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Derivation of mutant *t*-haplotypes of the mouse by presumed duplication or deletion

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SUMMARY

The genetic properties of some new mutant *t*-haplotypes derived from the naturally occurring haplotype t^6 have been investigated. Several mutant haplotypes gave taillessness with brachyury, *T*, were viable when homozygous, and when in compound with t^6, and all these expressed *tf* and appeared to have arisen by meiotic crossing-over between *T* and *tf* in the region of crossover-suppression of t^6. The haplotype t^{h17} arose by crossing-over in a stock of t^6 carrying the translocation T(1;17)190Ca, in which the translocation break in chromosome 17 is within the crossover-suppressing region; hence t^{h17} could not be separated from T190. It was of the complementary crossover type in that it did not interact with *T*, and was lethal in compound with t^6; its male segregation ratio was normal with heterogeneity. A further haplotype, t^{h20}, expressed *tf*, gave taillessness with *T*, and was lethal when homozygous and in compound with t^6. The expression of *tf* was attributed to a deletion, which also covered the loci of the nearby lethals knobbly, *Kb*, and t^{w5}. t^{h20} showed weak complementation of t^{w5}. The haplotype t^{h7} had previously been interpreted as carrying a duplication; it was shown that this duplication was semi-lethal when homozygous. It is suggested that lethal mutant *t*-haplotypes commonly arise by duplication or deletion but such structural changes are not necessarily at the end of the haplotype, and need not arise by unequal meiotic crossing-over.

1. INTRODUCTION

The haplotypes of the *t*-complex of the mouse display an unusual combination of properties (Bennett, 1975; Klein, 1975; Klein & Hammerberg, 1977):

(i) interaction with the mutant gene brachyury, *T*, to give a tailless phenotype in *T*/*t* heterozygotes;

(ii) homozygous lethality;

(iii) abnormal segregation from male *T*/*t* or +/*t* heterozygotes;

(iv) sterility of males heterozygous for two different lethal *t*-haplotypes;

(v) suppression of crossing-over in the segment of chromosome 17 between the *T* and *H-2* regions,

(vi) frequent mutation from one haplotype to another, commonly due to

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crossing-over in the region of crossover suppression (Dunn, Bennett & Beasley 1962; Lyon & Meredith, 1964*a*, *b*; Bennett, Dunn & Artzt, 1976).

The haplotype t^6, which will be discussed here, was discovered in a non-inbred laboratory stock (Carter & Phillips, 1950), and belongs to the t^0 lethal complementation group (Bennett, 1975; Klein, 1975). It strongly suppresses crossing-over

