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Information on the nature of *t*-haplotypes from the interaction of mutant haplotypes in male fertility and segregation ratio

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SUMMARY

The *t*-haplotype t^6 interacts with brachyury, *T*, to produce taillessness, and exhibits lethality, male sterility and high male segregation ratio. Mutant haplotypes derived from t^6 which have lost the lethality also lose the sterility. We here report that two mutants which have retained the lethality have also retained the sterility and suggest that the two properties of lethality and sterility are due to a factor or region of the *t*-haplotype located close to the locus of *tf*. Although t^6 has a high male segregation ratio, mutants derived from it may have low or normal ratios. In trans heterozygotes of two mutant haplotypes the ratios tended to be equalized, whereas in cis heterozygotes a high ratio like t^6 was obtained, at least in one case. On this evidence the low-ratio gene *Low* is reinterpreted as a mutant *t*-haplotype and designated t^{low}. It is suggested that abnormal ratio is due to an *A*-factor or region located between the loci of *T* and *tf*, so that t^6 carries T-, A- and LS-factors which can exist separately and retain their properties. t^6 may extend less proximally than other natural *t*-haplotypes, which in general may involve changes in interstitial heterochromatin.

1. INTRODUCTION

Naturally occurring haplotypes of the *t*-complex of the mouse exhibit a syndrome of characteristic properties:

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

(ii) homozygous lethality or semi-lethality,

(iii) aberrant segregation ratio of + or *T* versus *t* in male +/*t* or *T*/*t* heterozygotes,

(iv) sterility of males heterozygous for two complementing lethals, or homozygous for semi-lethals,

(v) strong suppression of crossing-over, over a region of chromosome 17, about 10–15 cM in length, extending from the locus of *T* to *H-2* (Hammerberg & Klein, 1975).

Mutations of *t*-haplotypes, involving a change in one or more of these properties, occur by crossing-over in the region of crossover suppression between *T* and *tf*, or by other means (Bennett, 1975; Klein, 1975).

Lyon & Meredith (1964) studied the genetics of a number of mutant haplotypes

arising from the haplotype t^6, and were able to identify and locate genetic factors responsible for the interaction with *T*, and the homozygous lethality. The T-interaction or T-factor showed no recombination with the locus of brachyury, *T*, whereas the lethal or L-factor clearly recombined with *T* and was located close to the locus of *tf*, the region between the two factors being occupied by abnormal *t*-chromatin of some kind. Several mutant haplotypes carried the T but not the

