Genet. Res., Camb. (1979), **33**, *pp.* 153–161
With 3 text-figures
Printed in Great Britain

Complementation reactions of a lethal mouse *t*-haplotype believed to include a deletion

By MARY F. LYON, SUSAN E. JARVIS, IRENE SAYERS
MRC Radiobiology Unit, Harwell, Didcot, Oxon., U.K.
AND D. R. JOHNSON
Department of Anatomy, University of Leeds, U.K.

(*Received* 27 *November* 1978)

SUMMARY

The lethal *t*-haplotypes of the mouse fall into several distinct complementation groups, and varying degrees of partial complementation occur when haplotypes from different groups are crossed. The haplotype t^{h20} arose as a mutant from the haplotype t^6, and is thought to include a small deletion covering loci near the *t*-lethals. In this paper the complementarity of t^{h20} was compared with that of its parent haplotype t^6. t^{h20} showed weaker complementation than t^6 of the haplotypes t^{w5}, t^{w32} and t^{w1}. Hence the loci of these lethals were probably exposed by the deletion in t^{h20}. However, t^{h20} showed full complementation of t^{w18} and showed full viability in compound with the dominant brachyury allele hairpin-tail, T^{hp}. Therefore, the t^{h20} deletion probably does not overlap the presumed deletion in T^{hp}, nor extend to the locus of the t^{w18} lethal. The significance of the weak complementation of t^{w5}, t^{w32} and t^{w1} is discussed.

1. INTRODUCTION

The naturally occurring *t*-haplotypes of the mouse present a group of enigmatic features (Table 1), one of which concerns the partial complementation of lethal *t*-haplotypes from different complementation groups. The present known lethals are divided into six groups (Table 2). Dunn (1957) showed that lethals within a group do not complement each other so that, in crosses of T/t^{lx}xT/t^{ly}, t^{lx}/t^{ly} young die. However, lethals of different groups show varying degrees of complementation, so that some t^{lx}/t^{ly} young are found, but fewer than would be expected if this genotype had full normal viability. Bennett (1975) in reviewing this problem, pointed out that crosses of particular groups show characteristic levels of complementation, but in addition to this, individual haplotypes within a group may differ from each other in complementation reactions, e.g. all haplotypes of the t^4 group show full complementation with haplotypes of all other groups. However, haplotypes of the t^{w1} group differ markedly in complementation of the t^o group, the relative viability of the t^o/t^x compounds at birth being: t^{w1}, 85%; t^{w3}, 90%, t^{w12}, 59%; t^{w20}, 12%; t^{w21}, 16% (Bennett & Dunn, 1964; Bennett, 1975). Lyon & Bechtol (1977) studied the mutant haplotype t^{h20}, derived from t^6 (a member of the

0016–6723/79/2828–7310 $01.00

t^0 complementation group). t^{h20} was thought to include a small deletion, which exposed the loci of tufted, *tf*, and a nearby lethal gene knobbly, *Kb* (Lyon, 1978) (Fig. 1). The loci of the *t*-lethals (except the t^4 group) lie close to the *tf* locus and it was thus of interest to know whether the deletion in t^{h20} covered the *t*-lethals. t^{h20}

Table 1. *Properties of naturally occurring* t-*haplotypes of the mouse*

Property
(1) Interaction with mutant gene brachyury, *T*, to produce taillessness in T/t heterozygotes
(2) Homozygous lethality or semi-lethality of t/t, the lethals falling into six known complementation groups
(3) Distorted transmission ratios of *t* from T/t or $+/t$ males
(4) Sterility of males homozygous for semi-lethals, or heterozygous for complementing lethals, t^x/t^y
(5) Suppression of crossing-over in the region of chromosome 17 between the loci of *T* and *H*-2
(6) High 'mutation' rate of one *t*-haplotype to another, usually accompanied by crossing-over in the region of crossover suppression.

