FURTHER STUDIES OF A MUTATION (*Low*) WHICH DISTORTS TRANSMISSION RATIOS IN THE HOUSE MOUSE^{1,2}

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A recently reported mutant (DUNN and BENNETT 1968), detected by its effect on lowering the male transmission ratio at a locus in the 9th linkage group, has now been mapped and shown to lie in the region associated with transmission ratio distortion and other effects brought about by many mutants known as *t* alleles (reviews in DUNN 1964; BENNETT 1964). In this paper we report the results of breeding experiments establishing its location, its effects on transmission of a segment of the 9th linkage group, and upon recombination. We then discuss questions suggested by the above data concerning ways in which genes may control the transmission of a segment or of a whole chromosome.

Transmission: The effect of the ratio distorter was first noted (DUNN and BENNETT 1968) in the progeny of a single male heterozygous for the dominant mutant gene *Brachy* (*T*), which produces a short tail when heterozygous and is lethal when homozygous. When mated with normal females, that male produced 42 normal offspring and two with *Brachy* tails. Sixteen *Brachy* male descendants of these produced, in four further generations of matings with a normal-tailed stock, about 13.7% *Brachy* offspring instead of the normal 50% in a total of 1881. *Brachy* daughters of the above males mated to normal males transmitted *Brachy* to half their progeny, but *Brachy* sons of these again transmitted *Brachy* to 13.6% of their offspring. It was assumed that a mutant gene linked to *T* was responsible for lowering the transmission of that locus in sperm only. We referred to that mutant as *Lr* but since that symbol had been pre-empted for a lens rupture mutation, we now refer to the ratio distorter as *Low*. It was noted that the locus of *Low* was linked with the loci of both *T* and the nearby marker *tf* (tufted) but the order of these three was not worked out. It appeared further that *Low* probably reduced the frequency of recombination in the *T*-*tf* region in males. Preliminary tests showed that males carrying *T* and *Low* on one homologue and a *t* allele with either a high or a low transmission ratio on the other (*T Low/tⁿ*) transmitted each homologue to half the offspring, suggesting a reciprocal modification or complementary interaction between the different ratio transmission distortion effects of *Low* and *tⁿ*.

In our further studies we have observed the maintenance of the transmission

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TABLE 1

Progenies* from Brachy Low ♂♂ mated with ++ ♀♀

Generation	Number of males tested	Normal tailed	Brachy	Average ratio <i>Br</i> /total	Range of ratios
F ₁ -F ₅ [†]	17	1623	258	13.7	.....
F ₆ [‡]	8	948	115	10.8	5.9-14.7
F ₇	14	1466	186	11.3	7.3-18.5
F ₈	8	975	153	13.6	5.5-22.0
F ₉	13	1277	165	11.4	3.9-15.5
F ₁₀	7	800	106	11.7	7.2-19.2
F ₁₁	9	936	136	12.7	8.1-17.3
F ₁₂	11	1210	199	14.1	7.9-20.5
Totals	70	7612	1060	12.2	3.9-22.0

* Progenies of less than 50 offspring have been omitted.

† These data are reprinted from DUNN and BENNETT (1968).

‡ One male in this generation produced litters showing normal ratios (0.5) as well as others with *Low* ratios. Results of breeding this male and his descendants have been omitted pending further study.

ratio effect for seven further generations of mating *T Low* males with ++ females. The results are shown in Table 1. The average transmission ratio remains close to 12.5% which we have adopted as the standard. Individual males show variation within a range of $\pm 8\%$ of the average, but the extremes are in males with less than 100 offspring. The low ratio appears to be under close genetic control.

Location: Since *Low* can be directly detected only in males by its effect in distorting transmission ratios, estimates of its recombination relations with nearby loci were first made from progenies produced by the testcross: $\delta \frac{T \text{ Low } tf}{++ +} \times \frac{++ + tf}{++ + tf} \text{♀}$. These repeated backcrosses of low ratio Brachy males to the standard stock of normal-tailed tufted females were in general the matings used to maintain the stock carrying *Low*, alternating with crosses $\delta \frac{T \text{ Low } tf}{++ +} \times \frac{++ +}{++ +} \text{♀}$ from our standard Brachy non-tufted stock. The latter cross was to reconstitute the genotype $\frac{T \text{ Low } +}{++ +}$ in which it was desired to maintain *Low*.