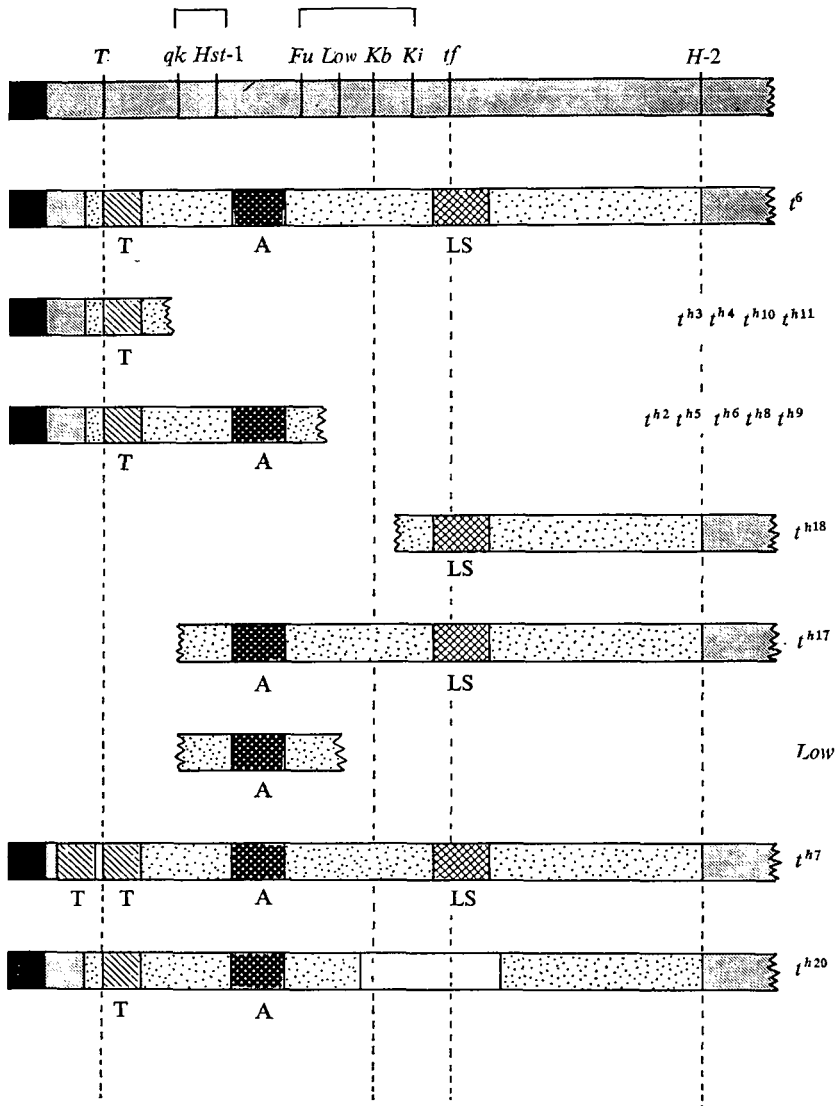

Fig. 1. Postulated structure of t^6 and its derived mutant haplotypes. The known loci in the corresponding region of chromosome 17 of normal mice are indicated in the top line. In the following lines the postulated extent of abnormal *t*-chromatin is shown by a dotted area. The hatched areas marked T, A and LS within the dotted area denote the approximate positions of the factors or regions of the haplotype concerned in T-interaction, abnormal ratio and lethality–sterility, respectively. The centromere is to the left and shown in black.

lethal factor, and thus were viable when homozygous (Fig. 1), and two haplotypes were of the complementary type, retaining the L-factor but having lost the T-factor, so that they were lethal but had no effect on the tail. We have since reported a third haplotype of this kind, t^{h17} (Lyon & Bechtol, 1977). Lyon and Meredith were unable to find the genetic basis for the abnormal male segregation ratio, the male sterility or the crossover suppression, although they did suggest that the abnormal segregation ratio and crossover-suppression were probably not due to factors at a single point, but to multiple factors or the abnormal *t*-chromatin of the whole haplotype.

Subsequently, Dunn & Bennett (1968) described a mutant gene which they called *Low*, which was located between *T* and *tf*, and which had the property of causing a low male segregation ratio of the chromosome on which it lay. *Low* arose in circumstances such that it could have been a mutant *t*-haplotype, derived indirectly from the haplotype t^6, but Dunn and Bennett rejected this explanation, on the grounds that the interactions of *Low* with segregation distorting *t*-haplotypes were different from those hitherto described for combinations of two *t*-haplotypes. We here present evidence that mutants derived from t^6 may in fact interact with other *t*-haplotypes in a way not unlike *Low*. We therefore suggest that *Low* should be reinterpreted as a mutant *t*, exhibiting the property of abnormal male segregation ratio only. If this is accepted it is then possible to analyse further the genetic basis of the segregation distortion, and we suggest the existence of an A-factor for abnormal segregation ratio, situated between the loci of *T* and *tf*, and therefore between the *T*-interaction and lethal factors already described (Fig. 1).

Concerning male sterility, Lyon and Meredith found that all mutants which had lost the lethality of the original allele t^6 had also lost the sterility. This seemed to suggest that the sterility might be due to a factor located close to the L-factor. To test this point we have studied the fertility of males heterozygous for a mutant haplotype including the L-factor (t^{h17} or t^{h18}) and a complementing lethal, t^{w5}.

On the basis of the information derived from these studies we have discussed the nature or structure of *t*-haplotypes and have suggested that t^6 may differ from other naturally occurring *t*-haplotypes at its proximal end, and have speculated that *t*-haplotypes in general involve abnormalities or differences in interstitial heterochromatin.

2. MATERIALS AND METHODS

The properties of the haplotypes derived from t^6 are summarized in Table 1. For convenience haplotypes carrying the T-factor will be referred to as T-end mutants, and those with the L- or LS-factor as L-end mutants. The haplotype t^{w5}, which is lethal with a male segregation ratio of 0·94, and forms male-sterile compounds with other lethals was kindly provided by Dr J. Archer, who obtained it from Dr D. Bennett.

To make animals carrying two mutant haplotypes in the cis-position (e.g. $T\ tf/t^{h2}\ t^{h18}+$) trans heterozygotes (e.g. $t^{h2}\ tf/t^{h18}+$) were crossed to $T\ tf/+tf$ or

$T\ tf/t^6+$. All the tailless offspring would carry the T-end mutant haplotype, in this case t^{h2}, and since t^{h18} shows no recombination with *tf*, all the non-tufted tailless offspring would also carry t^{h18}. Such offspring were kept and checked for t^{h18} by breeding tests, before their experimental use.

