INVESTIGATIONS OF THE NATURE OF t -ALLELES IN THE MOUSE

I. GENETIC ANALYSIS OF A SERIES OF MUTANTS DERIVED FROM A LETHAL ALLELE

MARY F. LYON and R. MEREDITH

Medical Research Council Radiobiological Research Unit, Harwell, Didcot, Berkshire

Received 16.i.64

THE t -alleles are a long series of recessive alleles in linkage group IX of the mouse which have a combination of unusual properties. These include:

1. A modifying effect on the expression of the gene T , so that Tt animals are tailless, rather than short-tailed like $T+$.
2. Homozygous lethality.
3. Male sterility in the genotypes t^*t^* or t^*t^y .
4. Abnormal male segregation ratio.
5. Suppression of crossing-over between T and the nearby locus of tufted, tf .
6. A high mutation rate to other t -alleles.

All t -alleles known at the start of this work had the T -modifying effect, but the various other properties were possessed by only some alleles. Moreover, different lethal alleles usually complemented each other (Dunn, 1956).

Another feature which makes the alleles of more than academic interest is that despite their deleterious nature they are very common both in the wild and in laboratory populations. Dunn (1956) studied 21 wild populations and found t -alleles in 15 of them, with only one population definitely negative. The frequency of heterozygotes in the populations containing an allele was quite high, about 40 per cent. Hence t -alleles, although apparently very deleterious, must be considered part of the normal genetic make-up of the mouse and therefore deserving of detailed study.

Lyon and Phillips (1959) showed that the "mutation" of one t -allele to another was in at least some cases the result of crossing-over in the region of crossover suppression. Hence these alleles were not "point" alleles but regions of abnormal chromosome. It followed that analysis of the properties of a series of mutants, all derived from the same parent allele, should reveal whether the various properties of this allele were due to separate linearly arranged factors or depended on the whole length of abnormal chromosome.

The present paper describes the analysis of such a series of mutants

derived from the lethal allele t^6 (Carter and Phillips, 1950). This allele is indistinguishable from one of the first known alleles t^0 and possesses all six of the properties listed above. Several new alleles were derived from it during its use in an investigation of the effect of X-rays on the mutation of t -alleles (Lyon, 1960), and others during study of its crossover-suppressing effect. Yet a third group were only indirectly derived from t^6 , since they arose during investigation of one of its mutants, t^{h7} , and these are discussed separately in a succeeding paper. Each allele was tested for the first five of the six properties possessed by t^6 .

1. ORIGIN AND GENETIC TESTS OF THE MUTANTS

In the experiment with X-rays mentioned above males of the genotype Ttf/t^6+ were irradiated and mated to females of the same genotype. Fig. 1 shows that in the absence of mutation all surviving

Parents Ttf/t^6+ $\times$ Ttf/t^6+
Tailless Tailless

Gametes	Normal		Crossovers		Mutation t^m+ ?
	Ttf	t^6+	$T+$	t^6tf	
Ttf	dies	tailless non-tufted	dies	tailless tufted	tailless ? tufted
t^6+	tailless non-tufted	dies	tailless non-tufted or dies	normal tailed or dies	normal tailed

FIG. 1.—Genotypes and phenotypes of offspring expected in a balanced lethal tailless line.

young were expected to be phenotypically tailless non-tufted (genotype Ttf/t^6+) like the parents; normal-tailed young would result from mutation of one t -allele to another, and tailless tufted ones from recombination between T and tf . Table 1 gives a list of the exceptional animals found. As a genetic test, the normal-tailed ones were mated to Ttf/tf animals, thus testing at the same time the two hypotheses that the animals carried two different t -alleles, and that the chromosome carrying the new allele also carried tf . The tailless tufted exceptions were mated to Ttf/t^6+ animals to test whether the t -allele that they carried was the same as or different from t^6 . Of the five normal-tailed exceptions tested four produced only tailless and normal-tailed young when mated to Ttf/tf , as would be expected from animals carrying two t -alleles with a T -modifying effect similar to that of t^6 , and proved

themselves to carry *tf*. Similarly, the five tailless tufted animals all threw normal-tailed young when mated to Tt^6 , thus showing that they carried *t*-alleles different from t^6 , since t^6t^6 zygotes die *in utero*. The fifth normal-tailed exception, potentially carrying t^{h7} , gave unexpected results in that she produced 15 short-tailed young and only one tailless offspring, which died early. She was later shown to carry an allele with a different *T*-modifying effect, and the breeding behaviour of her and her descendants will be considered separately in a later paper.