The results of backcrosses and testcrosses of the first 12 generations following the recognition of *Low* are shown in Table 2. The 93 males in the first three lines were all shown to be *T-Low* by the low transmission ratio of Brachy (Table 2) which varied between about 4 and 22%. The recombinant classes from the 72 males in the first two lines detectable by inspection were Brachy non-tufted! and normal-tailed tufted!; these occurred in about equal frequencies. After the first recombinant $\frac{T \text{ Low } +}{++ + tf}$ was detected, it was possible to test recombination with

TABLE 2

Recombination in the T-tf interval in males and females with and without Low
(testcrosses with ++tf)

Generation	Males tested	Genotypes	Progeny observed	Recombinants	Rec./Obs.
F ₁ -F ₅	17	$\frac{T \text{ Low } tf}{+ + +}$	477	22	0.0440
F ₆ -F ₁₂	55	$\frac{T \text{ Low } tf}{+ + +}$	1922	54	0.0281
F ₆ -F ₁₂	21*	$\frac{T \text{ Low } +}{+ + tf}$	768	14	0.0182
Totals	93		3167	89	0.0281 ± 0.0029
F ₆ -F ₁₀	15*	$\frac{T + tf}{+ + +}$	488	26	0.0533
F ₆ -F ₁₀	29†	$\frac{T + +}{+ + tf}$	436	11	0.0252
Totals	4†		924	37	0.0400 ± 0.0064
	Females tested				
Testcrosses	13‡	$\frac{T \text{ Low } tf}{+ + +}$	201	13	0.0647 ± 0.0174
Testcrosses	3*	$\frac{T + +}{+ + tf}$	65	8	0.1231 ± 0.0407

* Derived from recombinants.

† Sons of Brachy ♂♂ by $\frac{+ + tf}{+ + tf}$.

‡ Daughters of $\frac{T \text{ Low } tf}{+ + +}$ ♂♂.

T and *Low* in repulsion to *tf*. Recombinants detectable from these were Brachy tufted! and normal-tailed non-tufted! (Table 2, line 3). The recombination fractions estimated from all the above have a weighted average of 0.028 ± 0.003 . Although we have no proof of difference in recombination frequency between coupling and repulsion heterozygotes and such a difference is inherently unlikely, we decided to adopt as a standard the recombination fraction from genes in the coupling arrangement, as being obtained from the most homogeneous set of data. This turns out to be very close to 0.03 which we shall use in subsequent computations as the recombination fraction, *in males*, for the interval from *T* to *tf* when *Low* is present in single dose.

The second group of 44 Brachy males, derived from recombinants and other males of similar sources lacking *Low*, gave a recombination fraction higher than that when *Low* was present, viz., 0.04.

Brachy females, who as nonrecombinant daughters of $\frac{T \text{ Low } tf}{+ + +}$ males were

known to carry *Low*, gave a higher recombination fraction than the males, viz., 0.065; while a few females of the same stock, which were known to lack *Low* gave a still higher recombination fraction, 0.123.

The values previously reported for the *T-tf* interval in the absence of *Low* or other known influences affecting recombination have been 0.067 in males and 0.091 in females (DUNN and BENNETT 1968). It appears therefore that when heterozygous, *Low* interferes with recombination in this interval both in males, which always show less recombination in this region, and in females (Table 2, last two lines).

In order to get estimates of the order and relative spacing of the three loci *T*, *Low*, and *tf*, the progeny of both classes of recombinants, Brachy non-tufted! and normal-tailed tufted! from the heterozygous males in coupling were tested for the presence of *Low*. Of 24 Brachy non-tufted males tested by normal females, 20 gave progenies with equal numbers of Brachy and normal offspring while 4 gave progenies with the ratio of Brachy to normal characteristic of *Low* males

TABLE 3

Progenies produced by test matings of Brachy non-tufted recombinant males with normal females

Male No.	Progenies		Diagnosis of genotype of ♂
	Normal	Brachy	
1	99	94	<i>T</i> .+/+/+++ (<i>tf</i>)
2	15	15	<i>T</i> .+/+/+++ (<i>tf</i>)
3	16	19	<i>T</i> .+/+/+++ (<i>tf</i>)
4	43	40	<i>T</i> .+/+/+++ (<i>tf</i>)
5	64	49	<i>T</i> .+/+/+++ (<i>tf</i>)
7	46	40	<i>T</i> .+/+/+++ (<i>tf</i>)
8	26	36	<i>T</i> .+/+/+++ (<i>tf</i>)
9	20	23	<i>T</i> .+/+/+++ (<i>tf</i>)
10	32	51	<i>T</i> .+/+/+++ (<i>tf</i>)
12	38	32	<i>T</i> .+/+/+++ (<i>tf</i>)
14	50	53	<i>T</i> .+/+/+++ (<i>tf</i>)
16	29	19	<i>T</i> .+/+/+++ (<i>tf</i>)
17	36	43	<i>T</i> .+/+/+++ (<i>tf</i>)
18	41	31	<i>T</i> .+/+/+++ (<i>tf</i>)
19	28	39	<i>T</i> .+/+/+++ (<i>tf</i>)
23	42	43	<i>T</i> .+/+/+++ (<i>tf</i>)
24	13	18	<i>T</i> .+/+/+++ (<i>tf</i>)
25	23	26	<i>T</i> .+/+/+++ (<i>tf</i>)
26	39	36	<i>T</i> .+/+/+++ (<i>tf</i>)
27	8	13	<i>T</i> .+/+/+++ (<i>tf</i>)
Totals 20* ♂ ♂	708	730	<i>T</i> .+/+/+++ (<i>tf</i>)
11	116	16	<i>T Low</i> .+/+/+++ (<i>tf</i>)
13	110	3	<i>T Low</i> .+/+/+++ (<i>tf</i>)
21	58	19	<i>T Low</i> .+/+/+++ (<i>tf</i>)
28	91	10	<i>T Low</i> .+/+/+++ (<i>tf</i>)
Totals 4 ♂ ♂	375	48	<i>T Low</i> .+/+/+++ (<i>tf</i>)