Table 1. *Properties of mutant t-haplotypes derived from* t^6

Postulated factors present	Haplotype	Phenotype of T/t	Viability t^m/t^m	Male segregation ratio	Fertility: t^6/t^m or t^m/t^m males	Crossing over *T-tf*
TA.+	t^{h2}, t^{h5} t^{h6}, t^{h8} t^{h9}, t^{h12}	Tailless	Viable	Low	Fertile	Mild suppression
T.++	t^{h3}, t^{h4}, t^{h10} t^{h11}	Tailless	Viable	Normal	Fertile	Mild suppression
++.L	t^{h18}	Short-tailed	Lethal	Normal	—	Mild enhancement
+.AL	t^{h17}	Short-tailed	Lethal	Normal variable	—	Enhancement

To test segregation ratio, males were mated usually to F_1 hybrid females of the type C3H/HeH × 101/H, and allowed to breed continuously, about 40 offspring per male being collected if possible. In some cases males were mated instead to $+tf/+tf$ females.

The males tested for sterility were of the genotypes t^6/t^{w5}, $T(t^{h18})/t^{w5}$, t^{h17}/t^{w5} and t^{h2}/t^{w5}. They were obtained from crosses of $T\ tf/t^6+ \times T\ tf/t^{w5}+$ and $T(t^{h18})+/t^{h2}tf \times T\ tf/t^{w5}+$. In the latter type of cross crossing-over could occur between *T* and t^{h18} and hence the genotypes of the males from this cross were not certain. Males aged 2–3 months were placed with 3 or 4 normal outbred females each, and the females were dissected in mid-pregnancy, or after 1 month if they failed to show signs of being pregnant. The live and dead embryos and corpora lutea were counted.

3. RESULTS

(i) *Segregation ratio*

The viable T-end mutant haplotypes derived from t^6 were divided into those which gave low male segregation of *t* from T/t heterozygotes, and those which gave normal ratios. The two L-end mutants, t^{h17} and t^{h18}, both gave normal ratios, although in the case of t^{h17} there was considerable heterogeneity among males.

However, although t^{h18} had no effect on segregation ratio when alone, when with a low ratio viable allele it very clearly modified the ratio given by that allele (Table 2). Five low-ratio viables were tested, and with three of these t^{h2}, t^{h5} and t^{h6}, the segregation ratios of the trans heterozygotes did not differ from 50%. With t^{h9}, the proportion of t^{h9}-carrying offspring from $T(t^{h18})+/t^{h9}tf$ was well below 50% ($\chi^2_1 = 16{\cdot}9$, $P < 0{\cdot}001$). However, it was significantly higher than in the simple T/t^{h9} heterozygotes ($\chi^2_1 = 14{\cdot}3$, $P < 0{\cdot}001$). Similarly, with t^{h8} the

proportion of t^{h8}-carrying offspring from $T(t^{h18})+/t^{h8}tf$ was significantly higher than in T/t^{h8} heterozygotes ($\chi^2_1 = 21{\cdot}4$, $P < 0{\cdot}001$), although it was still non-significantly below 50% ($\chi^2_1 = 3{\cdot}1$, $P > 0{\cdot}05$). Thus, with all five low-ratio haplotypes t^{h18} modified the segregation ratio, in the direction of normality. By contrast, with the one normal-ratio haplotype tested, t^{h11}, there was no significant change in segregation ratio in the trans heterozygotes ($\chi^2_1 = 0{\cdot}4$, $P > 0{\cdot}5$), although the ratio was marginally above 50% ($\chi^2_1 = 4{\cdot}4$, $0{\cdot}02 < P < 0{\cdot}05$).

Table 2. *Segregation ratios of cis and trans-heterozygotes with* t^{h18}

	Male type and offspring								
	$T+/t^{hn}$			$T(t^{h18})/t^{hn}$			$T\ tf/t^{hn}(t^{h18})$		
Haplotype	T	t^{hn}	% t	T	t^{hn}	% t	T	t^{hn}	% t
t^{h2}	91	22	19·5	103	105	50·5	86	67	43·8
t^{h5}	127	21	14·2	185	184	49·9	—	—	—
t^{h6}	119	34	22·2	98	94	49·0	23	67	74·4
t^{h8}	158	47	22·9	124	98	44·1	—	—	—
t^{h9}	115	26	18·4	153	89	36·8	—	—	—
t^{h11}	91	116	53·8	100	132	56·9	94	89	48·6

Includes data of Lyon & Meredith (1964).