TABLE 1

Origin and genetic tests of the first mutant animal of each t-allele

Phenotype	Allele	Result of mating to $Ttf/+tf$			Genotype
		Tailless	Short-tailed	Normal	
Normal-tailed	t^{h3}	27	...	23	$t^6+ / t^{h3}tf$
	t^{h5}	32	...	35	$t^6+ / t^{h5}tf$
	t^{h6}	24	...	21	$t^6+ / t^{h6}tf$
	t^{h10}	13	...	11	$t^6+ / t^{h10}tf$
	t^{h7}	1	15	12	$Ttf/t^{h7}+$
Tailless tufted		Result of mating to Ttf/t^6+			
		Tailless	Short-tailed	Normal	
	t^{h2}	5	...	2	$Ttf/t^{h2}tf$
	t^{h4}	37	...	27	$Ttf/t^{h4}tf$
	t^{h8}	14	...	9	$Ttf/t^{h8}tf$
	t^{h9}	16	...	41	$Ttf/t^{h9}tf$
	t^{h11}	11	...	6	$Ttf/t^{h11}tf$

Having established that the exceptional animals carried mutant *t*-alleles and that the mutation had involved crossing-over, four properties of the alleles were then investigated: the viability of the homozygotes, the segregation ratio of *T:t* in male heterozygotes, the fertility of males of the types t^6t^{hn} and $t^{hn}t^{hn}$, and the effect on crossing-over between *T* and *tf*. At the same time these properties are reported for an allele, t^{21} , which arose in a different stock, carrying translocation T190, and which was briefly described by Lyon and Phillips (1959).

2. PROPERTIES OF THE MUTANT ALLELES

(i) Viability of the homozygotes

Animals of the type Tt^{hn} were mated together (table 2). Some normal-tailed young were obtained with all the alleles tested, showing that homozygotes for all these alleles were viable and normal-tailed. With some of the alleles the observed ratio of tailless to normal-tailed

young departed widely from the 2:1 ratio expected with normal Mendelian segregation. As shown below, however, these were alleles for which the proportion of *t*-carrying offspring sired by *Tt* males was well below 50 per cent., *i.e.* alleles with a low segregation ratio. It is not possible to calculate an expected tailless:normal ratio in the viability tests from the observed male segregation ratios since Bateman (1960) has shown that this ratio may depend on the genotype of the egg fertilised, and in this experiment the females used for the segregation ratio tests were from a stock different from that used in the viability tests. However, it seems reasonable to suppose that the anomalous male segregation ratio was the main factor responsible for the shortage

TABLE 2
The offspring of matings of the type $Tt^{hn} \times Tt^{hn}$

Allele	Offspring	
	Tailless	Normal
t^{h2} . . .	62	11
t^{h3} . . .	78	45
t^{h4} . . .	28	9
t^{h5} . . .	104	7
t^{h6} . . .	45	9
t^{h8} . . .	64	15
t^{h9} . . .	94	10
t^{h10} . . .	86	40
t^{h11} . . .	28	18
t^{21} . . .	121	69

of homozygotes in these crosses, rather than poor viability of the homozygotes, especially since normal numbers of homozygous offspring were obtained from matings of the type $Tt \text{♀} \times tt \text{♂}$.