Table 2. *Complementation groups of lethal* t-*haplotypes* (*Bennett*, 1975)

Group	Members of group
t^0	t^0, t^6
t^4	t^4, t^9, t^{w18}
t^{12}	t^{12}, t^{w32}
t^{w1}	t^{w1}, t^{w3}, t^{w12}, t^{w20}, t^{w21}
t^{w5}	t^{w5}, t^{w15}, t^{w17}, t^{w75} (and many others).
t^{w73}	t^{w73}

showed no complementation of t^6, from which it was derived, but it differed from t^6 in giving very weak complementation of t^{w5}. Lyon & Bechtol therefore concluded that the deletion covered the locus of the t^{w5} lethal factor. The concept of a deletion (rather than a crossover) in t^{h20} was further strengthened by the finding that its distal end retained the *H*-2 haplotype of its parent t^6 (Bechtol & Lyon, 1978). It was clearly of interest to study the complementation reactions of t^{h20} further.

We report here that t^{h20} gives weaker complementation than t^6 of t^{w1} and t^{w32}, as well as t^{w5}, suggesting that sequences involved in all three of these complementation groups are deleted in t^{h20}. On the other hand, t^{h20} shows full complementation of t^{w18}, a member of the t^4 group. The locus of this lethal is thought to lie a few units proximal to *tf* (Figs. 1, 3). It would appear that the presumed deletion in t^{h20} does not extend to the locus of t^{w18} (t^4) lethal. Crosses were also made between t^{h20} and the dominant allele at the brachyury locus, hairpin tail T^{hp} (Johnson, 1974, 1975) which is thought to involve a deletion covering the loci of *T* and *qk* (Bennett, 1975). Since at least one viable fertile T^{hp}/t^{h20} animal was obtained the deletions involved in T^{hp} and t^{h20} probably do not overlap.

2. MATERIALS AND METHODS

The haplotype t^{w32} was obtained from a breeding nucleus provided by Dr Lynn Fraser, MRC Clinical Research Centre, Harrow, and breeding nuclei of t^{w1} and t^{w18} were kindly given by Dr Jean Louis Guénet, Institut Pasteur, Paris. Dr David Johnson, Department of Anatomy, Leeds sent animals carrying hairpin-tail, $T^{hp}/+$, to Harwell. All these genes were maintained on essentially the same genetic

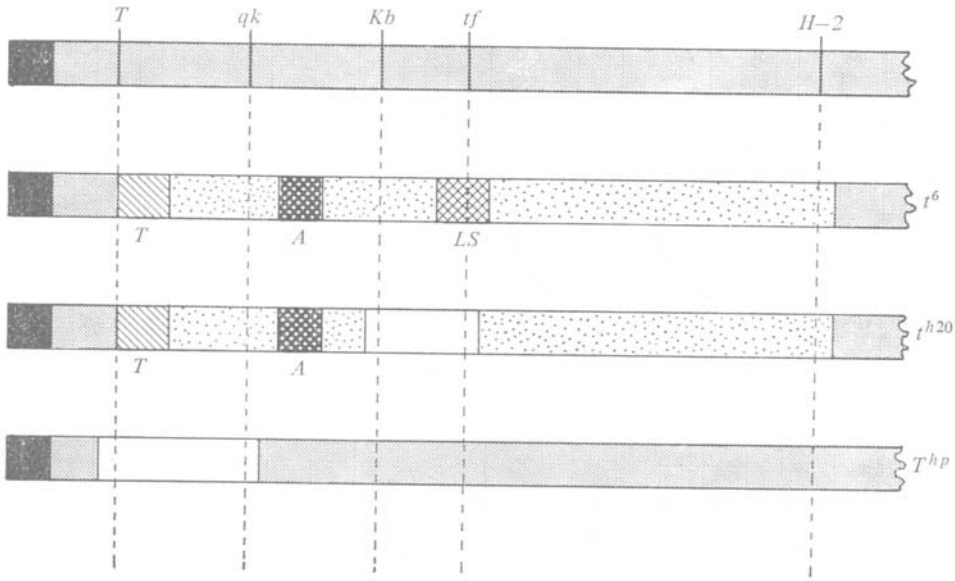

Fig. 1. Postulated structure of the haplotype t^6, its location on chromosome 17 relative to known loci, and the relation to it of t^{h20} and T^{hp}. The abnormal chromatin in t^6 is believed to extend approximately from the locus of brachyury, *T*, to *H*-2. The property of interaction with brachyury is believed to be due to a T-factor, located near the brachyury locus, lethality is due to the LS-factor located near *tf*, and abnormal male transmission ratio requires the presence of the A-factor located between *T* and *tf* (Lyon & Mason, 1977; Lyon & Bechtol, 1977). The presumed deletions in t^{h20} and T^{hp} are shown as white areas.