Table 3. *Segregation ratios of* T/t^{hn} *and* $T(t^{h18})/t^{hn}$ *males tested with different types of females*

Male ...	T/t^{hn}						$T(t^{h18})/t^{hn}$					
Female...	+/+			tf/tf			+/+			tf/tf		
Haplotype	T	t	% t	T	t	% t	T	t	% t	T	t	% t
t^{h5}	127	21	14·2	—	—	—	49	57	53·8	136	127	48·3
t^{h6}	53	20	27·4	66	14	17·5	98	91	49·0	—	—	—
t^{h9}	115	26	18·4	—	—	—	27	12	30·8	126	77	37·9

For the haplotypes t^{h2}, t^{h6} and t^{h11} the cis-type heterozygotes with t^{h18} were also studied. Here again, with the two low-ratio alleles t^{h2} and t^{h6} the segregation ratios from the cis double heterozygotes were significantly different from the simple heterozygotes (for t^{h2}, $\chi^2_1 = 17{\cdot}3$, $P < 0{\cdot}001$; for t^{h6}, $\chi^2_1 = 63{\cdot}6$, $P < 0{\cdot}001$). For t^{h2} the ratio from the cis heterozygote was close to 50%, but for t^{h6} it was significantly above normal ($\chi^2_1 = 21{\cdot}5$, $P < 0{\cdot}001$). With the normal ratio haplotype t^{h11} the ratio from cis heterozygotes remained unchanged, at a value close to 50%.

As mentioned earlier, some males were tested with F_1 hybrid females, and others with $+tf/+tf$ females. For some haplotypes both types of females were used with one type of heterozygote (Table 3) and the results showed clearly that the observed differences in segregation ratio did not depend on the type of female used, but on the male.

More limited data on interactions in trans heterozygotes were obtained with

t^{h17} (the other L-end mutant from t^6), and with t^6 itself (Table 4). Like t^{h18}, t^{h17} interacted with the low-ratio viable haplotype t^{h2}, so that the two haplotypes were transmitted with approximately equal frequency from trans heterozygotes. Data from control sibs of the trans heterozygotes, carrying t^{h2} only, show that the changed ratio of t^{h2} was not due to any extraneous effects, e.g. of the genetic background. With t^6, some data were obtained from heterozygotes with the normal

Table 4. *Segregation ratios of trans-heterozygotes with* t^6 *and* t^{h17}

	Male types and offspring					
	T/t^{hn}			t^6/t^{hn}		
	T	t^{hn}	% t^{hn}	t^6	t^{hn}	% t^{hn}
t^{h3}	46	41	47·1	36	9	20·0
t^{h4}	80	90	52·9	31	12	27·9
t^{h6}	119	34	22·2	20	19	48·7
t^{h8}	158	47	22·9	13	10	43·5
t^{h12}	117	28	19·3	35	19	35·2
	$+/t^{hn}$			t^{h17}/t^{hn}		
	+	t^{hn}	% t^{hn}	t^{h17}	t^{hn}	% t^{hn}
t^{h2}	42	7	14·3	37	40	51·9

Includes data of Lyon & Meredith (1964).

Table 5. *Fertility of males carrying* t-*haplotypes*

	Males		Females		Live embryos / Total females
	Total	Fertile	Total	Preg.	
t^6/t^{hn}*	58	58	236	229	5·64
t^{hn}/t^{hn}*	57	55	251	219	4·35
t^6/t^{w5}	6	0	21	0	—
$T(t^{h18})/t^{w5}$	7	2	23	6	—
t^{h2}/t^{w5}	7	6	22	19	4·45
t^{h17}/t^{w5}	2	0	6	0	—

* Ten haplotypes.

ratio haplotypes t^{h3} and t^{h4}, as well as with low-ratio ones. In the case of t^{h3} and t^{h4}, the haplotypes were transmitted with unequal frequencies such that t^6 retained approximately the same segregation ratio as it showed in T/t^6 or $+/t^6$ heterozygotes. With the low ratio haplotypes, however, the effect was similar to that seen with t^{h17} and t^{h18}, in that the segregation ratios approached equality. For t^{h12}, the change in ratios was relatively small and equality was not reached, but with t^{h6} and t^{h8}, although the numbers of offspring were small, it did appear that the segregations were approximately 50%, as found previously in the trans heterozygotes of these two haplotypes with t^{h18}.