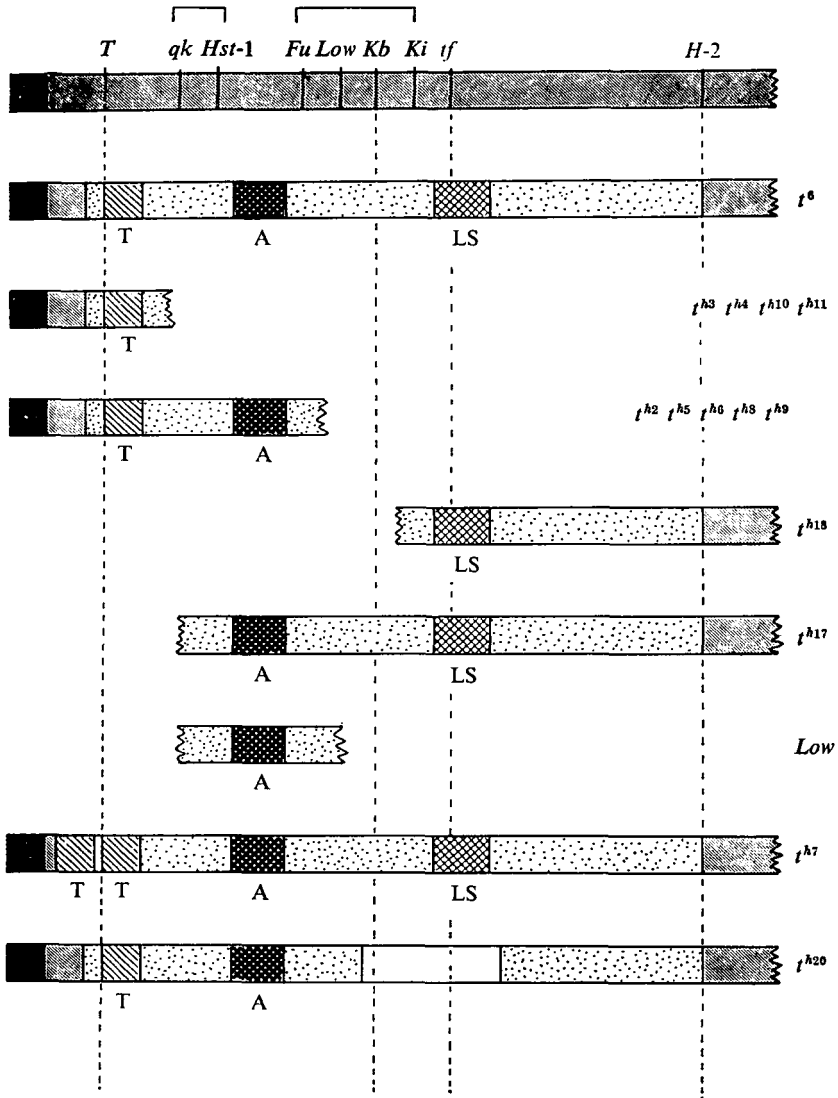

Fig. 1. Representation of the postulated structure of t^6 and its derived haplotypes. The corresponding region of a normal chromosome 17 is depicted on the top line. The abnormal chromatin of the t^6 haplotype (stippled) is believed to extend approximately from the locus of *T* to *H-2*, and includes three factors or regions (hatched): the T-factor responsible for taillessness in *T*/*t* heterozygotes; the A-factor, responsible for abnormal ratio; and the LS-factor, responsible for lethality and male sterility. In the derived haplotypes, arising by crossing-over, duplication or deletion, different combinations of factors are present, and the abnormal chromatin ends at varying points. (First published in *Genetical Research*).

in the *T–tf–H-2* region of chromosome 17 (Lyon & Phillips 1959; Klein & Klein, 1972; Hammerberg & Klein, 1975*b*), and unlike other members of the t^0 group it carries the *H-2* haplotype $H\text{-}2^{tw1}$ (Hammerberg & Klein, 1975*a*). It has a segregation ratio in males of 0·6–0·65, and males carrying t^6 and a complementing lethal are sterile (Bennett, 1975; Klein & Hammerberg, 1977).

Lyon & Meredith (1964*a*, *b*) by studying crossovers derived from the t^6 haplotype were able to locate some of the genetic factors responsible for the various properties of the haplotype. The property of interaction with the mutant gene brachyury, *T*, to produce a tailless phenotype was due to a factor showing no recombination with *T* (the T-factor), whereas homozygous lethality was due to a factor located near the locus of tufted, *tf* (Fig. 1). Mutant haplotypes derived from t^6 could carry either of these factors separately and hence certain mutants could recombine with each other.

Recent further work has led to the view that a factor close to or identical with the lethal factor is responsible for male sterility, so that this factor is now called the LS-factor. In addition, a factor concerned in abnormal male segregation ratio, the A-factor, has been located somewhere between the T- and LS-factors (Fig. 1) (Lyon & Mason, 1977).

One mutant haplotype, t^{h7}, appeared to have arisen not by meiotic crossing-over but by duplication of the T-factor, possibly as a result of unequal sister-strand crossing-over (Lyon & Meredith, 1964*b*). Several further haplotypes were derived from t^{h7} (Fig. 2), presumably by crossing-over either between the halves of the postulated duplication or between the T- and the LS-factor. One of the latter type, t^{h15}, was of interest in that, by crossing-over between the loci of *T* and *tf*, it had lost the t^6 lethal factor, which t^{h7} still carried (Fig. 2). This meant that by studying t^{h7}/t^{h15} or t^{h15}/t^{h15} animals one could determine the phenotypic effect of the postulated duplication of the T-factor. In Lyon & Meredith's (1964*b*) work this phenotype was left in some doubt, and we here present further data, indicating that the duplication had a clear-cut phenotypic effect and acted as a semi-lethal.

There have been further mutant haplotypes derived from t^6 since Lyon & Meredith's report, and these are described here. One, t^{h17}, was a further example of the relatively uncommon type of mutant haplotype, carrying the LS-factor, but not the T-factor. A further one, t^{h20}, was apparently derived by crossing-over between *T* and *tf*, but retained the lethality of its parent haplotype t^6, whereas all other similar crossover haplotypes had lost the lethality. In this paper we present evidence from crosses with nearby lethal loci, that t^{h20} in fact probably includes a small deletion, which may have arisen through unequal sister-strand crossing-over, and carries other lethalities in addition to that of t^6.

2. MATERIALS AND METHODS

The majority of the mutants reported here occurred in the t^6 balanced lethal stock. Animals of genotype $T\ tf/t^6+ \times T\ tf/t^6+$ were mated and offspring were scored at birth for taillessness and at 28 days for tufted (*tf*). Since two of the

expected genotypes, T/T and t^6/t^6, are lethal, and since t^6 suppresses crossing-over between T and tf, then all surviving non-mutant offspring were expected to be genetically T tf/t^6+ like their parents, and phenotypically tailless non-tufted. If a crossover occurred between T and tf this could be detected in a tailless tufted offspring, T $tf/t^m tf$, whereas a mutation to a t-haplotype giving a viable compound with t^6 could be detected in a normal-tailed offspring t^6+/t^m?

