Genet. Res., Camb. (1979), **33**, *pp.* 269–277
Printed in Great Britain

Recombinational analysis of the viable *t*-haplotype t^{38}

BY DOROTHEA BENNETT, KAREN ARTZT, JANICE COOKINGHAM AND CATHERINE CALO

Laboratory of Developmental Genetics, Sloan-Kettering Institute for Cancer Research, New York, New York 10021, U.S.A.

(*Received* 6 *March* 1979)

SUMMARY

A viable *t*-haplotype, t^{38}, has been shown to distort linkage relations in the *T–tf* region of mouse chromosome 17. Separate experiments were done with two different interstitial markers, *Low* and *qk*, which map respectively about 5 and 4 centimorgans (cM) from the locus of *T* in female $T+tf/+$(*Low* or *qk*)+ heterozygotes. In females that carried t^{38}, however (i.e. $t^{38}++/+Low\ tf$ or $t^{38}++/+qk\ tf$), there was virtually no recombination between t^{38} and either interstitial marker, although the t^{38}–*tf* distance was normal. These observations suggest that t^{38} suppresses recombination in the lefthand part of that region and strongly enhances it further to the right.

1. INTRODUCTION

Naturally occurring recessive lethal or semilethal haplotypes of the *T/t* complex in the mouse share several attributes in addition to their effects on the viability of homozygotes. All of them (1) interact with the dominant mutation *T* to produce taillessness in *T/t* animals, (2) suppress regular meiotic crossing over in the 14 centimorgan distance from the locus of *T* to the locus of *H–2*, (3) are transmitted from heterozygous males at far higher than Mendelian frequencies, and (4) produce sterility in males that are either homozygous for semilethal haplotypes or are doubly heterozygous for complementing lethals (Bennett, 1975 for review). This bizarre set of observations has led to suggestions that these haplotypes comprise a long segment of abnormal chromatin in which factors related to these functions are somehow integrated (Lyon & Bechtol, 1977 for review). Yet, each of these wild haplotypes can be shown to break down into at least two quite clearly separable elements by a process that involves genetic recombination within the 9 cM region between *T* and *tf*. Thus, in heterozygotes carrying appropriate markers ($T\ tf\ H\text{–}2^x/t^n+{}^{tf}\ H\text{–}2^y$), rare ($\approx 1\text{–}5\times10^{-3}$) crossovers can be seen to occur in the usually restricted region between *T* and *tf;* these separate the t^n-haplotype into two sharply definable components, one at the proximal (centromeric) end of the region that is responsible for producing taillessness by interaction with *T* but is viable, and one near *tf* that contains the lethal factor but does not interact with *T* (Bennett, 1975; Lyon & Meredith, 1964; Lyon & Mason, 1977).

0016–6723/79/2828–7470 $01.00

Approximately 75 viable factors (referred to as 'T–int' by Lyon and as t^T by us) thus derived from the various lethal haplotypes have been studied. All of them not only interact with T to produce taillessness but also permit substantially normal recombination between T and *tf*. Furthermore, all t^T factors act as true genetic alleles of T since T/t^T genotypes never show recombination between T and t^T. Nevertheless, the t^T factors that have been isolated and characterized appear not to be simple 'tail interaction' elements since they are a heterogeneous group which can be divided into subsets on the basis of transmission ratio distortion and male sterility interactions. As a general rule, t^T factors show either normal or low transmission from male heterozygotes rather than the markedly enhanced rate typical of their parent allele. For example, in a sample of 65 independently isolated viable t^T factors analysed by Bennett, Dunn & Artzt, (1976), 21 had ratios significantly lower than 50% (usually 20–35%) and 44 were normal. Likewise, Lyon & Mason (1977) reported on 10 different viable derivatives of t^6 of which 6 had low ratios and 4 were normal. t^T factors also reveal complexity in their interactions with lethal or semilethal haplotypes. Interestingly, although males homozygous for any single viable factor (t^T/t^T) are fully fertile, males that carry one such viable factor and a lethal *t*-haplotype often have some impairment in fertility. A study by Dunn & Bennett (1969) showed that all of 8 different viable factors paired against 4 different lethal or semilethal haplotypes resulted in t^T/t^{lethal} males whose fertility (as measured by offspring/female/unit of time) ranged from almost nil to about 75% of normal. This means, incidentally, that this quasi-sterility is dependent on some function of the distal part of the lethal haplotype since t^T factors derived by recombination produce infertility with their parent allele, although t^T/t^T males are fertile. The infertility of t^T/t^{lethal} compounds is not a completely general rule, however, since the viable haplotypes derived from t^6 that were studied by Lyon & Mason (1977) did not interact with lethal haplotypes to lower fertility.