(ii) *Male sterility*

All the genetically t^6/t^{w5} males tested were completely sterile (Table 5), as were two t^{h17}/t^{w5} males, but with $T(t^{h18})/t^{w5}$ and t^{h2}/t^{w5} the results were not so clear cut. Five out of seven presumed $T(t^{h18})/t^{w5}$ males were sterile and two fertile; six out of seven t^{h2}/t^{w5} males were apparently normally fertile, but the seventh was sterile; three further t^{h2}/t^{w5} males mated to one female each and allowed to breed continuously were fertile. The probable explanation for these inconsistencies is that the genotypes of the $T(t^{h18})/t^{w5}$ and t^{h2}/t^{w5} males were not certain; but only probable, on account of recombination between t^{h18} and t^{h2} in the parental mating. If one assumes that t^{w5} has a male segregation ratio of 0·9, that t^{h18}/t^{w5} has a viability

	Male	
	$T\ tf$ 0·1	$t^{w5}+$ 0·9
Female		
$T(t^{h18})+$ $\frac{1}{2}\times 0{\cdot}91$	TT dies	Tailless
$t^{h2}+tf$ $\frac{1}{2}\times 0{\cdot}91$	Tailless tufted	Normal tailed
$T+tf$ $\frac{1}{2}\times 0{\cdot}09$	TT dies	Tailless
$t^{h2}(t^{h18})+$ $\frac{1}{2}\times 0{\cdot}09$	Tailless	Normal tailed
Offspring		
Tailless	$T(t^{h18})+/t^{w5}+$ $\frac{1}{2}\times 0{\cdot}91\times 0{\cdot}9\times 0{\cdot}5$	$T+tf/t^{w5}+$ $\frac{1}{2}\times 0{\cdot}09\times 0{\cdot}9$ $t^{h2}(t^{h18})+/T\ tf$ $\frac{1}{2}\times 0{\cdot}09\times 0{\cdot}1$
Normal tailed	$t^{h2}+tf/t^{w5}+$ $\frac{1}{2}\times 0{\cdot}91\times 0{\cdot}9$	$t^{h2}\ (t^{h18})+/t^{w5}+$ $\frac{1}{2}\times 0{\cdot}09\times 0{\cdot}9\times 0{\cdot}5$

Fig. 2. Expected offspring of a mating of $T(t^{h18})/t^{h2}tf$ female by $T\ tf/t^{w5}+$ male.

of 0·5 (Lyon & Bechtol, 1977), and that 9% recombination occurs between t^{h2} and t^{h18} (Lyon & Meredith, 1964) the expected frequencies of the various types of offspring from the mating of $T(t^{h18})+/t^{h2}tf$♀ × $T\ tf/t^{w5}+$♂ are shown in Fig. 2. Of the phenotypically tailless young, 1/5·55 or 18% would be crossover types carrying either t^{h18} or t^{w5} but not both. Thus, to find two such animals in a sample of seven would not be unexpected, and indeed one of the two on genetic test proved to be $T\ tf/t^{w5}+$. Among the normal tailed offspring, however, only 4·7% would be expected to be crossovers carrying both t^{h18} and t^{w5} and hence the one sterile normal-tailed animal out of ten was again not unexpected.

Dunn & Bennett (1969) found that male t^x/t^y compounds which were not fully sterile typically had a very reduced fertility, and they expressed the fertility in terms of offspring per mating unit, where a mating unit was 4 weeks of mating with one known fertile female. In our work the males were left with 3–5 females for 4 weeks, unless the females became pregnant earlier. Thus, the fertility in our work has been expressed in terms of live embryos per female mated, and this

measure should be roughly comparable to Dunn and Bennett's index. A normal figure in our stocks would be approximately five embryos per female. Thus, the t^{h2}/t^{w5} males, even including the sterile animal, had a fertility not appreciably below normal, and the three further animals allowed to breed continuously produced a mean of 4·0 young per mating unit. Also included in Table 5 are data collected previously by Lyon and Meredith on the fertility of viable mutants derived from t^6, both when homozygous t^{hn}/t^{hn} or when heterozygous with t^6. Again the number of live embryos per female, even including sterile animals, was not appreciably below normal.

4. DISCUSSION

This work has shed further light on the genetic bases of both the segregation ratio distortion and the male sterility produced by the natural *t*-haplotype t^6.