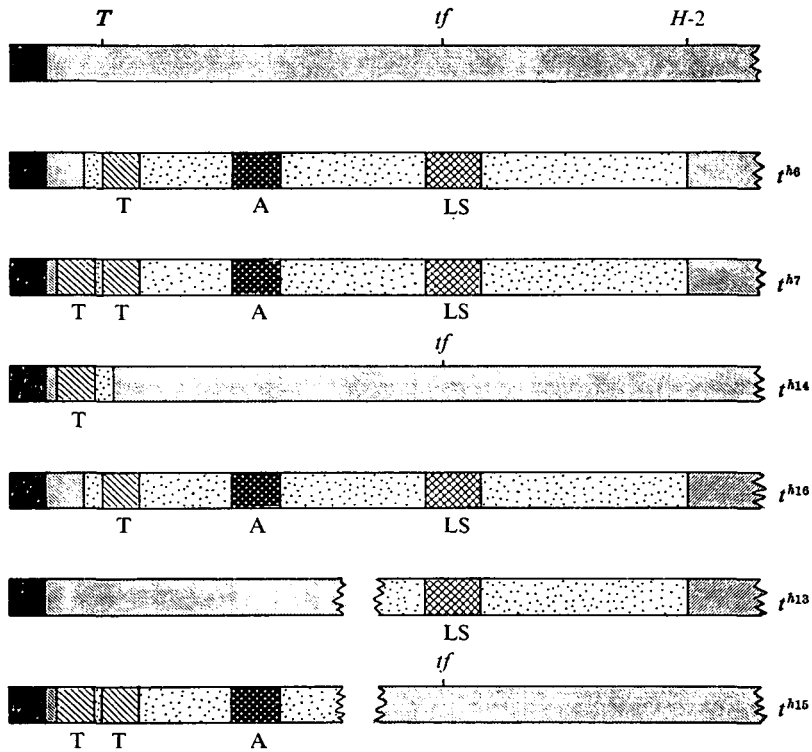

Fig. 2. The postulated structure of t^{h7} and its derived haplotypes. t^{h7} carries a duplication including the T-factor present in t^6. t^{h14} and t^{h16} arose from t^{h7} by crossing-over between the two halves of the duplication. t^{h13} and t^{h15} arose by crossing-over between the A- and LS-factors.

Some additional mutant haplotypes were found in other types of crosses made for a variety of specific purposes, such as linkage tests, or the construction of particular compound genotypes. These mutants were all discovered as unexpected crossovers between T and tf. Three other mutants t^{21}, t^{h17} and t^{h19}, again recovered as unexpected crossovers, were all found in a stock carrying the reciprocal translocation T(1;17)190Ca (Carter, Lyon & Phillips, 1955, 1956). This translocation was induced by X-rays in an animal carrying t^6, and chromosome 17 was broken within the cross-over suppressing region of t^6. The properties of t^6 were retained in the translocation heterozygotes, including the property of mutation through crossing-over (Lyon & Phillips, 1959).

All apparent mutants or crossovers were tested for the presence of tf (if this was not already evident) by crossing to tf/tf. The viability of mutant haplotypes was

tested by crossing T/t^m by T/t^6, and $T/t^m \times T/t^m$ and looking for normal tailed offspring, genetically t^6/t^m or t^m/t^m. Crossover-suppression was tested by mating $T+/t^m tf$ or $Tt^m/+tf$ to $+tf/+tf$. To test segregation ratio in males, T/t^m males were crossed either to $+tf/+tf$ females or to wild-type F_1 hybrids of the cross C3H/HeH × 101/H, and offspring were scored for short ($T/+$) or full length ($+/t$) tails.

The haplotype t^{h20} was also tested for viability against the haplotype t^{w5}, kindly given by Dr J. Archer, who obtained stock from Dr D. Bennett, of Cornell University. In addition, it was crossed to the mutant gene, knobbly, *Kb*, a previously unpublished mutant which arose in a mutagenesis experiment in this laboratory. Knobbly is lethal when homozygous, the Kb/Kb embryos dying soon after implantation, and the locus of *Kb* lies between *T* and *tf*, 1–3 map units from *tf*. Allelism with *Ki* has not been tested.

3. RESULTS

Since the data of Lyon & Meredith (1964*c*) a further 7 mutant haplotypes have been discovered in the t^6 balanced lethal stock; another one, t^{h17}, occurred in the T190 translocation stock, and the haplotype t^{h20} occurred in an experimental cross (Table 1).