(ii) *Segregation ratio of $T:t$ in male heterozygotes*

It has long been known from the work of Dunn (1956) and his colleagues that the segregation ratio of *t*-alleles in males heterozygous for them may depart widely from normal, varying with the particular allele tested from a large excess of *t*-carrying gametes to a great deficiency of them. In this experiment males of genotype Tt^{hn} were put with one or two normal females (F_1 hybrids between strains C3H and 101) and left to breed continuously. From two to five males were used for each allele, and about 40 young were raised from each male. Table 3 shows that t^{21} gave a segregation ratio above normal and five alleles, t^{h2} , t^{h5} , t^{h6} , t^{h8} and t^{h9} , gave a ratio below normal. For each allele the individual males consistently showed deviations in the same direction. There was some variation among the low ratio alleles in

TABLE 3

Segregation ratio of T:t in Tt males mated to ++^t females

Allele	T	t	Per cent. t	$\chi^2_{1:1}$
t^{21} . . .	53	97	64.7	12.91
t^{h8} . . .	46	41	47.1	0.29
t^{h4} . . .	80	90	52.9	0.59
t^{h10} . . .	114	128	52.9	0.81
t^{h11} . . .	91	106	53.8	1.14
t^{h2} . . .	91	22	19.5	42.13
t^{h5} . . .	127	21	14.2	75.92
t^{h6} . . .	119	34	22.2	47.22
t^{h8} . . .	158	47	22.9	60.10
t^{h9} . . .	142	38	21.1	60.09

the exact proportion of *t*-carrying young, but a heterogeneity test showed that this variation was not statistically significant ($\chi^2 = 4.77$ for 4 d.f. $0.3 < P < 0.5$).

(iii) *Fertility of t^6t^{hn} and $t^{hn}t^{hn}$ males*

Individual 8-week-old males were placed with boxes of 3 or 5 females of a fertile hybrid stock and were left with them for four weeks. The females were examined regularly for signs of pregnancy. When 13 or more days pregnant they were killed and dissected and the numbers of live and dead implants in the uterus and of corpora lutea in the ovary were counted (table 4).

All the genotypes tested were fertile. The next question was to measure the fertility and compare it with that expected of normal mice. Two measures of fertility which were available were the number of females which became pregnant, and the ratio of number of implants to number of corpora lutea, a value which would be reduced if the number of eggs fertilised was reduced. In previous experiments, with females of the same stock mated to normal males, the number of implants was about 75 per cent. of the number of corpora lutea, but, since these controls were not simultaneous, only large differences from the figure of 75 per cent. implantation can be considered meaningful in the present experiment. The table shows that for all alleles the fertility of t^6t^{hn} males was virtually equal to that of normal males, only t^6t^{h4} and t^6t^{h10} showing a rather low proportion of implants. The homozygous males, however, tended to have a slightly reduced fertility. With $t^{21}t^{21}$, $t^{h4}t^{h4}$, $t^{h8}t^{h8}$ and $t^{h9}t^{h9}$ males several females failed to become pregnant, and one $t^{h8}t^{h8}$ and one $t^{h9}t^{h9}$ male sired no litters. Moreover, for t^{h2} , t^{h5} , t^{h9} and t^{21} , the proportion of corpora lutea represented by implants was markedly below normal.

TABLE 4
Fertility of $t^{a/bn}$ and $t^{bn/bn}$ males

Allele	$t^{a/bn}$						$t^{bn/bn}$					
	No. of males	Pregnant total females	Corpora lutea	Implants	Live implants	Implants corpora lutea (per cent.)	No. of males	Pregnant total females	Corpora lutea	Implants	Live implants	Implants corpora lutea (per cent.)
t^{a2}	7	21/21	196	125	114	63.8	5	19/19	183	101	86	55.2
t^{a3}	5	15/15	154	100	90	64.9	8	23/24	236	169	158	71.6
t^{a4}	10	29/30	304	179	160	58.9	5	16/19	165	106	96	64.2
t^{a5}	5	23/25	211	128	118	60.7	5	25/25	271	153	139	56.5
t^{a6}	5	23/25	244	168	142	68.9	5	25/25	205	131	117	63.9
t^{a8}	5	23/25	229	178	160	77.7	5	16/25	147	90	85	61.2
t^{a9}	5	30/30	275	187	163	68.0	6	23/30	184	81	69	44.1
t^{a10}	5	25/25	233	134	115	57.5	5	23/25	213	131	118	61.5
t^{a11}	5	25/25	239	172	156	72.0	5	22/25	206	139	116	67.5
t^{a1}	5	15/15	154	123	112	79.9	8	27/34	264	122	108	46.2

(iv) Recombination with tufted

The normal recombination between T and tf , using $T+ / +tf$ heterozygotes, is about 8 per cent. (Lyon, 1956) but t^6 suppresses this crossing-over so that with $Ttf/t^6 +$ heterozygotes only about 1 in 500 young are recombinants, and these are found to carry mutant t -alleles.