We had shown previously that all of ten mutant haplotypes derived from t^6 which had lost its lethality had also lost the male sterility (Lyon & Meredith, 1964). We have now demonstrated that a mutant which retained the lethality, but lost some other effects, also retained the male sterility. This evidence suggests that the male sterility is due to a genetic factor located close to the lethal factor, and perhaps identical with it. It would require the analysis of many more mutants to determine whether the factors for lethality and sterility are indeed identical, but for simplicity it seems appropriate to consider them as so until they are proved separable. A suitable symbol for the lethality–sterility factor would thus be the LS-factor.

Other naturally occurring *t*-haplotypes are also lethal or semi-lethal and give sterile male compounds with other lethals (Bennett, 1975; Klein, 1975), and again derived mutant haplotypes which have lost the lethality have also lost the male sterility. However, there is a major difference between t^6 and other natural haplotypes in that, as we have shown here, mutants from t^6 show full or nearly full male fertility, whereas mutants derived from other haplotypes usually permit only partial fertility, with only 0·1–1·8 offspring per 4 weeks of mating, in contrast to a normal value of 4·7 (Dunn & Bennett, 1969; Bennett & Dunn, 1971). The mutant *Low* discovered by Dunn & Bennett (1968) also permits full male fertility, in trans heterozygotes with a variety of *t*-haplotypes. Bennett & Dunn (1971) therefore considered that it differed in this respect from other *t*-haplotypes studied by them, and this was one of two items of evidence leading them to believe that *Low* was not a *t*-haplotype. However, as previously mentioned, if *Low* was a mutant *t* then it was derived indirectly from t^6. We have just seen that t^6-derived mutants do permit full male fertility in compounds with other *t*-haplotypes and thus the behaviour of *Low* in this respect was in fact fully consistent with it being a mutant *t* derived by crossing-over from t^{h17}, which in turn was derived from t^6.

The second piece of evidence which led Bennett and Dunn to consider *Low* as not a *t*-haplotype concerned its interactions with other haplotypes in segregation ratio. However, this is again a case in which our present work has shown that mutant haplotypes derived from t^6 behave differently from those studied by

Bennett and Dunn. From their data they postulated that the ratios in which two haplotypes were transmitted from a trans heterozygote depended on the ratios they showed in T/t or $+/t$ heterozygotes, such that a *high*-ratio haplotype in a trans heterozygote was transmitted in the same relative ratio as from a T/t or $+/t$ heterozygote, e.g. if the transmission of t^x from T/t^x is 0·65 and of t^y is 0·20 then the ratio of t^x from a t^x/t^y heterozygote will be given by $0{\cdot}65/0{\cdot}5 = x/0{\cdot}20$ or $x = 0{\cdot}26$. In other words, the normally 'high' haplotype will become the 'low' one in a t^x/t^y heterozygote.

In our work the haplotype t^{h18} showed a consistently normal male segregation ratio in $T(t^{h18})/+$ heterozygotes, and hence on the basis of Bennett and Dunn's formula it would be expected not to modify the ratio of other haplotypes in trans heterozygotes. Similarly, t^{h17} had a relatively minor abnormality in segregation ratio in $T(t^{h17})/+$ animals, whereas the haplotype t^6 itself showed a high ratio. Yet in trans heterozygotes all behaved similarly in tending to give equal, or more nearly equal, ratios in heterozygotes with low ratio haplotypes; t^6, by contrast, maintained its own ratio against normal ratio haplotypes. Thus, t^6 and more particularly its mutants t^{h17} and t^{h18} do not follow Bennett and Dunn's rule, and hence the fact that *Low* also departs from the rule does not preclude it from being a t-haplotype. *Low*'s behaviour was basically similar to that of t^6 and its mutants in that in heterozygotes with normal-ratio haplotypes it maintained its own (low) ratio, but with either low-ratio or high-ratio haplotypes the segregations became normalized. It thus seems that *Low*'s behaviour is again fully in accord with that of other t-haplotypes derived from t^6. Since we know that by its origin it could have been a t-haplotype and since it seems highly improbable that a gene independent of the t-complex but having such very similar properties should have mutated in the same stock, we consider that it would be more appropriate from now on to consider *Low* as a mutant t-haplotype, and to give it the new designation t^{low}.