Table 1. *Origin of mutant haplotypes from* t^6

		Original animal		Viability	
Stock of origin	Haplotype	Phenotype	Genotype	t^6/t^{hn}	t^{hn}/t^{hn}
$T+/t^6$T190	t^{h17}	Short-tailed, semi-sterile	$T(t^{h17})$T190/++	Lethal	Lethal
$t^{h11}tf/t^{h11}(t^{h18})+ \times Ttf/t^6+$	t^{h20}	Normal tailed, tufted	$t^{h11}tf/t^{h20}tf$	Lethal	Lethal
Ttf/t^6+	t^{h33}	Normal tailed	$t^6+/t^{h33}tf$	Viable	Viable
	t^{h34}	Normal tailed	$t^6+/t^{h34}tf$	Viable	Viable
	t^{h35}	Normal tailed	$t^6+/t^{h35}tf$	Viable	Viable
	t^{h37}	Tailless, tufted	$T\ tf/t^{h37}tf$	Viable	Viable
	t^{h38}	Normal tailed	$t^6+/t^{h38}tf$	Viable	Viable
	t^{h39}	Normal tailed	$t^6+/t^{h39}tf$	Not tested	
	t^{h40}	Normal tailed	$t^6+/t^{h40}tf$	Viable	Viable

The 7 haplotypes found in the $T\ tf/t^6+$ stock had all arisen by crossing-over between *T* and *tf*. All enhanced the expression of *T*, so as to produce tailless T/t heterozygotes, and 6 of the 7 proved viable when homozygous and in compound with t^6 (the remaining one was not tested for viability). These haplotypes therefore agreed well with our earlier results, in which crossing-over within the t^6 haplotype between the loci of *T* and *tf* always separated the *T*-interacting and the lethal properties of t^6; they were not studied further.

The mutation rate in the t^6 stock, including these new mutants, was approximately 5×10^{-3} (Table 2). This is considerably higher than figures found by others for mutation rates of other *t*-haplotypes, which are usually of the order of $1–2 \times 10^{-3}$ (Bennett, 1975; Bennett *et al.* 1976).

Haplotype t^{h17}

This haplotype, which has previously been briefly reported but not numbered (Lyon, 1967), appeared in an exceptional *T* T190/+ + male offspring of a cross of + +/+ + × *T* +/t^6 T190, the T190 break lying within the crossover-suppressing region of t^6. It proved impossible to separate t^{h17} from T190, and thus all tests with this haplotype are complicated by the presence of the translocation. Linkage tests between *T* and *tf* in the presence of t^{h17} (Table 3) indicated that recombination in this region was enhanced in both sexes, the normal figures being 8 % recombination in females, and 6 % in males. A slight enhancement of recombination was also seen in the presence of the similar haplotype t^{h18} (Lyon & Meredith, 1964*a*), but in the present case it seems more likely that the enhancement was due to the translocation T190, since a similar effect occurs with another translocation affecting chromosome 17, T(9;17)138Ca, in the absence of any *t*-haplotypes (Lyon & Phillips, 1959; Klein & Klein, 1972).

Table 2. *Mutant haplotypes derived from* t^6

Source	Mutant type: N-tail	Mutant type: Tufted	Mutant type: Total	Total mice	Mutants per 1000
Stock	8	7	15	2927	5·12
X-ray expt	5	3	8	—	—
Other	—	—	9	—	—
Total			32		

Table 3. *Properties of the haplotype* t^{h17}

Property	Parents: Female	Parents: Male		Offspring: OC	Offspring: RC	Offspring: Recomb.
Crossover suppression	*T*(t^{h17})T190/+*tf*+	+*tf*+/+*tf*+		715	163	17·8 %
	+*tf*+/+*tf*+	*T*(t^{h17})T190/+*tf*+		704	158	18·3 %
Male segregation ratio	+ +/+ +	*T*(t^{h17})T190/+ +		*T*(t^{h17})	+ +	t^{h17}
				399	463	46·3 %
Viability	*T tf*+/t^6+ +	*T*(t^{h17})T190/+*tf*+	*T* t^6+^{tf}	*T*+ +^{tf}	*T*+*tf*	+*t*+^{tf}
			3	3	20	16

OC = parental combinations; RC = recombinations.

Tests of segregation ratio in males indicated that t^{h17} should be regarded as having 'normal ratio with heterogeneity' (Table 3). The overall ratio was near to 0·5, but several individual males gave ratios clearly below 0·5, and others a ratio significantly above 0·5. Haplotypes of the t^4 group also show this type of variability (Bennett, 1975).

If our views on the structure of the *t*-complex are correct, since t^{h17} has lost the proximal and retains the distal end of t^6, it would be expected to carry the t^6 lethal factor. In order to test this a cross of *T tf*+/t^6+ + × *T* +(t^{h17})T190/+*tf*+ + was made. If the t^6/t^{h17} genotype was lethal this cross should yield very few tailless

offspring, these few having arisen after crossing-over between T and t^{h17} in the T190 male parent. Table 3 shows that only 3 tailless offspring were found. These 3 were all proved by genetic test not to carry T190 and since it has proved impossible to separate t^{h17} and T190 it is assumed they did not carry t^{h17}. Thus, these animals had arisen by crossing-over, and the t^6/t^{h17} genotype was presumed to be lethal. It was not possible to make a meaningful test of the viability of t^{h17}/t^{h17}. No viable homozygotes for T190 could be obtained in appropriate crosses, but it is theoretically possible that this was due to a lethal effect of the translocation break, rather than the effect of a t-haplotype. Nevertheless, we felt justified in concluding that t^{h17} carried the t^6 lethal factor.