To test the present t -alleles for crossover-suppression male and female $Tt+tf$ heterozygotes (mainly $T+ / ttf$ but a few $Ttf/t+$) were mated with $++'ttf$ homozygotes. All alleles permitted recombination between T and tf , i.e. the crossover-suppressing effect of t^6 had been

TABLE 5

Percentage recombination between T and tf in heterozygotes for viable t -alleles

Female heterozygotes

Allele	Offspring			Recombination per cent.	Heterogeneity χ^2
	non-crossover	crossover	Total		
t^{21} . . .	183	24	207	11.59	4.08*
t^{h2} . . .	151	15	166	9.04	0.34
t^{h3} . . .	42	6	48	12.50	1.45
t^{h4} . . .	148	13	161	8.07	0.014
t^{h5} . . .	201	2	203	0.99	13.17*
t^{h6} . . .	206	23	229	10.04	1.56
t^{h8} . . .	154	8	162	4.94	1.87
t^{h9} . . .	147	11	158	6.96	0.163
t^{h10} . .	156	17	173	9.83	0.96
t^{h11} . .	167	13	180	7.22	0.091
Total . .	1555	132	1687	7.82	23.70

$$\chi^2 = 23.70 \quad \text{d.f.} = 9 \quad P = 0.01 - 0.001$$

lost (tables 5 and 6). There was then the question whether the percentage of recombination was normal, or whether any partial crossover-suppression still remained. Since the alleles were believed to have arisen through crossing-over in the region of crossover-suppression of t^6 it might have been possible to estimate the length of abnormal chromosome still present by noting the amount of crossover-suppression remaining.

In general the male heterozygotes gave less recombination than the females. This is in contrast to the original figures for recombination in $T+ / +tf$. However, in more recent data from heterozygotes of this type such a sex difference is present (table 7). The reason for the difference between the two bodies of data from $T+ / +tf$ is not known, but it is clear that the sex difference in Tt heterozygotes cannot be taken as evidence of crossover-suppression. There still remained the possibility of differences between alleles, considering heterozygotes of

the same sex. χ^2 tests (for heterogeneity of a $2 \times n$ table) showed statistically significant heterogeneity among the alleles for both sexes of heterozygote. Among the females the largest contributions to χ^2 were made by t^{h5} with a very low recombination of less than 1 per cent., and t^{21}

TABLE 6
Percentage recombination between T and tf in heterozygotes for viable t-alleles
Male heterozygotes

Allele	Offspring			Recombination per cent.	Heterogeneity χ^2
	non-crossover	crossover	Total		
t^{21} . . .	180	8	188	4.26	0.35
t^{h2} . . .	191	6	197	3.05	0.10
t^{h3} . . .	196	7	203	3.45	0.0001
t^{h4} . . .	147	8	155	5.16	1.34
t^{h5} . . .	222	2	224	0.89	4.43*
t^{h6} . . .	158	7	165	4.24	0.30
t^{h8} . . .	203	9	212	4.25	0.39
t^{h9} . . .	185	12	197	6.09	4.07*
t^{h10} . . .	181	2	183	1.09	3.07
t^{h11} . . .	205	6	211	2.84	0.24
Total . . .	1868	67	1935	3.46	14.29

$\chi^2 = 14.29$ d.f. = 9 $P = 0.02 - 0.01$

TABLE 7
Percentage recombination between T and tf in the absence of t-alleles

Type of heterozygote	Offspring				Per cent. recombination
	T+	Ttf	++	+tf	
$T+ / +tf$ ♀ .	167	21	15	180	9.22 ± 1.30
$Ttf / ++$ ♀ .	5	47	59	5	
$T+ / +tf$ ♂ .	167	17	10	168	6.02 ± 1.07
$Ttf / ++$ ♂ .	1	66	67	2	

