

Lethality of heterozygotes between *t*-haplotype complementation groups of mouse: sex-related effect on lethality of t^6/t^{w5} heterozygotes

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SUMMARY

The various lethal *t*-haplotypes are divided into several complementation groups defined by lethality of t^x/t^y heterozygotes, and only partial complementation is usually observed in heterozygotes between *t* complementation groups. Several effects of the *t* region have been found to be preferentially or exclusively detectable in males. This paper describes results showing that the viability of t^6/t^{w5} heterozygotes is influenced by the sex of the embryo; female viability is only slightly reduced (viability ratio approximately 0.80), while male viability is far below normal (0.25).

1. INTRODUCTION

Over 100 *t*-haplotype mutants of the 17th chromosome of *Mus musculus* have been isolated from wild and laboratory mouse populations around the world. Most mutants were initially recognized by their ability to interact with the Brachyury mutant, *T*, of the 17th chromosome to produce tailless *T/t* mice (Dobrovolskaia-Zavadskaia & Kobozeff, 1932), while others were derived from these mutants by recombination, duplication, or deletion (Lyon & Phillips, 1959; Dunn, Bennett & Beasley, 1962; Lyon & Bechtol, 1977; Bechtol & Lyon, 1978). The various lethal *t*-haplotypes have been divided into several complementation groups defined by lethality of t^x/t^y heterozygotes by the time of birth (Dunn, 1956; Bennett, 1975). Those pairs which produce no live long-tailed t^x/t^y are by definition members of the same complementation group; those which produce at least some long-tailed progeny are of different complementation groups. However, only partial complementation is usually observed, that is, t^x/t^y heterozygotes show reduced viability relative to their *T/t^x* or *T/t^y* sibs (Dunn, 1956; Dunn & Gluecksohn-Schoenheimer, 1943; Smith, 1956; Silagi, 1962).

The *t* region has been shown to encompass a large region of the 17th chromosome. It includes *T* and its effect on tail length to the left and *H-2* to the right. Several of the effects of the region, are found to be preferentially or exclusively detectable in males. For example, males, but not females, homozygous for the semilethal *t*

haplotypes (t^{w^2} , t^{w^8} , $t^{w^{36}}$) are sterile (Bennett & Dunn, 1967; Johnston, 1968), with testes reduced in weight, many areas of degeneration in their spermatogenic tubules, and few or no sperm apparent in ejaculates (Johnston, 1968). Many lethal t haplotypes are preferentially transmitted by male but not female heterozygotes (Chesley & Dunn, 1936; Dunn & Gluecksohn-Schoenheimer, 1939), and only males heterozygous for haplotypes of two different lethal complementation groups are sterile (Dunn, 1937). The testes of these sterile males, unlike those of the homozygous semilethals, are of normal weight and sperm are formed in approximately normal numbers, but apparently fail to mature (Bennett & Dunn, 1967; Bennett & Dunn, 1971; Bryson, 1944; McGrath & Hillman, 1980). In contrast to the fertility of the females, males heterozygous for a lethal and a semilethal t haplotype are sterile and produce sperm with reduced motility (Bennett & Dunn, 1967). The quaking (qk) mutation, located approximately 3 centimorgans to the right of T , provides yet another male-specific effect within the t region. Male, but not female, qk/qk homozygotes are sterile, with testicles smaller than normal and an apparent defect in spermatid differentiation resulting in no sperm in their genital tract (Bennett *et al.* 1971). A very different example of a male-influenced effect is that on the expressivity of T on various genetic backgrounds. As shown by Mickova & Ivanyi (1974), the effect of T on tail length is modified by both $H-2$, or a linked gene, and the sex of the embryo. Thus, while the mean tail-length to body-length ratio of $H-2^{b/b}$ and $H-2^{b/k}$ backcross progeny was not different among the female offspring with these two genotypes, there was significant difference in mean tail/body ratio between the two groups of males.

As will be shown here, reduced viability of t^6/t^{w^5} heterozygotes is another trait of the t region unequally expressed among males and females. Female viability among long-tailed t^6/t^{w^5} offspring is only slightly reduced (viability ratio approx. 0.80), but male viability is far below normal (0.25).