Haplotype t^{h20}

The haplotype t^{h20} was discovered in an exceptional normal-tailed tufted animal, from a mating of $t^{h11}\ tf/t^{h11}\ (t^{h18})+ \times T\ tf/t^6+$. Assuming that only one mutational event had occurred the original animal would have carried $t^{h11}tf$ and a mutant $t^m\ tf$ haplotype. It was crossed to $T\ tf/++$ and several offspring of the type $T\ tf/t\ tf$ were mated with $T\ tf/t^6+$, in order to test for the occurrence of viable normal-tailed offspring. Of six tested animals 4 produced several normal-tailed as well as tailless offspring, and were assumed to be $T\ tf/t^{h11}tf$. The fifth tested animal was sterile, and the sixth gave tailless offspring only. This last animal was presumed to be genetically $T\ tf/t^mtf$, where t^6/t^m is lethal. The t^m was named t^{h20}, and the present t^{h20} stock is descended from this $T\ tf/t^{h20}tf$ animal. Although the parent mating which gave rise to t^{h20} was between animals carrying different t haplotypes, it is assumed that the mutation occurred in the $T\ tf/t^6+$ parent, since otherwise it would be necessary to postulate two mutational events to explain the normal-tailed tufted phenotype of the original animal.

Table 4. *Viability tests of genotypes involving* t^{h20}

Genotype tested	Parents		Offspring					Conclusion
	Female	Male	Tailless			N-tailed		
t^{h20}/t^{h20}	T/t^{h20}	T/t^{h20}	65			0		Lethal
t^6/t^{h20}	T/t^6	T/t^{h20}	55			0		Lethal
			$T+$	$++$	$Tt+$	$Tt\ tf$	$+tf$	
t^{h18}/t^{h20}	$T(t^{h18})+/t^{h2}tf$	$+tf/t^{h20}tf$	12	0	0	2	32	Lethal
t^{h2}/t^{h20}	$T+/t^{h2}tf$	$++/t^{h20}tf$	0	11	0	17	20	Viable

Table 5. *Properties of the haplotype* t^{h20}

Property	Test mating		$T+$	$+t^{h20}$	t^{h20}
	Female	Male			
Male segregation ratio	$++/++$	$T\ tf/t^{h20}tf$	207	312	60·1 %
	$+tf/+tf$	$T\ tf/t^{h20}tf$	130	249	65·7 %
			OC	RC	Recomb.
Crossover suppression	$T+/t^{h20}tf$	$+tf/+tf$	174	0	0·0 %
	$+tf/+tf$	$T+/t^{h20}tf$	249	1	0·4 %

OC = parental combinations; RC = recombinations.

Further tests on t^{h20} (Table 4) showed that it was lethal both when homozygous and when in compound with t^6 and t^{h18}, but viable with t^{h2}. It strongly suppressed crossing-over between *T* and *tf* (Table 5), and its male segregation ratio (0·60–0·65) was indistinguishable from that of the original haplotype t^6.

In other words t^{h20} appeared up to this point to be an unchanged t^6 haplotype, carrying *tf*. This seemed somewhat surprising, however, in view of evidence that *t*-haplotypes suppress crossing-over up to the *H-2* complex and therefore possibly extend to *H-2*. It seemed possible that t^{h20} had arisen through a small deletion exposing the locus of *tf*, rather than a crossover between *T* and *tf*. To explore this possibility t^{h20} was crossed with two lethals near to the locus of *tf* (and therefore near the postulated deletion), namely the *t*-haplotype t^{w5}, and the mutant knobbly, *Kb*. If a small deletion had occurred in t^{h20}, these recessive lethals might be exposed in heterozygotes with t^{h20}. To serve as controls, t^{w5} was also tested with t^6 and its mutants t^{h18} (with the lethal factor) and t^{h2} (viable), and *Kb* with other lethal *t*-haplotypes.

Heterozygotes between lethal *t*-haplotypes, t^x and t^y, from different complementation groups are characteristically not fully viable and the ratio of the observed to the expected number of t^x/t^y which survive in any cross is defined as the 'complementation factor' (Bennett, 1975). For the cross of t^0 (the complementation group of t^6) with t^{w5} Bennett has found a complementation factor of 0·47. Assuming a male segregation ratio for t^{w5} of 0·9 (Dunn, 1960), Table 6 shows that crosses of t^6 and t^{h18} with t^{w5} gave complementation factors close to Bennett's value. However, in crosses of t^{h20} with t^{w5} very few normal-tailed young (presumed t^{h20}/t^{w5}) were obtained, and the complementation factor was only one seventh of the expected value.

Table 6. *Complementation tests of* t^6 *and derived alleles with* t^{w5}

Females	Offspring			Complementation factor
	Tt+	*Ttf*	*tt*	
Ttf/t^6+	51	—	29	0·63
T(t^{h18})/t^{h2}*tf*	14	2	27*	0·44†
Ttf/t^{h20}*tf*	80		6	0·08

All females mated to *Ttf*/t^{w5} + males

* These animals prove the genotype t^{h2}/t^{w5} to be viable.