background as when received by us, and hence must be considered to differ from each other in background. Similarly, the haplotypes t^6 and t^{h20}, obtained from our own stocks, were maintained on non-inbred background. Although t^{h20} was originally derived from t^6, this event had occurred many generations earlier and the two stocks must by now have diverged in background.

Animals were classified for tail-length within a few days (usually 1 day) after birth, and for tufted, *tf*, at approximately 4 weeks. Any doubtful animals were re-examined, and their genotypes were if necessary checked by breeding tests.

In calculating complementation factors it is necessary to know the transmission ratio of the haplotype carried by the male parent. Bennett (1975) gives the transmission ratios characteristic of each complementation group, but also states that these ratios can at times vary unpredictably. Therefore, in this work the transmission ratios were checked, either in the actual males used for the tests, or in

closely related males from the same stock. For t^{w5} and t^{w32} some tests were made by crossing $Ttf/t^{w5}+$ or $Ttf/t^{w32}+$ males to normal $+/+^t$ females and counting the proportions of $T/+$ and $+/t$ offspring. In addition, for t^{w5}, t^{w32} and t^{w1} transmission ratio was estimated within the complementation tests themselves by comparing the frequencies of tailless tufted and tailless non-tufted young. In a cross of $Ttf/t^{h20}tf$ by $Ttf/t^{w1}+$, since both t^{h20} and t^{w1} strongly suppress crossing-over, the tailless tufted young will carry t^{h20} and the tailless non-tufteds t^{w1}. Hence the transmission of t^{w1} can be calculated.

3. RESULTS

As previously mentioned t^6 belongs to the t^0 group. Although the complementarity of this with other groups was already known, t^6 itself had apparently not been tested in this way. Hence, in each test the behaviour of t^6 and t^{h20} was compared.

The male transmission ratios of t^{w5}, t^{w32} and t^{w1} found in our tests were within the range of published values (Table 3) (Bennett, 1975; Dunn, 1960; Bennett & Dunn, 1964). In choosing a value to use in calculating complementation factors, the figures were rounded to the nearest 5%, since any greater precision would probably be unrealistic.

Varying percentages of t^6/t^n offspring were obtained in crosses with the different haplotypes (Table 4). The complementation factor, also shown in Table 4, was calculated on the basis that it corresponded with the viability of the t^6/t^n type. Figure 2 shows that if the male segregation ratio of the haplotype under test is r and the viability of t^6/t^n is v, then v is given by no. of t/t young/(no. of $T/t \times r$). The values for the complementation factor in Table 4 are obtained by equating this factor to v. The complementation factors of t^6 with t^{w5}, t^{w1} and t^{w18} were reasonably close to values given previously by Bennett (1975) for t^0 with these haplotypes (47, 85 and 88% respectively), particularly in view of the uncertainties imposed by variation among males in transmission ratio. The high complementation of t^6 and t^{w1} was confirmed in reciprocal crosses of $Ttf/t^{w1}+$ females with Ttf/t^6+ males, which gave $24T/t{:}14t/t$ offspring ($v = 0{\cdot}90$). However, with t^{w32} the complementation factor of 0·61 was considerably higher than the figure of 18% given by Bennett for complementation of t^0 and t^{12}. In fact, the percentage t/t young (and hence the complementation) in the crosses of t^6 with t^{w32} did not differ significantly from that in t^{w5} crosses ($\chi^2_1 = 0{\cdot}0007$).