(for ♀:♂ difference, $\chi^2 = 3.61$, $0.05 < P < 0.1$)

with a high value. Among the males t^{h5} again made a high contribution to χ^2 owing to its low recombination, and t^{h9} gave a high value. It is doubtful whether any importance should be attached to the high recombination values of t^{21} females and t^{h9} males, since their χ^2 values were only just over the 5 per cent. significance level, but it is clear that t^{h5} partially suppresses crossing-over in both males and females. This suggests that t^{h5} has lost only a small part of the abnormal chromosome region present in t^6 , whereas all the other alleles have lost a major part.

3. SUMMARY OF PROPERTIES OF THE MUTANT ALLELES

These studies showed that all ten of the alleles which had obviously arisen by crossing-over were viable when homozygous, and all were fertile. They were divided into three groups on the basis of their segregation ratio and the low ratio group was further subdivided, since t^{h5} showed marked crossover-suppression. The alleles within a group

FIG. 2.—Diagrammatic representation of the abnormal chromosome region present in t^6 and of the crossover alleles derived from it.

were indistinguishable and may have been repeats of each other, but the fact that there were four distinguishable types, all the result of crossing-over, showed that this crossing-over was not restricted to a particular point in the abnormal region, but could occur at various points, as indicated in fig. 2. The lethality and male sterility of t^6 had been lost in every case. This suggested two main possible explanations: either these two properties were due to factors lying at a particular point on the chromosome at a distance from T and close to tf , or they were due to a certain minimum length of abnormal chromosome. These two hypotheses should be distinguishable by studies of

crossovers of the type complementary to those already studied. If the lethality and sterility were due to factors lying at a distance from T the complementary crossovers should possess these properties, whereas if due to a certain minimum length of chromosome they should not. One animal of such a crossover type was found (t^{h18}), and tests of its descendants for the presence of the t^6 lethal factor are described below. Tests for the sterility factor were not possible.

4. THE PROPERTIES OF t^{h18}

Among the offspring of a cross of the type $+tf/t^6+ \times Ttf/tf$, made in the course of linkage studies with t^6 , there was one $T+$

Parents $T(t^{h18})+/+tf \times Ttf/t^6+$

gametes	Non-crossover		Crossover	
	$T(t^{h18})+$	$+tf$	Ttf	$+(t^{h18})+$
Ttf	dies	short-tailed tufted	dies	short-tailed non-tufted
t^6+	tailless or dies	normal tailed	tailless	normal tailed or dies

FIG. 3.—Phenotypes of offspring expected from a cross made to test for lethality of t^6t^{h18} .

animal which was non-tufted. This must have arisen through crossing-over in the $+tf/t^6+$ parent and was of a type complementary to that of all the other crossovers detected. This animal, $Ttf/(t^{h18})+$, was mated to $+tf/tf$. The new allele t^{h18} permitted recombination between T and tf and from this cross descendants of genotype $T(t^{h18})+/+tf$ were obtained. Males of this genotype were mated to Ttf/t^6+ females. Fig. 3 shows that if t^6t^{h18} animals were viable one-third of the offspring of such a cross should be tailless. If on the other hand t^6t^{h18} animals died, only very few tailless young would be expected, which would be the product of crossing-over in the $T(t^{h18})+/+tf$ parent. In fact, out of 159 progeny only 5 (or 3.1 per cent.) were tailless and χ^2 testing deviation from the expectation of one-third tailless was 65.2. If the tailless young actually found were due to crossing-over between t^{h18} and T , the percentage recombination was 6.2, a value consistent with that given by linkage studies described below. This constitutes the evidence that animals of type t^6t^{h18} do not survive and hence that t^{h18} carries the t^6 lethal factor.

t^{h18} was next tested, as far as possible, for the other properties possessed by t^6 . It was already clear that it had no T -modifying effect; both $T/(t^{h18})$ and $T(t^{h18})/+$ animals were short-tailed like $T+$. It was not possible to test the fertility of $t^{h18}t^{h18}$ or t^6t^{h18} since both these genotypes were lethal. The remaining properties were male segregation ratio and crossover-suppression; table 8 shows the