2. MATERIALS AND METHODS

Young adults were mated in the combinations shown in Table 1. The phenotype of offspring was determined at 1 to 3 days after birth and was confirmed before weaning. The T/t^{w^5} tailless males were gifts of Dr M. Sherman, Hoffman-LaRoche (last two males Table 1*a* and all males Table 1*b*) and L. Shevinsky, The Wistar Institute (all other males Table 1). These lines were maintained by brother-sister mating and one stock (L.S.) had come from the other (M.S.) two to four generations earlier. Except in three cases the tf/tf long-tailed and T/t^6 tailless females originated from a small closed random-bred colony maintained at the Wistar Institute. The exceptions are the B10.6R tf/tf (tufted) (Lyon, 1956) female in mating 1 and the T/t^6 females in matings 2 and 18 which were sibs from the mating: random-bred $T/tf \times t^6/C3H.SW_N$. Data are recorded (see Table 1) as the numbers of female and male progeny showing each t phenotype: tailless (T/t^6 , T/t^{w^5}), short tailed (T/tf), long tailed (t^{w^5}/tf , t^6/t^{w^5}). T/T is embryonic lethal and is therefore not recorded. The frequency (0.43) of males among T/tf short-tailed progeny is not

significantly different ($P_a > 0.2$) from 0.5. The frequency of males among long-tailed (t^6/t^{w5}) progeny is significantly different from that among their tailless (T/t^6 and T/t^{w5}) sibs ($P_b < 0.001$) and from the frequency (0.43) among T/tf ($P_c < 0.005$). The transmission frequency of t^{w5} from male heterozygotes was calculated from the total recovered progeny of the crosses $tf/tf \times T/t^{w5}$. For determining the viability ratios for t^6/t^{w5} , the expected number of t^6/t^{w5} mice (supposing full viability and a transmission frequency for t^{w5} of 0.81, see Table) was calculated by assuming the number of T/t^6 and T/T progeny to be equal and the number of T/t^{w5} and t^6/t^{w5} progeny to be equal, since there is no distortion of the 50-50 transmission of haplotypes from the female parent. The equation for transmission frequency of t^{w5} thus becomes $(t^6/t^{w5})/(T/t^6 + T/t^{w5}) = (\text{expected number of long-tailed progeny})/(\text{observed number of tailless progeny}) = 0.81$. The viability ratios (observed number of long-tailed progeny/expected number of long-tailed progeny) of t^6/t^{w5} males and females are significantly different ($P_d < 0.001$).

3. RESULTS AND DISCUSSION

While breeding for t^6/t^{w5} heterozygotes for another experiment, it was observed that significantly fewer male heterozygotes than female heterozygotes were recovered. As shown in Table 1, T/t^{w5} males were mated to both tf/tf (tufted) and T/t^6 females. If the viability of males and females is equal within each of the resulting phenotypes of offspring, then it follows that the frequencies of males within each phenotype should be approximately 0.5. This prediction is fulfilled with the exception of the t^6/t^{w5} (long-tailed) progeny of $T/t^6 \times T/t^{w5}$, where the frequency of males is 0.25 ± 0.05 (Table 1c).

The reduced viability of t^x/t^y heterozygotes between two different lethal complementation groups is well known (Bennett, 1975). For each pair of t haplotypes the viability ratio of the t^x/t^y genotype can be calculated by comparing the observed number of long-tailed newborns to the number expected based on the known transmission frequency of the t from the male parent. The high transmission of t^{w5} from males is well recognized (Dunn, 1960; Lyon, Jarvis & Sayers, 1979), and in the present population its mean transmission frequency is 0.81 (Table 1c). The viability ratio for t^6/t^{w5} is, therefore, 0.52 ± 0.06 (Table 1c). This is comparable with the viability ratio of 0.63 previously reported for t^6/t^{w5} by Lyon & Bechtol (1977). If the viability ratio for t^6/t^{w5} is recalculated considering only the female or only the male offspring of the $T/t^6 \times T/t^{w5}$ cross, then a clear difference between male and female survival emerges. Among the female progeny the viability ratio of t^6/t^{w5} is 0.80 ± 0.03 , while among the males the ratio is 0.25 ± 0.04 . The difference between male and female survival is highly significant, with $P < 0.001$.