The apparent complexity of *t*-viable (t^T) haplotypes is borne out by mapping experiments that we report here. A t^T factor derived from t^0 (t^{38}), with a low transmission ratio and the ability to interact with lethal haplotypes to produce sterility, appears to map not as a point mutation but as a regional change that distorts normal linkage relationships with interstitial markers over much of the distance between T and *tf*.

2. MATERIALS AND METHODS

t^{38} is a viable *t*-haplotype derived from t^0 by recombination. The transmission ratio of t^{38} from heterozygous males is low (15%); t^{38} permits apparently normal recombination in the *T–tf* interval when measured in $T\ tf/t^{38}+$ or $T+/t^{38}\ tf$ animals, 8/188 or $4{\cdot}3 \pm 1{\cdot}5$% in males and 10/83 or $12{\cdot}0 \pm 3{\cdot}6$% in females (Bennett *et al.* 1976).

Two different interstitial markers, *Low* (or t^{Low}) and *qk*, were used in separate crosses. Recombination was measured in females heterozygous for t^{38} and one of

these markers in order to take advantage of the enhanced rate of crossing over in female as opposed to male mice (Dunn & Bennett, 1967).

1. The mutant *Low* has two observable characteristics: (1) to reduce transmission of the chromosome on which it resides in heterozygous males to about 15% and (2) to reduce recombination between *T* and *tf* in both male and female heterozygotes to about one-half standard values, i.e. to $2{\cdot}8 \pm 0{\cdot}3\%$ in males and $6{\cdot}5 \pm 1{\cdot}7\%$ in females. About 5/6 of the crossovers in *T Low*+/++*tf* animals occur between *T* and *Low*, and only 1/6 distal to *Low;* thus, *Low* maps about 5 units distal to *T* and 1 unit proximal to *tf* in females, although the physical distances involved presumably are 10 and 2 units respectively because of *Low's* inherent crossover suppression. Fertility and recombination are entirely normal in *Low/Low* homozygotes, which are indistinguishable from wild-type (Dunn & Bennett, 1971).

There are some grounds for thinking that *Low* may represent a transmission distorting factor derived from a mutant *t*-haplotype. It was detected in a chromosome derived by recombination from one carrying the lethal factor t^{h17}, which in turn was derived from t^6. Bennett & Dunn (1971) found no evidence that *Low* resembled a viable *t*-factor as its interactions with defined lethal *t*-haplotypes with respect to both transmission ratio distortion and sterility differed sharply from interactions typical of the viable *t*-factors they studied. Later, however, Lyon & Mason (1977) showed that the behaviour of the recombinant derivatives of t^6 (which represent the one class not studied by Dunn & Bennett) not only differed from all the *t*-haplotypes studied by Dunn & Bennett, but was also similar to that of *Low*. Lyon & Mason concluded that t^6 differs structurally from most other natural *t*-haplotypes, and that *Low* appears to be an isolated transmission distorting factor that should be redesignated t^{Low}. We have retained the original designation in this paper.

The experimental design for mapping t^{38} relative to *Low* was as follows:

Cross. $\text{♀} \dfrac{+\ Low\ tf}{t^{38}+\ +} \times \text{♂} \dfrac{T+tf}{+\ +tf}$.

Offspring expected. Only males that carry *T* are informative.

