length OT	% $t^{w100}tf$	χ^{2}*	χ^{2}
$t^{w100}tf/T+$	+/+	6	91	409		18	202·24	—
$t^{w100}tf/t^{low}tf$	$T/+$	4	—	58	82	59	4·12	13·85†
			Tufted	Non-tufted				
$t^{w100}tf/t^{6}+$	$+tf/+tf$	3	76	99		43	3·02	—
$t^{w100}tf/t^{0}+$	$+tf/+tf$	2	69	89		44	2·54	118·87‡
$t^{w100}tf/t^{w5}+$	$+tf/+tf$	4	34	18		65	4·92	0·16§
			H-2					
			$H\text{-}2^{d}$	$H\text{-}2^{q/k}$				
$t^{w100}H\text{-}2^{d}/t^{h2}H\text{-}2^{qk}$	$H2^{b}/H\text{-}2^{b}$ or $H\text{-}2^{b}/H\text{-}2^{k}$	4	67	43		61	5·24	13·51†

* Comparison based upon normal Mendelian rates of 50 %.
† Comparison based upon the transmission of $t^{w100}tf$ from t^{w100}tf/t^{6}+ males (line 3).
‡ Chi-square based upon a value of 0·21 for transmission rate of t^{0}.
§ Chi-square based upon a value of 0·32 for transmission rate of t^{w5}.

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0·9:0·5 = X:0·18 or 0·32, which is not significantly different from the observed value of 0·35 (Table 4, line 5). However, if the same formula is applied to the $t^{w100}tf/t^0+$ combination where the normal transmission rate of t^0 is 0·58 (Hammerberg, unpublished data), then the expected rate for t^0 would be 0·21 (observed is 0·56).

Transmission rates of $t^{w100}tf$ are thus dependent upon the *t*-haplotype placed *trans*. The *t*-haplotypes examined so far have had complete *t*-complexes. In order to determine the effects of only the T region upon $t^{w100}tf$ transmission rates, two t^6 recombinants, $t^{low}tf$ and $t^{h2}tf$ differing in the T region were placed in *trans* with $t^{w100}tf$. The *H-2* antigens served as markers for the segregating $t^{w100}tf$ and $t^{h2}tf$ haplotypes: $t^{w100}tf$ was typed as H-2^d, while t^{h2} is a *H-2* recombinant consisting of *H-2* K^q and *H-2* D^k (Table 2). $t^{w100}tf/t^{h2}tf$ males were outcrossed to *H-2*^{b} or *H-2*^{b}/*H-2*^{k} females. The segregation of $t^{w100}tf$ and $t^{h2}tf$ was followed with the appropriate antisera listed in Table 2. Segregation of $t^{w100}tf$ and $t^{low}tf$ could be followed by using the tail-determining factor on $t^{w100}tf$ which is absent from $t^{low}tf$: $T/t^{w100}tf$ mice are tailless while $T/t^{low}tf$ mice are short-tailed. As expected from published results (Bennett & Dunn, 1971) $t^{w100}tf/t^{low}tf$ males transmit both *t*-haplotypes in Mendelian fashion (Table 4, line 2). The combination $t^{w100}tf/t^{h2}tf$ also shows $t^{w100}tf$ being transmitted close to the expected Mendelian values (Table 4, line 6).

4. DISCUSSION

The three region model of the *t*-complex proposed by Lyon & Mason (1977) was based upon work with t^6-recombinants. A mid-region, A, was proposed, which, when by itself, is transmitted by the male in low numbers. The L region (distal end) by itself is transmitted normally, but when combined in *cis* with the A region, variable transmission of the *t*-haplotype is obtained. Only when all three regions, T, A and L, are in *cis* is moderately high transmission of the *t*-haplotype obtained, indicating that all three regions are necessary for high transmission ratio distortion. When both seventeenth chromosomes carry A regions from a t^6-haplotype with or without other regions, each A region is transmitted equally. A recessive sterility factor was located in the L region of the t^6-complex, while the T–A regions of t^6 were found to be devoid of such effects upon fertility.

Using a deletion of the T region, T^{Orl} (Erickson *et al.* 1978; Hammerberg, 1981), it was demonstrated that the T region of t^6 differs from other *t*-haplotypes in that it lacks the quasi-sterility factor. In addition, it was shown that the T region can interact with the A region to modify transmission ratio distortion. Because the various T regions were in *trans* with a deletion, these loci were expressed in the hemizygous state. Using a non-t^6-recombinant, $t^{w100}tf$, the interaction between different regions, *cis* and *trans*, was measured.