When $Ttf/t^{h20}tf$ females were used the complementation with t^{w5}, t^{w32} and t^{w1} was in each case lower than in the corrresponding test with t^6 and the differences in percentage t/t were statistically significant (t^{w5}, $\chi^2_1 = 21{\cdot}3$; t^{w32}, $\chi^2_1 = 50{\cdot}9$; t^{w1}, $\chi^2_1 = 9{\cdot}3$). With t^{w5} and t^{w32} only a very small number of t/t offspring were born, so that the complementation factors were very low, below 0·10 in each case. Although the percentage t/t appeared lower with t^{w32} the difference from the results with t^{w5} was not statistically significant ($\chi^2_1 = 1{\cdot}9$, with Yates correction). Thus, t^{w5} and t^{w32} essentially did not differ from each other in complementation of t^6 and t^{h20}. However, t^{w1}, which as mentioned above gave higher complementation

Table 3. *Male transmission ratios of haplotypes used in complementation tests*

Female parent	Male parent	Tailless tufted (T/t^{h20})	Tailless non-tufted (T/t^{n})	Proportion t^{n}
$Ttf/t^{h20}tf$	$Ttf/t^{w5}+$	4	30	0·88
	$Ttf/t^{w32}+$	1	67	0·98
	$Ttf/t^{w1}+$	13	52	0·80
		Short-tailed	Normal tailed	
$+/+$	$Ttf/t^{w5}+$	7	21	0·75
	$Ttf/t^{w32}+$	12	138	0·92

Table 4. *Offspring of complementation tests of* t^{6} *and* t^{h20} *with various* t-*haplotypes, using* $Ttf/t^{6}+$ *or* $Ttf/t^{h20}tf$ *females by* $T\ tf/t^{n}+$ *males*

		Female $Ttf/t^{6}+$				Female $Ttf/t^{h20}tf$			
Haplotype	Segregation ratio	Offspring *Tt*	Offspring *tt*	Percentage *tt*	Comp. factor	Offspring *Tt*	Offspring *tt*	Percentage *tt*	Comp. factor
t^{w5}*	0·90	51	29	36	0·63	80	6	7·0	0·08
t^{w32}	0·95	101	57	36	0·59	131	3	2·2	0·024
t^{w1}	0·80	55	50	48	>1·00	75	28	27	0·47
t^{w18}	0·50	32	22	41	>1·00	—	—	—	—

* Data for this haplotype includes that of Lyon & Bechtol (1977).

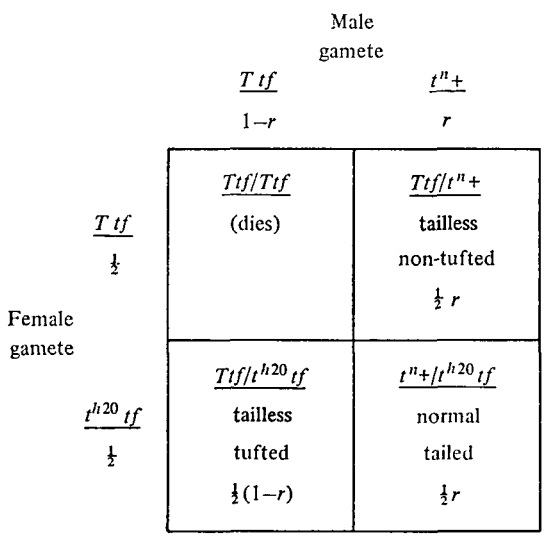

Fig. 2. Gametes and zygotes produced in a cross of $Ttf/t^{h20}tf$ female by $Ttf/t^{n}+$ male if the male transmission ratio of t^{n} is r. The total proportion of tailless offspring is $\frac{1}{2}(1-r+r)$ and hence if the viability of t^{n}/t^{h20} is v the ratio of $t/t:T/t$ offspring is $\frac{1}{2}rv:\frac{1}{2}$.

https://doi.org/10.1017/S0016672300018280 Published online by Cambridge University Press

of t^6 ($v = >1{\cdot}0$) also gave statistically significantly higher complementation of t^{h20}, with $v = 0{\cdot}45$ (t^{w5} *v.* t^{w1}, $\chi^2_1 = 13{\cdot}0$; t^{w32} *v.* t^{w1}, $\chi^2_1 = 31{\cdot}9$).