Dunn & Bennett (1971) carried out extensive work on the genetics of t^{low} (calling it *Low*), from which it is clear that it constitutes a previously unknown kind of t-haplotype. It is viable and male-fertile when homozygous, does not interact with brachyury (T), and permits crossing-over between T and *tf*. Thus, of the various properties of the t-complex, it possesses only the segregation distortion, being transmitted in a low ratio from heterozygous males. Linkage studies with T and *tf* showed t^{low} to be located between these loci, and also showed that there was some mild reduction of crossing-over between T and *tf* in t^{low} heterozygotes.

Our interpretation of this is that t^{low} carries a factor for abnormal ratio or A-factor, separated from the T-factor and LS-factor (Fig. 1). The possibility that there might be such a factor, located between T and *tf*, was already indicated by the earlier work (Lyon & Meredith, 1964), which showed two types of viable mutants from t^6, with either normal or low segregation ratios. Those with low ratio may be supposed to carry both the T- and the A-factors, and the remainder would carry the T-factor only. The two LS-end mutants t^{h17} and t^{h18} probably also

differ in this way. Since t^{low} was derived from t^{h17} then t^{h17} must carry the A-factor as well as the LS-factor, and this is consistent with the abnormal segregation ratios shown by some t^{h17} heterozygotes. No t^{h18} heterozygotes have ever been shown to give abnormal ratios, however, and hence this haplotype probably carries the LS-factor only.

Table 6. *Interactions in segregation ratio of* t*-haplotypes carrying various combinations of factors*

Factors present		Haplotypes	Ratios
No A	T. + +/+ + +	t^{h3}, etc./+	Equal
	+ + .L/+ + +	t^{h18}/+	Equal
	T. + +/+ + .L	t^{h11}/t^{h18}	Equal
	T. + .L/+ + +	t^{h11} t^{h18}/+	Equal
A/+	+ .A. +/+ + +	t^{low}/+	*t* low*
	TA. +/+ + +	t^{h2}, etc./+	*t* low
	+ .AL/+ + +	t^{h17}/+	Variable
	TAL/+ + +	t^{6}/+	*t* high
	+ .A. +/T. + +	t^{low}/t^{w62}	t^{low} low*
	TA. +/+ + .L	t^{h2}/t^{h18}	Equal
	TA.L/+ + +	t^{h2} t^{h18}/+	Equal
		t^{h6} t^{h18}/+	High
	TAL/T. + +	t^{6}/t^{h3}, etc.	t^{6} high
A/A	+ .A. +/TA. +	t^{low}/t^{38}	Equal*
	+ .A. +/TAL	t^{low}/t^{1}	Equal*
	TA. +/+ .AL	t^{h2}/t^{h17}	Equal
	TA. +/TAL	t^{h6} etc./t^{6}	Equal

* Bennett & Dunn (1971).

The interactions of the various haplotypes in segregation ratio, both in Bennett and Dunn's work and in our own, provide further insight into the genetic mechanisms by which segregation distortion is produced at least in the t^6 haplotype. The A-factor is essential for the occurrence of an abnormal ratio and in haplotypes, or combinations of haplotypes, which lack this factor the ratios are always normal (Table 6). Examples of such combinations of haplotypes are the cis and trans heterozygotes involving t^{h11} and t^{h18}. If A is present and heterozygous, however, the ratio observed depends on the other factors present, and whether they are in the cis or trans configuration. The A-factor alone gives a low ratio, as does the TA combination in t^{h2}, t^{h5}, t^{h6}, etc. This suggests that the LS-factor, in combination with A, might be responsible for the high ratio seen in t^6 itself. However, t^{h17}, in which A- and LS-factors are combined, does not consistently show a high ratio, but merely a variable one. Thus, it appears that both the T-factor (or some factor close to it) and the LS-factor can affect the observed ratio.

The equalisation of ratios in trans heterozygotes of t^{low} and other haplotypes might be attributed to homozygosity for the A-factor. However, the same effect appears in trans heterozygotes with t^{h18}, which is thought to carry the LS-factor only. Thus, the LS-factor (or nearby region) can interact with other factors to vary the ratio not only when in the cis but also when in the trans position. By

contrast, although as previously mentioned the T-factor does seem to be important in determining the high ratio of t^6 itself, when it is isolated as in t^{h11}, t^{h3} and t^{h4} it produces no effect in trans heterozygotes, either in our own work or in Bennett and Dunn's work with t^{low}.