† For t^{h18}/t^{w5}.

This shortage of the t^{h20}/t^{w5} type was confirmed by reciprocal crosses of T/t^{w5} females by a T/t^{h20} male, and by crosses of t^6/t^{w5} females by a T/t^{h20} male. Again, few animals of the t^{h20}/t^{w5} class were found. This suggests that t^{h20} indeed involved a deletion of the t^{w5} lethal locus. It would be valuable to establish the genotype of the few surviving presumed t^{h20}/t^{w5} animals by breeding test: unfortunately, however, only one such animal has so far survived beyond the first few days of life, and this was a male which proved sterile. Such poor postnatal viability is characteristic of compounds of weakly complementing *t*-haplotypes (Silagi, 1962).

When $T\ tf/t^{h20}tf$ animals were crossed with $Kb\ tf/+\ +$, no $Kb\ tf/t^{h20}tf$ offspring at all were found (Table 7), whereas crosses of t^{h17}, t^{h18} and t^{w5} with Kb gave the expected number of Kb/t^{h17}, Kb/t^{h18} and Kb/t^{w5} young, which were phenotypically indistinguishable from $Kb/+$. Hence t^{h20} differed from other t^6-mutants in this respect. This suggests that Kb/t^{h20} is lethal as is Kb/Kb, and that t^{h20} involves a deletion covering the locus of Kb.

Table 7. *Viability of compounds of* Kb *with various* t*-haplotypes*

Haplotype tested	Parents	Offspring				Conclusion
		*Kb tf**	*Kb T tf*	*T+*	*++*	
t^{h20}	$+Kb\ tf/+\ +\ +\times T+tf/t^{h20}+tf$	0	4	2	13	
	$T+tf/t^{h20}+tf\times +Kb\ tf/+\ +\ +$	0	20	12	13	Lethal
		Kb tf	*Kb+**	*+tf*	*++*	
t^{h17}	$+t^{h17}$T190$/+tf+\times Kb\ tf+/+tf+$	14	10	9	15	Viable
t^{h18}	$Kb+tf/+\ +tf\times +t^{h18}+/+\ +tf$	11	11	10	12	Viable
		Kb T tf	*Kb+**	*T+*	*++*	
t^{w5}	$Kb\ tf+/+t^{h17}$T$190\times T+tf/t^{w5}+\ +$	1	10	6	1	Viable

* Class of which viability is under test.

In view of this evidence that t^{h20} carried at least three lethalities it was of interest to find the time of death of t^{h20}/t^{h20} embryos. Bennett *et al.* (1959) found that homozygotes for t^{w20} which belongs to the t^{w1} complementation group (death at 11–21 days) but poorly complements the t^0 group (death 6–7 days), died earlier than homozygotes for other haplotypes of the t^{w1} group. To test for a similar effect, $+/t^{h20}$ females, pregnant by $+/t^{h20}$ males, were dissected and the numbers of live and dead embryos were counted (Table 8). There was a marked excess of embryos dead at the 'small mole' stage and no excess loss at any other stage. Thus, one may conclude that t^{h20}/t^{h20} embryos die soon after implantation, as do t^6/t^6, t^{w5}/t^{w5} (Bennett, 1975) and Kb/Kb, i.e. there is no evidence from time of death that t^{h20} carries any lethalities acting earlier than those it is already known to carry.

Table 8. *Time of death of embryos homozygous for* t^{h20} *or* t^{h15}

	Parents	CL	Embryos						Moles
			Imp.	Live	Dead	Large moles	Small moles	Others	Imp. (%)
Test	$+/t^{h20}\times +/t^{h20}$	80	70	38	—	—	32	—	45·7
Control	$+/+\ \times +/t^{h20}$	72	66	61	1	—	4	—	6·1
Test	$T/t^{h15}\times T/t^{h15}$	39	34	17	1	10	6	1*	
	$+/t^{h15}\times T/t^{h15}$	40	28	23	—	1	4	2*	
	$\left.\begin{matrix}t^{h2}/t^{h15}\\ t^{6}/t^{h15}\end{matrix}\right\}\times T/t^{h15}$	15	11	10	—	—	—	—	

CL = corpora lutea; Imp = implanted embryos; * Embryos with cranioschisis.

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Viability of t^{h15}/t^{h15}

Lyon & Meredith (1964*b*) reported that when T/t^{h7} animals were crossed with $T\ tf/t^{h15}tf$ 9 out of 59 offspring showed a typical 'drunken' gait. These animals were small and runted, with reduced eye aperture, and appeared sexually immature, except for one female, which produced a single litter. The genotype of these animals could therefore not be established, but they were presumed to represent the t^{h7}/t^{h15} type. It was postulated that the phenotypic effect of this genotype was due to homozygosity for a duplication including the T-interacting factor of t^6. If this were so, then animals of genotype t^{h15}/t^{h15} should show the same effect.