To provide further information on the weak complementarity of t^{h20} some reciprocal crosses were made using Ttf/t^{h20} males with females carrying t^{w5}, t^{w32} or t^{w1} (Table 5). In each case the complementation factor was similar to that found in the reciprocal cross. With t^{w5} some data were available from crosses of $t^6+/t^{w5}+$ females with $Ttf/t^{h20}tf$ males; 46 T/t and 4 t^{w5}/t^{h20} offspring were born, again giving a very low complementation factor.

Table 5. *Further data on complementation tests of* t^{h20} *with various* t*-haplotypes, using* Ttf/t^n+ *females with* Ttf/t^{h20} *males* (*segregation ratio* 0·65)

Female	Offspring *Tt*	Offspring *tt*	Percentage *tt*	Comp. factor
$Ttf/t^{w5}+$	30	3	9·1	0·15
$Ttf/t^{w32}+$	30	1	3·2	0·05
$Ttf/t^{w1}+$	55	24	30	0·67
$Ttf/t^{w18}+$	28	28	50	>1·00

Lyon & Bechtol (1977) reported that t^{h20} gave no complementation of t^6. However, only 55 offspring of $Ttf/t^6 \times Ttf/t^{h20}+$ matings were studied. In view of the very few t/t young born in crosses of t^{h20} with t^{w5} and t^{w32} it seemed advisable to extend the data with t^6. Accordingly, a further 78 young were obtained in crosses of $Ttf/t^6+ \times Ttf/t^{h20}+$ and no live t/t animals were found.

The complementation test between t^{w18} and t^{h20} was made in only one direction, using $Ttf/t^{w18}+$ females and $Ttf/t^{h20}tf$ males (Table 5). This test indicated full complementation between these two haplotypes, with 50% of young born being t/t. Thus, t^{w18} differs from the other haplotypes tested in giving as high a complementation of t^{h20} as of t^6.

The t^{h20} haplotype was crossed with hairpin-tail, T^{hp}, in order to test whether T^{hp}/t^{h20} animals were viable, and hence to deduce whether the presumed deletions included in T^{hp} and t^{h20} overlapped. As a control the viability of T^{hp} with other *t*-haplotypes was tested also (Table 6). In all cases, including t^{h20}, viable T^{hp}/t^n offspring were obtained. In the case of t^{h20}, in addition to crosses of Ttf/t^{h20} by $T^{hp}+/+tf$, 5 $+tf/t^{h20}tf$ females were used. (Table 6, last two lines). The offspring of these matings were expected to be T^{hp}/t^{h20}, $T/+$ and $+tf/t^{h20}tf$ or $+tf/+tf$ in the ratio 1:1:2. Considering all five females together there was a deficiency of T^{hp}/t^{h20} young. However, this shortage was entirely accounted for by two females which produced no T^{hp}/t^{h20} offspring. The simplest explanation would appear to be an error in the ascription of genotype to these two females. If they were $+tf/+tf$ rather than $+tf/t^{h20}tf$ then no T^{hp}/t^{h20} offspring would be expected and the ratio of short-tailed ($T/+$) to normal tailed ($+/+$) offspring would be 1:1, close to the observed value of 16:21. Thus, it seems probable that the T^{hp}/t^{h20} genotype has full viability to the time of birth. The females used in these crosses

had rather poor mothering ability and relatively few young of any genotype were reared to weaning age. However, one T^{hp}/t^{h20} male was reared to maturity and proved fertile. When mated to normal (+/+) females he produced 16 short-tailed ($T^{hp}/+$) and 66 normal-tailed ($+/t^{h20}$) offspring, a transmission ratio of 80·5% t^{h20}. This is higher than the ratio of 65% usually found with t^{h20} but, as individual males of any *t*-haplotype are known to vary somewhat in segregation ratio, no importance can be attached to this.

Table 6. *Occurrence of* T^{hp}/t^{n} *offspring after matings of various females with* $T^{hp}+/+tf$ *males*

		Offspring		
Female		$T^{hp}t^{n}$	$T+$	$+t^{n}$
$Ttf/t^{6}+$		4	4	8
$Ttf/t^{w5}+$		5	4	4
$Ttf/t^{w32}+$		5	15	7
$Ttf/t^{w18}+$		3	6	6
$Ttf/t^{h20}tf$		4	5	5
$+tf/t^{h20}tf$	A(3*)	16	16	44
	B(2)	—	16	21

* no. of females.