	Genotype	Phenotype	Progeny classes expected in crosses to +++/+++ female
Parental types	$+\ Low\ tf/T+tf$	Short-tail, tufted	0·15 nt: 0·85 short tail
	$t^{38}+\ +/T+tf$	Tailless, non-tufted	0·15 nt: 0·85 short tail
Recombinants (t^{38}-*Low* region)	$+\ +\ +/T+tf$	Short-tail, non-tufted	0·5 nt: 0·5 short tail
	$t^{38}\ Low\ tf/T+tf$	Tailless, tufted	?
Recombinants (*Low-tf* region)	$+\ Low+/T+tf$	Short-tail, non-tufted	0·15 nt: 0·85 short tail
	$t^{38}+tf/T+tf$	Tailless, tufted	0·15 nt: 0·85 short tail

Although recombinants arising from crossovers in the two different regions are not distinguishable by phenotype, progeny tests could clearly discriminate among the short-tailed non-tufted class, since those that retained *Low* would

produce few normal-tailed offspring while those that lost it would have normal segregation. Likewise, one class of tailless male should segregate in favour of the *T*-bearing chromosome, this time because of t^{38} on the homologue. We could not predict the behaviour of the other expected class of tailless tufted animals (t^{38} *Low tf*/*T*+*tf*) because nothing is known about the *cis* interaction of *t*-factors and *Low*. There were three obvious possibilities for segregation of the t^{38} *Low tf* chromosome:

(1) normal, because of complementary *cis* interaction between *Low* and t^{38},
(2) more severely impaired than when either t^{38} or *Low* were present alone,
(3) indistinguishable from that imposed by t^{38} alone.

Only the first two of these possibilities would permit the two classes of tailless animals to be distinguished.

II. The mutation *quaking* (*qk*) produces defective central nervous system myelination, and abnormal spermatogenesis and sterility in male homozygotes (Bennett *et al.* 1971). Heterozygotes appear entirely normal, without observable abnormalities in either sperm or recombination. Although the homozygous effects of *qk* on spermatogenesis are reminiscent of the homozygous effects of *t*-haplotypes, the two mutations appear not to interact at all in *trans* heterozygotes. *qk* maps at 3 cM distal to *T* in samples that include equal numbers of males and females (Bennett, unpublished). Since recombination in female mice is usually approximately double that in males (Dunn & Bennett, 1967), the *T*–*qk* distance in females must approximate 4 cM.

The experimental design here was as follows:

Cross. $\text{♀}\frac{+qk\ tf}{t^{38}++} \times \text{♂}\frac{T^{Hp}+tf}{++tf}$.

Offspring expected. Only animals carrying T^{Hp} are informative. T^{Hp} is a deletion covering the locus of *qk*; thus *qk* is pseudodominant in T^{Hp}/*qk* heterozygotes.

	Genotype	Phenotype
Parental types	$+qk\ tf/T^{Hp}+tf$	Short-tailed, quaking, tufted
	$t^{38}++/T^{Hp}+tf$	Tailless, non-quaking, non-tufted
Recombinants	$+++/T^{Hp}+tf$	Short-tailed, non-quaking, non-tufted
(t^{38}-*qk* region)	$t^{38}\ qk\ tf/T^{Hp}+tf$	Tailless, quaking, tufted
Recombinants	$+qk+/T^{Hp}+tf$	Short-tailed, quaking, non-tufted
(*qk*-*tf* region)	$t^{38}+tf/T^{Hp}+tf$	Tailless, non-quaking, tufted

3. RESULTS

I. *Mapping with* Low

Eighty-nine females of genotype $+Low\ tf/t^{38}++$ mated to $T+tf/++tf$ males produced 1188 short-tailed and tailless offspring that were scored for the tufted phenotype. Ninety-two of these were recombinants between t^{38} and *tufted;* 48 were short-tailed but not tufted and therefore carried a recombinant chromosome that was either $+++$ or $+Low+$; 44 were tailless tufted animals whose recombinant chromosome had to be either t^{38} *Low tf* or $t^{38}+tf$. The overall recombination fraction is $7{\cdot}7 \pm 0{\cdot}8$ %.