During the writing of this paper, a literature search uncovered results which show a trend similar to that observed here of excess male lethality. Dunn & Gluecksohn-Schoenheimer noted in 1943 that 'the most reasonable assumption was that some of the normal-tailed zygotes die before birth and that this mortality falls

Table 1. *The t phenotype and sex of offspring from tf/tf × T/t^{w5} and T/t⁶ × T/t^{w5}*

Progeny tail length:	<i>tf/tf</i> ♀ × <i>T/t^{w5}</i> ♂				<i>T/t⁶</i> ♀ × <i>T/t^{w5}</i> ♂					
	Short		Long		(Mating number)		Tailless		Long	
Progeny sex:	♀	♂	♀	♂			♀	♂	♀	♂
(a)	0	2	9	10	(1)	(2)	8	3	3	2
	2	2	10	11	(3)	(4)	16	31	16	5
	4	3	17	9	(5)	(6)	22	23	9	4
	11	8	27	33	(7)	(8)	10	7	3	2
	2	2	8	11	(9)	(10)	5	3	10	1
	15	9	38	34	(11)	(12)	1	1	1	1
	1	2	21	28	(13)	(14)	10	10	3	2
	35	28	130	136			72	78	45	17
	63		266		= 329	212 =	150		62	
(b)	8	4	20	13	(15)					
	1	1	13	7	(16)					
	0	0	2	8	(17)					
	9	5	35	28						
	14		63							
					(18)		5	5	5	0
							5	5	5	0
							10		5	
(c)	44	33	165	164			77	83	50	17
	77		329		= 406	227 =	160		67	
Frequency of males:	0.43 ± 0.05 (0.20 < P _a < 0.25)		0.50 ± 0.03				0.52 ± 0.04		0.25 ± 0.05 (P _b < 0.001 P _c < 0.005)	
Transmission frequency of <i>t^{w5}</i> :	(329/406) = 0.81						Viability rates: Total: 0.52 ± 0.05 ♀: 0.80 ± 0.03 ♂: 0.25 ± 0.04 (P _d < 0.001)			

Each row under a given genotype of mating represents the progeny of a single mating pair, and mating pairs of different genotypes listed in a single row share the same *T/t^{w5}* male. Part c of the Table gives the sum of results including both paired (part a) and unpaired (part b) matings. (See Materials and Methods for description of calculations and significance tests.)

most heavily on the males'. Calculating from their Table 3, the frequency of males among long-tailed, $t^0/t^{1\text{old}}$ offspring of the cross Line 29 (T/t^0) \times Line A ($T/t^{1\text{old}}$) was $93/235 = 0.40 \pm 0.03$, while the frequency of males among the tailless sibs was $222/488 = 0.45 \pm 0.02$. (For explanation of $t^{1\text{old}}$ vs. $t^{1\text{new}}$ see Silagi, 1962.) In the reciprocal matings of Line A \times Line 29, the frequency of males among $t^0/t^{1\text{old}}$ progeny was $48/116 = 0.41 \pm 0.05$, while that among the tailless sibs was $177/353 = 0.50 \pm 0.04$. These results, while showing a much smaller difference in viability of male and female t^x/t^y heterozygotes, nevertheless, support the trend of lower male viability documented in the present publication.

The present study shows that the viability of t^6/t^{w5} heterozygotes is influenced by the sex of the embryo. Silagi (1962) has published a detailed morphologic study of lethality among t^0/t^{12} heterozygotes; however, it is not known whether t^6/t^{w5} resembles t^0/t^{12} . It likewise remains unknown what molecular mechanism(s) is responsible for the lethality of the t^x/t^y heterozygotes or to what extent the autosomal genetic background influences expressivity of this (these) trait(s).

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