4. DISCUSSION

This work extends and confirms our earlier finding (Lyon & Bechtol, 1977) that t^{h20} differs from t^{6} in the complementation of *t*-haplotypes of other groups. This helps to confirm that in t^{h20} some alteration, such as a deletion, has occurred which involves the loci of *tf*, *Kb* and the *t*-lethal factors t^{w5}, t^{w1} and t^{w32}.

Although the change is presumed to be a deletion the present work provides no further evidence on this point. Since T^{hp}/t^{h20} apparently had normal viability it is probable that the t^{h20} deletion does not overlap the presumed deletion in T^{hp}. The T^{hp} deletion extends from the locus of brachyury, *T*, to beyond quaking, *qk* (Bennett, 1975). Therefore, if T^{hp} and t^{h20} do not overlap, then the t^{h20} deletion does not extend proximally as far as *qk*.

The complementation of t^{w18} by t^{h20} was complete, and equal to that of its parent haplotype, t^{6}. Haplotypes of the t^{4} group, to which t^{w18} belongs, all fully complement *t*-lethals of other groups. Furthermore, they permit recombination between *T* and *tf*. It is therefore thought that the lethal factor of the t^{4} group lies at a different locus from the other lethals, somewhere between *T* and *tf*. The full complementation of t^{w18} and t^{h20} suggests that the deletion in t^{h20} does not extend to the t^{w18} lethal locus. As T^{hp}/t^{w18} also is viable it seems probable that the T^{hp} deletion too does not cover the t^{w18} lethal locus, which thus lies between the T^{hp} and t^{h20} deletions, and the position of the various factors is as shown in Fig. 3.

A further point of interest is that t^{h20} gave *some* complementation of t^{w1}, t^{w5} and t^{w32}. This means that, if the presumption that t^{h20} involves a deletion (or at least

a 'null allele') is accepted, then t^{w1}, t^{w5} and t^{w32} cannot themselves be 'null alleles'. This point was of course already fairly clear from the known complementation reactions of the various lethal groups, but is further confirmed by the work with t^{h20}. Similarly, since t^6 (from which t^{h20} was derived) behaves differently from t^{h20} in complementation tests, it too cannot be a 'null allele'.

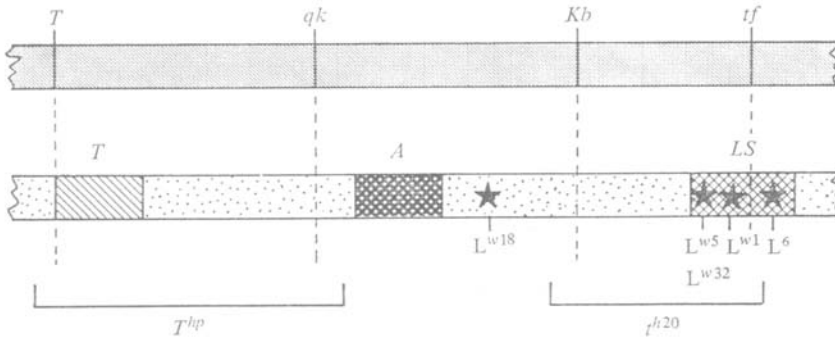

Fig. 3. Postulated relative locations on chromosome 17 of the various *t*-lethal factors, indicated by L^6, L^{w5}, L^{w32}, L^{w1} and L^{w18}, and of the deletions involved in T^{hp} and t^{h20}.

A surprising point is that t^{h20} apparently gave no complementation of t^6, although more data are still needed on this question. Only 133 offspring were raised in complementation tests between t^6 and t^{h20}, and the result of 0/133 t/t animals in this test does not differ significantly from the 4/165 found in the tests of t^{h20} with t^{w32}. Thus, it is still possible that very weak complementation of t^6 by t^{h20} does occur.