TABLE 8
Tests of the properties of the allele t^{h18}

Property	Test mating		Offspring		
	♀	♂	Short-tailed	Normal	Tailless
Viability of t^6t^{h18}	Ttf/t^6+	$T(t^{h18})+/+tf$	75	79	5
Segregation ratio	♀	♂	Short-tailed	Normal	Per cent. t^{h18}
	$++$	$T(t^{h18})+$	81	78	50.9
Recombination between T and tf	♀	♂	Non-crossover	Crossover	Per cent. recombination
	$T(t^{h18})+/+tf$ $+tf/+tf$	$+tf/+tf$ $T(t^{h18})+/+tf$	272 531	44 22	13.9 4.0

results of tests of these. The only statistically significant effect was a raised recombination in females. An increase in recombination was the opposite of expectation but had to be considered real since it was highly significant statistically and a large effect in relation to the control value.

Thus the properties of t^{h18} were: a lethal effect with t^6 , a crossover-enhancing effect in females, no tail-modifying effect and no effect on male segregation ratio.

5. DISCUSSION

At the start of this work it was already known that the properties of t -alleles were due in some way to an abnormal chromosome region, rather than a "point" locus, and that these properties were separable, at least to some extent, by crossing-over within the region.

These points, put forward by Lyon and Phillips (1959) and Lyon (1960, 1961) were discussed in full by Dunn, Bennett and Beasley (1962). They pointed out that the lethal property of t -alleles could be lost by crossing-over and the T -modifying property retained. In the

present work with the allele t^6 the complementary type of crossover was obtained, carrying the lethal property but not the T -modifying one. This showed that the lethal property was due, not to the whole length of the abnormal region, but to some factor at a definite position on the chromosome. This position was very close to the locus of tufted, tf , no crossovers between the lethal and tf having yet been found. Information about the genetic basis of the T -modifying property was obtained from another group of alleles, described in a later paper, and for this reason fuller discussion of the evidence about the nature of t -alleles will be deferred until this second group of alleles has been described.

6. SUMMARY

The properties of t -alleles were already known to be due to an abnormal chromosome region rather than a "point" locus. A series of mutant alleles derived from the lethal t -allele t^6 have been genetically analysed in an attempt to find the exact genetic basis of some of the properties of this allele.

Ten alleles had arisen by crossing-over in the abnormal chromosome region. These could be divided into three groups on the basis of male segregation ratio, and the low ratio group could be further subdivided, since one allele in this group showed partial crossover-suppression and the others did not. Thus there were four groups of alleles, and all of these had lost the lethality of t^6 , suggesting that the lethality was due to a specific factor lying at a distance from the T -locus. This was confirmed by the finding of the complementary type of crossover, t^{h18} , carrying the lethal factor but lacking the T -modifying effect of t^6 . The lethal factor's position was very close to the locus of tufted, tf .

7. REFERENCES

- BATEMAN, N. 1960. Selective fertilisation at the T -locus of the mouse. *Genet. Res. Camb.*, **1**, 226-238.
- CARTER, T. C., AND PHILLIPS, R. J. S. 1950. Three recurrences of mutants in the house mouse. *J. Hered.*, **41**, 252.
- DUNN, L. C. 1956. Analysis of a complex gene in the house mouse. *Cold Spring Harbor Symp. Quant. Biol.*, **21**, 187-195.
- DUNN, L. C., BENNETT, D., AND BEASLEY, A. B. 1962. Mutation and recombination in the vicinity of a complex gene. *Genetics*, **47**, 285-303.
- LYON, M. F. 1956. Hereditary hair loss in the tufted mutant of the house mouse. *J. Hered.*, **47**, 101-103.
- LYON, M. F. 1960. Effect of X-rays on the mutation of t -alleles in the mouse. *Heredity*, **14**, 247-252.
- LYON, M. F. 1961. The nature of t -alleles in the mouse. *Ann. hum. Genet.*, **25**, 263.
- LYON, M. F., AND PHILLIPS, R. J. S. 1959. Crossing-over in mice heterozygous for t -alleles. *Heredity*, **13**, 23-32.