It was interesting that in the cis heterozygote involving t^{h6} and t^{h18} the high segregation ratio of the original haplotype t^6 was restored, providing some evidence that in t^6 the ratio distortion is indeed produced by cis interactions among the various regions of the haplotype. However, in t^{h2}–t^{h18} cis heterozygotes the high ratio was not restored, although the T-, A- and LS-factors were all present in the cis configuration. This seems to indicate that these three known factors are not the only parts of the t^6 haplotype which are involved in segregation ratio distortion. t^{h2} and t^{h6} presumably differ in the point at which the abnormal t-chromatin ends, between the A- and LS-factors, and it must be this that affects their behaviour in cis heterozygotes with t^{h18}.

It is also necessary to find some explanation for the rather different interactions of the t^6-derived haplotypes, from those of the haplotypes studied by Bennett and Dunn. First, we may point out that isolated A-factors and LS-factors, as in t^{low} and t^{h18}, have not yet been obtained from other haplotypes and therefore we cannot say whether the behaviour of these particular factors is in any way unusual. The interactions to be considered are those between T-end viable factors such as t^{h2} to t^{h12} with t^6 or other lethal haplotypes. In trans heteroyzgotes of this kind involving t^6-derived factors the tendency was towards equalization of ratios, and the males were fully fertile, whereas in the trans heterozygotes involving viable haplotypes studied by Bennett and Dunn the males were in general semi-sterile and the segregation ratio distortions were in some cases exacerbated and in some other cases reversed. This suggests that the proximal or T-end of t^6 (which constitutes t^{h2} to t^{h12}) differs in some way from the proximal end of other natural haplotypes. Such a difference might be qualitative, i.e. in the actual nature of the DNA sequences involved, or quantitative, in the extent of t-chromatin present to the left of the T-locus. Hammerberg & Klein (1974) have suggested that different natural haplotypes may extend for slightly different distances beyond the *H-2* locus at their right or distal ends; there is at present no evidence concerning the exact positions of the left or proximal ends of any of the t-haplotypes. We should like to suggest as a working hypothesis that most natural t-haplotypes have a region or factor to the left of the T-locus, which is involved in male sterility and segregation distortion. We further suggest that t^6 has normal chromatin in this region, and that this difference accounts for its lower segregation ratio (0·6–0·65) than other natural haplotypes, and the different behaviour of its mutants in interactions.

The molecular abnormality in the chromatin of t-haplotypes remains unknown, but the finding that in t^{low} a central or non-terminal region of the haplotype can exist alone and retain its properties (segregation distortion and crossover suppression) helps to reduce the possibilities for conjecture. There is no evidence for major chromosomal structural changes (Womack & Roderick, 1974; E. P. Evans,

personal communication), and thus the mind turns to functional changes such as those produced by heterochromatin. However, since the haplotypes, or at least t^6, can be broken into parts which retain their properties the effects cannot be explained by a single long range position effect, nor even by two such effects, operating from each end. Short-range effects, operating over only a few functional units (and hence needing to be multiple) cannot of course be ruled out. At present the most likely basis for the abnormality in *t*-haplotypes would seem to be a change in interstitial heterochromatin, in the form of moderately repetitious DNA. The change is presumably repeated at intervals many times over the length of the haplotype, but it would be premature to speculate any further concerning the exact nature of the change in repetitious DNA, or the means by which it brings about the observed genetic properties of the haplotype.

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REFERENCES

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

BENNETT, D. & DUNN, L. C. (1971). Transmission ratio distorting genes on chromosome IX and their interaction. In *Proc. Symp. Immunogenetics of the H-2 System*, pp. 90–103. Basel: Karger.

DUNN, L. C. & BENNETT, D. (1968). A new case of transmission ratio distortion in the house mouse. *Proceedings of the National Academy of Sciences* **61**, 570–573.

DUNN, L. C. & BENNETT, D. (1969). Studies of effects of *t*-alleles in the house mouse on spermatozoa. II. Quasi-sterility caused by different combinations of alleles. *Journal of Reproduction and Fertility* **20**, 239–246.

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

HAMMERBERG, C. & KLEIN, J. (1975). Linkage relationships of markers on chromosome 17 of the house mouse. *Genetical Research* **26**, 203–211.

KLEIN, J. (1975). *Biology of the Mouse Histocompatibility-2 Complex*. Berlin: Springer-Verlag.

LYON, M. F. & BECHTOL, K. (1977). Derivation of mutant *t*-haplotypes of the mouse by presumed duplication or deletion. *Genetical Research* (in the Press).

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.

WOMACK, J. E. & RODERICK, T. H. (1974). T-alleles in the mouse are probably not inversions. *Journal of Heredity* **65**, 308–310.