The breeding behaviour of male recombinants was analyzed, with results for short-tailed non-tufted males shown in Table 1. In all but one of these 18 recombinants crossing over had occurred between *Low* and *tf*. Twenty-seven tailless tufted recombinants were also progeny tested. All gave low ratios of normal tailed offspring typical of those expected from T/t^{38} males. As pointed out in Materials and Methods, we could not completely ascertain genotype in these males for lack of knowledge about *cis* interaction of t^T and *Low*. However, our failure to find any anomalous ratios suggests that either t^T and *Low* do not interact either positively or negatively, or that no crossovers between t^{38} and *Low* had occurred. The fact that only one such crossover was found in the short-tailed recombinants suggests that the latter possibility may be the case. The observation that 17 of 18 crossovers between T and *tf* occurred in the *Low–tf* region, which represents only 1/6 of the total region, was surprising. This led us to suspect that perhaps the T-interaction factor of t^{38} was physically separate from its low ratio factor so that the t^{38}/Low heterozygotes might have this genetic constitution:

$$\frac{+^{T}\ Low\ tf}{t^{38(T)}\ t^{38(LR)}\ +}$$

and, thus, that the majority of recombinant chromosomes we had tested were either $+^{T}\ t^{38(LR)}\ +^{Low}\ +^{tf}$ or $t^{38(T)}\ +\ t^{38(LR)}\ Low\ +^{tf}$, with only one of the 18 short-tailed recombinants resulting from a crossover between $t^{38(LR)}$ and *Low* to produce a $+^{T}\ +^{t^{38(LR)}}\ +^{Low}\ +^{tf}$ chromosome. We tried to test this notion by crossing these recombinants to $T\ tf/t^{w12}\ tf$ females and progeny testing the resulting normal-tailed males in crosses by $+tf/+tf$ females as follows:

	Expected breeding behaviour	
Genotype	Transmission of *tf v* +	Fertility
$\frac{+^{T}\ t^{38(LR)}\ +^{Low}\ +}{t^{W12}\ +\quad\quad +tf}$	Distorted	Quasi-sterile
or		
$\frac{+^{T}\ +^{38(LR)}\ Low\ +}{t^{W12}\ +\quad\quad +tf}$	Equal	Normal

The expectations outlined are based on the assumption that the putative $t^{38(LR)}$ factor would interact with t^{w12} in the same way as established by data (Bennett & Dunn, 1971) for intact *t*-viable haplotypes. Five different recombinant males produced sons that were tested; all of these had normal fertility and normal ratios, and therefore presumably carried chromosomes with *Low* but not the hypothetical $t^{38(LR)}$ factor. These data suggest strongly that t^{38} does not contain two readily separable factors, one for tail interaction with T and one for transmission ratio, and consequently that the recombinants we obtained were in fact largely between *Low* and *tf*.

Table 1. *Results of progeny tests by + + + / + + + females of short-tailed non-tufted recombinant sons of* + Low tf/t^{38} + + *mothers crossed to* T + tf/ + + tf *males*

Male no.	Offspring		Diagnosis of recombinant chromosome
	Normal-tailed	Short-tailed	
1	17	58	+ Low +
2	3	12	+ Low +
3	8	20	+ Low +
4	5	50	+ Low +
5	5	39	+ Low +
6	12	65	+ Low +
7	58	62	+ + +
8	11	53	+ Low +
9	18	91	+ Low +
10	33	69	+ Low +
11	7	60	+ Low +
12	19	76	+ Low +
13	12	55	+ Low +
14	14	25	+ Low +
15	13	47	+ Low +
16	7	35	+ Low +
17	1	62	+ Low +
18	3	57	+ Low +

II. *Mapping with* qk

Linkage data from 27 females heterozygous for *qk* and t^{38} are shown in Table 2. The overall recombination fraction between t^{38} and *tf* is 14/147 or 9·2 ± 2·4 % but as can be seen in the Table, no crossovers occurred between t^{38} and *qk*. Likewise, in an additional 86 offspring whose mothers were homozygous for *tf*, none were recombinant in the t^{38}–*qk* region. So, in the total sample of 233 progeny, no crossovers in the 4 cM distance between the *T*-locus and *qk* were observed.