The normal fertility achieved by $t^{w100}tf/t^0+$ males is unexpected because T^{Orl}/t^0 males are quasi-sterile. However, $t^{w100}tf$ does possess the ability to cause quasi-sterility as $t^{w100}tf/t^{w5}$ males are quasi-sterile. If a single gene within the T region is responsible for quasi-sterility, then the alleles of t^6, t^0 and t^{w5} are different. Only

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the t^6 allele resembles the wild-type, in that it is never quasi-sterile. The t^0-haplotype (a member of the same complementation group as t^6) has an allele which is intermediate in its effect; in the hemizygous state (T^{Orl}/t^0) it is quasi-sterile, but when placed in *trans* with $t^{w100}tf$, a non-*t*-recombinant, normal fertility results. The t^{w5} haplotype has the same T region as $t^{w100}tf$ since the original *t*-haplotype of $t^{w100}tf$ was t^{w5}-like (Bennett & Dunn, 1960). Thus, $t^{w100}tf/t^{w5}+$ males are homozygous for the same quasi-sterile allele and are quasi-sterile.

A polymorphic system could exist for quasi-sterility, where each member of a complementation group sharing the same *H-2* haplotype would have the same allele at the quasi-sterile locus. When such alleles are found in the hemizygous (T^{Orl}/t^0) or homozygous state ($t^{w100}tf/t^{w5}+$), male quasi-sterility will result. Quantitative variation probably exists in the ability of these different alleles to interact to cause quasi-sterility as $t^{w100}tf/t^0+$: males have normal fertility, whereas males with a t^0-haplotype in combination with t^0-recombinants are sterile (Dunn & Bennett, 1969).

The effect of $t^{w100}tf$ upon the transmission ratios of complete *t*-haplotypes is the same in one respect: the transmission ratio of $t^{w100}tf$ is drastically increased. In the case of moderate distorters, t^0 and t^6, the increase is such that Mendelian values are almost obtained. The high distorter, t^{w5}, has a more extreme effect upon $t^{w100}tf$ transmission: $t^{w100}tf$ is transmitted in excess of 50%. This increase in transmission of low distorters has also been observed for other high distorters-low distorters combinations (Bennett & Dunn, 1971). This interaction between *t*-haplotypes must be a property of the degree of distortion that they produce. High distorters could carry some enhancing factor(s) that causes their high distortion. These factors could be lost (t^0 and t^6) resulting in moderate distortion. When a moderate distorter, t^0 or t^6, is in *trans* to an A region, the L region interacts with both A regions to allow Mendelian transmission. However, when high distorters are placed across from a low distorter *t*-recombinant, the enhancing factors will increase the transmission of the *t*-recombinant. The enhancing factors of a high distorter must alter the modifying factors within the T region (Hammerberg, 1981) *trans* to it, such that when *trans* to wild-type T region, extreme distortion is seen but when *trans* to a *t*-haplotype consisting of T–A regions, increased segregation of *trans* *t*-haplotypes occurs.

The interaction of $t^{w100}tf$ with t^6-recombinants, $t^{h2}tf$ and $t^{low}tf$, again demonstrate the similarity between the T end of t^6 and the wild-type at the modifying gene(s) within the T region. The A region of $t^{h2}tf$ and $t^{low}tf$ are similar, while $t^{h2}tf$ maintains the T region of t^6 and $t^{low}tf$ has the wild-type T region. $t^{w100}tf$, in repulsion with both $t^{low}tf$ and $t^{h2}tf$ is transmitted close to Mendelian values. The transmission rates of $t^{w100}tf$ by $t^{w100}tf/t^{h2}tf$ or $t^{w100}tf/t^{low}tf$ may not be significantly different from normal Mendelian values, but are significantly different from $t^{w100}tf$ rates in $t^{w100}tf/t^6+$ or $t^{w100}tf/t^0+$ males. The difference among these *t*-haplotypes lies in the presence or absence of a L region. The presence of a L region causes the *t*-haplotype (t^0 or t^6) carrying it to be transmitted in a higher frequency than the $t^{w100}tf$ haplotype. Removal of the L region ($t^{h2}tf$ or $t^{low}tf$) results in an increase in the

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transmission of $t^{w100}tf$. The L region would thus appear to interact better with an A region that is *cis* to it, causing it to be transmitted slightly better than the *t*-haplotype in repulsion.

Quantative interactions within the *t*-complex can be found among its different regions. There exists within the T region a polymorphic system that influences quasi-sterility in the male, with different allelic combinations resulting in quasi-sterility. Transmission ratio distortion appears to be influenced by the presence of enhancing factors that are present in high distorters but lacking in moderate distorters. These enhancing factors influence the *trans* T region modifying factors such that a trans T region derived from a *t* complex is transmitted greater than normal. The L region would appear to influence the *cis* A region, better than a *trans* A region, when two A regions are combined, resulting in higher transmission of the chromosome bearing the L region. These observations suggest that preferential interactions between regions can occur both *cis* and *trans*.

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