To test this, matings of $T\ tf/t^{h15}tf \times T\ tf/t^{h15}tf$ have been made. From these matings 6 out of 85 young were of the expected 'drunken' phenotype, small and with reduced eye aperture. In addition no non-drunken t^{h15}/t^{h15} animal was found among various offspring of T/t^{h15} parents which were genetically tested. Bearing in mind the low male segregation ratio of t^{h15} (about 0·20) the expected ratio of T/t^{h15} to t^{h15}/t^{h15} animals from these crosses was $0{\cdot}5:(0{\cdot}2 \times 0{\cdot}5)$, so that one-sixth of the 85 offspring or 14·2 t^{h15}/t^{h15} should have been found. In the crosses to produce t^{h7}/t^{h15}, where $T\ tf/t^{h7}+$ males, with a normal 0·5 segregation ratio, were used the expected number of t^{h7}/t^{h15} was $0{\cdot}33 \times 59$ or 19·7. Thus, the total observed number of 15 'drunken' animals from the two types of mating is approximately half of the expected 33·9 and it is probable that a proportion of the affected animals died prenatally or perinatally.

In crosses expected to produce t^{h15}/t^{h15} some pregnant females were dissected, in an attempt to find the missing affected animals (Table 8). In the crosses of $T/t^{h15} \times T/t^{h15}$ one would expect some T/T embryos, which would die at about 10 days gestation. These are probably represented by the 'large moles' and 'dead embryos' found in this cross. Excluding these, the only characteristic abnormal type found were the three embryos with cranioschisis. These embryos represent 6 % of the total of 50 embryos alive at mid-gestation. Since, owing to the low segregation ratio of t^{h15}, only 17 % of t^{h15}/t^{h15} were expected, these animals with cranioschisis may well represent the missing affected t^{h15}/t^{h15}.

Thus the evidence indicates that the genotypes t^{h7}/t^{h15} and t^{h15}/t^{h15} are both affected by the same semi-lethal syndrome, due to a presumed duplication of a segment of chromosome carrying the T-interaction factor.

4. DISCUSSION

On the basis of the genetics of the mutant *t*-haplotypes until then derived from t^6, Lyon & Meredith (1964*b*) proposed a speculative model of the mode of origin of new *t*-haplotypes in general. It is of interest to consider whether the new haplotypes since discovered provide further support for this model.

It was suggested that all mutants arose by crossing-over, viables by equal crossing-over and lethals by unequal crossing-over, resulting in duplications or deficiencies. Six further viable mutants arising by crossing-over between *T* and *tf* are reported here and Bennett *et al.* (1976) have listed many others. This close

association between loss of lethality and gain of the *tf* marker confirms our earlier finding that the lethality of the t^6-haplotype was due to a factor located near the locus of *tf*. Since Bennett *et al.* found these viable crossover alleles arising from haplotypes of the t^{12}, t^{w1}, t^{w5} and t^{w73} complementation groups also, this suggests that the lethal factors of these groups also lie near the *tf* locus. In contrast the t^9 group, in which all haplotypes permit recombination between *T* and *tf*, probably has a lethal factor lying between these two loci, so that loss of lethality always involves crossing-over, but crossing-over does not always cause loss of lethality.

Our two lethal mutants, t^{h7} and t^{h20}, both appear to have had a somewhat more unusual origin. We postulated earlier that t^{h7} had arisen through a duplication including the T-factor, and we have now confirmed that this postulated duplication produced a phenotypic effect, which could be regarded as semi-lethal, in that some affected animals apparently died pre- or perinatally, and few were fertile. t^{h20} we have postulated to include a small deletion, which covers the loci of *tf*, *Kb* and the t^{w5}-lethal factor. Further work is needed to determine if other *t*-lethal factors are also covered. No visible chromosomal change could be detected in Giemsa banded mitotic preparations (E.P. Evans, personal communication), and therefore the existence of this deletion must remain presumptive. The birth of a few presumed t^{h20}/t^{w5} animals in the complementation tests in fact raises an element of doubt whether a deletion is the correct interpretation. One might expect that as t^{w5}/t^{w5} is lethal in early embryogeny then t^{w5-} would also be lethal, since there can be no molecule to correct the t^{w5} defect. However, the molecular nature of the t^{w5} defect is unknown, and it could possibly be of a type which would be partially corrected by a homologous deletion, and thus our hypothesis of a deletion in t^{h20} is not yet ruled out.

Our data do not indicate the extent of the deletion, since our tests have been limited to a small cluster of loci. However, t^{h20} carries an *H-2* haplotype which does not differ from that of its parent haplotype t^6, and does differ from that of the homologous *tf* chromosome carried in our stocks of *t*-haplotypes (Bechtol & Lyon, in preparation). We therefore conclude that the *H-2* region is not deleted, and furthermore there is no evidence that crossing-over with the homologous chromosome was involved in the formation of t^{h20}. This is the justification for supposing that the expression of tufted by t^{h20} is due to the deletion of the locus of *tf*, rather than the presence of the mutant *tf* allele. Whether the locus of the t^6 lethal factor is also deleted we cannot say.