Summary of Results

Table 3 gives recombination frequencies measured in females between *T* (or t^{38}) and *tf*, and the two interstitial markers *Low* and *qk*. Within the limits of error, the *T*–*tf* or t^{38}–*tf* distance is equivalent in all groups sampled regardless of what combinations of *Low*, t^{38}, *qk* their genotypes contained. *Low* heterozygotes had consistently less recombination in this region than any other genotypes, again suggesting that *Low* may impede crossing over although the differences are not clearly statistically significant. On the other hand, it is quite clear that t^{38} does not alter the total recombination distance between *T* and *tf* but that it does dramatically reduce the distance between *T* and either of the two interstitial markers (*qk* or *Low*). The conclusion that these data require is that t^{38} enhances recombination in the region distal to *Low* and *qk*, and that t^{38} (which is clearly genetically allelic to *T*) either suppresses recombination in a 4–5 cM region distal to *T* or physically occupies that region.

Table 2. *Recombination in females heterozygous for* qk *and* t[38]

	Offspring from + *qk tf*/*t*[38] + + ♀♀		Offspring from +*qk tf*/*t* +*tf* ♀♀	
	Chromosome	Number	Chromosome	Number
Parental classes	+ *qk tf*	86	+ *qk* (*tf*)	42
	t[38] + +	47	*t*[38] + (*tf*)	44
Recombinants (*t*[38]–*qk*)	+ + +	0	+ + (*tf*)	0
	t[38] *qk tf*	0	*t*[38] *qk* (*tf*)	0
Recombinants (*qk*–*tf*)	+ *qk* +	9	—	—
	t[38] + *tf*	5	—	—

Table 3. *Comparisons of map distances within the* T–tf *interval in the presence or absence of* t[38]

	Reference	*T*–*tf* or *t*[38]–*tf* distance	*T*–*m* or *t*[38]–*m* distance	*m*–*tf* distance
♀ 'Standard' (*T tf*/+ +)	Dunn & Bennett, 1967	9·1 ± 1·0	—	—
In ♀ *T* + +/*t*[38] + *tf*	Bennett *et al.* 1976	12·0 ± 3·6	—	—
In ♀ *T Low* +/+ *Low tf*	Dunn & Bennett, 1971	10·0 ± 2·2	—	—
In ♀ *T Low tf*/+ + +	Dunn & Bennett, 1971	6·5 ± 1·7	5·4	1·1
In ♀ *t*[38] + +/+ *Low tf*	This paper	7·7 ± 0·8	0·4	7·3
In ♀ *T qk*/+ +	Unpublished	—	≈ 4	≈ 5
In ♀ *t*[38] + +/+ *qk tf*	This paper	9·2 ± 2·4	0	9·2 ± 2·4

4. DISCUSSION

The cross-over suppression between *T* and *H–2* that is a constant feature of naturally occurring recessive *t*-haplotypes has until now appeared to be primarily related to the region of the haplotype responsible for lethality or semilethality. When wild haplotypes separate by recombination into their two typical derivatives (a proximal portion carrying t^T and viable when homozygous, and a distal region carrying the lethal factor) the proximal region has at most showed 'mild' cross-over suppression in the *T–tf* region, whereas the distal region with its *t*-lethal factor thought to map near *tf* continues to prevent recombination between *tf* and *H–2* although recombination is freely permitted or even enhanced in the *T–tf* region (Bechtol & Lyon, 1978). The data in this paper show that the apparent lack of effect of *t*-viables on recombination in the *T–tf* interval may be spurious. At least one such factor, *t*[38], strongly suppresses recombination in the proximal end of the *T–tf* interval, but apparently compensatory recombination in the distal part restores crossing over to normal over the total distance measured. A hint of a similar situation was reported by Pizarro & Dunn (1970) who found that another viable, *t*[w35], probably suppressed recombination between *T* and *tf* in males but not females and also appeared to enhance crossing over between *T* and *H–2*. The mechanism by which *t*-factors alter the frequency of recombination is completely unknown, although Lyon & Bechtol (1977) have speculated on a 'change in interstitial heterochromatin, in the form of moderately repetitious DNA'.