On the other hand if it should prove that there is indeed no complementation of t^6 by t^{h20} this would not be the first example of a mutant *t*-haplotype which gave zero complementation of one lethal group and very weak complementation of another. Bennett & Dunn (1964) described two mutant haplotypes, t^{w20} and t^{w21}, both derived from haplotypes of the t^{w5} group, which showed zero complementation of t^{w1} and very weak complementation of t^0 (12 and 16% respectively). t^{w21} showed quite strong complementation of t^{w5} and t^{w32}. No data appear to be given for t^{w20} but presumably it complemented t^{w5} (from which it was derived).

Bennett & Dunn suggested that t^{w20} and t^{w21} might include a duplication or deletion accounting for their unusual complementation effects. As we suggest that t^{h20} may include a deletion, and the evidence for this includes loci other than the *t*-lethals, i.e. *Kb* and *tf*, it is tempting to speculate that t^{w20} and t^{w21} by contrast included duplications. One could go on to suggest that t^{w1}-lethal, which acts relatively late in development, is a hypermorph and not complemented by a duplication, whereas t^{w5} and t^{w32} would be hypomorphs and strongly complemented. Conversely, the hypermorph t^{w1} would be relatively strongly complemented by the deletion in t^{h20}, and the hypomorphs t^{w5} and t^{w32} weakly complemented. However, an explanation of this type does not really seem adequate. It is not clear why a deletion should complement a hypomorph at all, and the fact that t^0 or t^6 are weakly or zero complemented in both cases is not explained. It is necessary to

introduce an additional *ad hoc* hypothesis that the duplicated or deleted regions covered different sequences of DNA.

The lethals of the different complementation groups, may represent different 'allelic forms', in the loosest sense of this term, of the same functional unit, or may be forms of different functional units (discussed by Klein & Hammerberg, 1977). In either case it is possible that the lethals of the t^0 (and t^6) group might involve a separate part of the DNA sequence. One could postulate that this part of the sequence was duplicated or deleted in t^{w20} and t^{w21} (in which complementation of t^0 was altered) and unaffected in t^{h20} (in which complementation of t^6 was unchanged). This would mean that t^0 (or t^6) involved an end part of the t-lethal sequence. Furthermore, the fact that the *tf* and *Kb* locus are affected in t^{h20} would require that the t^6-unaffected part is distal to *tf* (Fig. 3). However, our data are inadequate to establish firmly that such an explanation is appropriate, and one must await finding of further informative mutant haplotypes.

We are very grateful to Dr Lynn Fraser for the gift of a breeding nucleus of t^{w32}, to Dr Jean-Louis Guénet for t^{w1} and t^{w18}.

REFERENCES

Bechtol, K. B. & Lyon, M. F. (1978). H-2 typing of mutants of the t^6 haplotype in the mouse. *Immunogenetics* **6**, 571–583.

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

Bennett, D. & Dunn, L. C. (1964). Repeated occurrences in the mouse of lethal alleles of the same complementation group. *Genetics* **49**, 949–958.

Dunn, L. C. (1957). Studies of the genetic variability in populations of wild house mice. II. Analysis of eight additional alleles at locus T. *Genetics* **42**, 299–311.

Dunn, L. C. (1960). Variations in the transmission ratios of alleles through egg and sperm in *Mus musculus*. *American Naturalist* **94**, 385–393.

Johnson, D. R. (1974). Hairpintail: a case of post-reductional gene action in the mouse egg? *Genetics* **76**, 795–805.

Johnson, D. R. (1975). Further observations on the hairpintail (T^{hp}) mutation in the mouse. *Genetical Research* **24**, 207–213.

Klein, J. & Hammerberg, C. (1977). The control of differentiation by the *T* complex. *Immunological Reviews* **33**, 70–104.

Lyon, M. F. (1978). Private communication. *Mouse News Letter* **59**, 21.

Lyon, M. F. & Mason, I. (1977). Information on the nature of *t*-haplotypes from the interaction of mutant haplotypes in male fertility and segregation ratio. *Genetical Research* **29**, 255–266.

Lyon, M. F. & Bechtol, K. B. (1977). Derivation of mutant *t*-haplotypes of the mouse by presumed duplication or deletion. *Genetical Research*, **30**, 63–76.