Our postulate is thus that t^{h20} arose from t^6 by a small deletion near the centre of the abnormal t^6 chromatin, if one assumes that this stretches approximately from the *T* to the *H-2* region. The t^{h20} haplotype thus seems to be a departure from our previous speculative model, which suggested that deletions or duplications would occur at the junction of *t* and normal chromatin, as a result of unequal meiotic crossing-over with the normal homologue. There is no simple mechanism by which a central deletion could arise by meiotic crossing-over. Various possible mechanisms of origin would include chromosome breakage and reunion, or unequal sister-chromatid exchange. This latter suggestion is attractive, since the duplication in

t^{h7} is postulated to have arisen by a similar mechanism, and one could thus explain both our unusual mutants by the same means.

Classically, in mutation from one lethal *t*-haplotype to another, the lethality of the original haplotype is lost simultaneously with the gain of a new lethality. In this respect t^{h7} and t^{h20} are atypical, in that although both have gained new lethalities or semi-lethalities, they have retained the original t^6-lethal factor. Thus, one must consider whether they provide good models for the origin of new lethals. In fact the data on mutation of one lethal to another are relatively sparse. Bennett *et al.* (1976) point out that only 8 cases have arisen in their work, 5 to the t^9, 2 to the t^{w1} and one to the t^{12} group. Of these only two, t^{w30} and t^{w52}, both to the t^9 group, occurred after the introduction of the *tf* marker into the stocks, and hence only in these two cases it is known that recombination with *tf* was involved. It is only since the introduction of *tf* that mutants (such as t^{h20}) which retain the original lethality have been detectable, and it is interesting that Bennett (1975) reported a tufted recombinant from t^{w12} (t^{w1} group) which was not distinguishable from t^{w12}, i.e. the $t^{w12}tf$ haplotype resembled t^{h20} in carrying the lethality of its parent, and t^{h20} is thus in this sense not unique. It is also of interest that the mutant haplotypes t^{w20} and t^{w21} (Bennett *et al.* 1959; Bennett & Dunn, 1964) resemble t^{h20} in another way, by carrying more than one lethality or near-lethality. These two haplotypes arose independently from different stocks of the t^{w5} group, and are both regarded as members of the t^{w1} group, but they complement t^0 very weakly (complementation factors of 12 % and 16 % respectively). This is reminiscent of t^{h20}'s weak complementation of t^{w5}, and Bennett & Dunn (1964) suggest t^{w20} and t^{w21} may involve deletions. Thus, t^{h20} may be by no means unusual as a lethal mutant haplotype. t^{h7} does appear unusual in that it is the only *t*-haplotype so far described which has an altered *T*-interaction factor.

Thus, our studies of t^{h20} and further work on t^{h15} lend support to our speculative model of the origin of new mutant lethal *t*-haplotypes by duplication or deletion, resulting from crossing-over. However, in other respects the data do not fit the model. We suggested that the duplications and deletions were always at the ends of the *t*-haplotypes, which were of various lengths. Any haplotype could only mutate to one shorter than itself, thus leading to polarity of mutation. In fact, except for the t^9 group, there is little evidence for any variation in length of naturally occurring *t*-haplotypes. Hammerberg & Klein (1975*a*) reported characteristic *H-2* haplotypes associated with particular *t*'s together with genetic data (1975*b*), indicating that crossover-suppression extends to *H-2*. Thus probably the *t*-haplotypes physically cover the *T* to *H-2* region, and some may extend for a short distance beyond *H-2* (Hammerberg & Klein, 1975*b*). Since the lethal factors are in all cases (except t^9) apparently located near *tf* they are clearly not terminal, and our postulated deletion in t^{h20} was not terminal.

A new model of *t*-haplotypes and their mutation is therefore needed. Mutant viable haplotypes clearly arise by meiotic crossing-over with the homologous chromosome. Lethal haplotypes may arise, as in the t^9 group, by unequal meiotic crossing-over, or by unequal sister-strand crossing-over, giving duplications or

deletions in any part of the haplotype, or by other means. Some polarity of mutation will result from this, since a haplotype with a particular DNA sequence deleted cannot mutate to one in which this sequence is present. However, the evidence for the existence of polarity now appears rather weak. The t^9 group should be excluded in consideration of polarity, since it seems a clearly shorter haplotype, so that longer haplotypes obviously can mutate to it, but it cannot revert to them. We are then left only with four mutations from one lethal *t*-haplotype to another: from t^{w5} to the t^{12} group in t^{w32}; from t^{w5} to t^{w1} and weak complementation of t^0 in t^{w20} and t^{w21}; and from t^6 to weak complementation of t^{w5} in t^{h20}. Clearly these are too few for polarity of *t*-haplotype mutation to be regarded as an established fact.

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