The information we have collected here also bears on the model proposed by Lyon and her colleagues (Lyon *et al.* 1979, for review and references). Briefly, this model suggests that at least three separate factors make up a naturally occurring *t*-haplotype: from proximal to distal in the *T–tf* region (and thought to be separated by '*t*-chromatin') these are postulated to be T–int (the tail-interaction factor), A (a factor involved in transmission ratio distortion) which produces a low ratio when it is present alone (as is supposed for t^{Low}) or coupled only with T–int and, usually but not always, a high ratio when in *cis* configuration with a third factor, LS (an element postulated to be responsible for both the lethality of homozygous embryos and sterility in males carrying two such factors, as well as for the above mentioned interaction with the A factor). If we have interpreted this model correctly, the A factor is thought of as possibly existing in allelic forms in different haplotypes, and *Low* thought to represent an isolated A factor (and was thus called t^{Low}) (Lyon & Mason, 1977). However, we have shown here that crossing over apparently can occur between *Low* and the t^{38} ratio factor, and this is incompatible with the idea that both contain A factors that are allelic. On the other hand, since the apparent recombination between *Low* and t^{38} occurs so rarely, it could also represent mutation. We are also unable to come to grips with this model in terms of incorporating other data previously obtained with t^{38}. t^{38} interacts with *T* to produce taillessness, gives a low transmission ratio, but is fully viable in homozygotes; so according to the Lyon model, it should have both T-int and A factors, but not LS; yet male compounds carrying t^{38} and any one of three different wild *t*-haplotypes tested are quasi-sterile (Dunn & Bennett, 1969). Lyon & Mason (1977) have also noted this discrepancy, and proposed that the proximal end of t^6 differs in some way from other known *t*-haplotypes. In any case, it is abundantly clear that further genetic and biochemical analysis will be necessary before the structure and relationship of genetic factors in the T/t complex is understood.

This work was supported by DOE Contract EE-77-S-02-4159, NSF Grant PCM 77-17835, and NIH Grants CA-08748 and CA-00276. We are grateful to Dr Lee Silver for valuable discussion.

REFERENCES

BECHTOL, K. & 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. (1971). Transmission ratio distorting genes on chromosome IX and their interactions. In *Proc. Symp. Immunogenetics of the H–2 System*, pp. 90–103. Basel: Karger.

BENNETT, D., DUNN, L. C. & ARTZT, K. (1976). Genetic change in mutations at the T/t locus. *Genetics* **83**, 361–372.

BENNETT, W. I., GALL, A. M., SOUTHARD, J. L. & SIDMAN, R. L. (1971). Abnormal spermiogenesis in quaking, a myelin-deficient mutant mouse. *Biology of Reproduction* **5**, 30–58.

DUNN, L. C. & BENNETT, D. (1967). Sex differences in recombination of linked genes in animals. *Genetical Research* **9**, 211–220.

DUNN, L. C. & BENNETT, D. (1969). Studies of the 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 mutant (*Low*) which distorts transmission ratios in the house mouse. *Genetics* **67**, 543–558.

FOREJT, J. (1972). Chiasmata and crossing-over in the male mouse; suppression of recombination and chiasma frequencies in the ninth linkage group. *Folia Biologica* (Praha) **18**, 161–170.

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.

LYON, M. F., EVANS, E. P., JARVIS, S. E., SAYERS, I. (1979). t-haplotypes of the mouse may involve a change in intercalary DNA. *Nature* **279**, 38–42.

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. & MEREDITH, R. (1964). Investigations of the nature of t-alleles in the mouse; I, II, III. *Heredity* **19**, 301–312, 313–325, 327–330.

PIZARRO, O. & DUNN, L. C. (1970). A study of recombination between the H–2 (histocompatibility) locus and loci closely linked with it in the house mouse. *Transplantation* **9**, 207–218.