* In addition, 4 ♂ ♂ giving normal ratios of Brachy were discovered by chance in testing offspring of ♂ *T Low tf*/+ + *tf* × + + + ♀. Since the number of nonrecombinants from this cross is unknown, these cannot be included in recombination frequency estimates.

(Table 3). The former appeared to be $\frac{T + +}{+ + tf}$, the latter $\frac{T Low +}{+ + tf}$. This would best be explained by assuming that *Low* lies between *T* and *tf* at about $20/24 = 5/6$ of the interval from *T*, and $1/6$ from *tf*. If we apply these relations to the recombination found between *T* and *tf* in $\frac{T Low tf}{+ + +}$ males, viz., 0.030, then assuming that no double crossover occurs in this short distance, we reach a map of this region as: *T*-0.025-*Low*-0.005-*tf*.

The recombinant class normal-tailed tufted! was tested to separate it into the two genotypes expected (1) $\frac{+ \cdot Low tf!}{+ + tf}$ arising from recombination between the loci of *T* (Brachy) and *Low*; and (2) $\frac{+ + \cdot tf!}{+ + tf}$ arising from recombination between the loci of *Low* and *tf*. Males and females of the recombinant class were mated with our standard Brachy stock which at these loci is $\frac{T + +}{+ + +}$, and a sample of sons of such matings was tested by mating with $\frac{+ + +}{+ + +}$ or $\frac{+ + tf}{+ + + tf}$ females. Of the 79 sons of male normal-tailed tufted! exceptions so tested with progenies of 20 and over, each of 77 produced offspring with a normal Mendelian ratio of Brachy to normal. The total offspring numbered 1221 normal-tailed (.505):1197 Brachy (.495). The remaining two males produced progenies of 12 normal and 98 Brachy and of 3 normal and 20 Brachy, respectively. If these exceptional males are $\frac{T + +}{+ Low tf}$, then the chromosome with the normal allele of *T* is being transmitted to 15/118 or 0.127 of the progeny. This is the transmission ratio shown to be characteristic of *Low* when in coupling with *T*.

In a similar way, 25 Brachy sons of female normal-tailed tufted! exceptions were tested by mating with $\frac{+ + +}{+ + +}$ or $\frac{+ + tf}{+ + +}$ females. Each of twenty-four sons gave normal ratios of Brachy and normal offspring; one produced 20 Brachy and 2 normal offspring. The latter male was thus probably $\frac{T + +}{+ Low tf}$. Proof will be provided below that the recombinant chromosome marked by tufted (+ *Low tf*) is in fact transmitted by male heterozygotes to about 12.5% of their progeny.

This evidence that *Low* may undergo regular recombination with retention of its effect on transmission ratio in sperm now permits it to be treated as a mutant gene which, in heterozygous males, controls at least that segment of the 9th chromosome which extends from *T* to *tf*. The proof of recombination of *Low* also raises the question whether *Low* exerts its effect on the transmission ratio of sperm before or after recombination has occurred. The essential question here is whether *Low* has already determined the transmission ratio of the *Low*-containing homologue before being separated from it (that is, from its centromere) or

TABLE 4

Analysis of recombination between the loci of T, Low, and tf in test matings

$\delta \delta T Low tf/+++ \times ++ tf/++ tf \text{ } \varnothing \varnothing$

The transmission ratio of *Low* in males is taken as 0.125; the recombination fraction *T-Low* as 0.025, *Low-tf* as 0.005

Gamete (sperm) classes		<i>Low</i> effective after recombination δ Trans- mission* Phenotype Frequency expected*		<i>Low</i> effective before recombination δ Trans- mission† Phenotype Frequency expected†	
Parental	<i>T Low tf</i>	0.125	Br tf	0.12125	0.12125
Parental	+ + +	0.875	+ +	0.84875	0.84875
Recomb.	<i>T . + +</i>	0.875	Br +!	0.021875	0.125
region 1	+ . <i>Low tf</i>	0.125	+ tf!	0.003125	0.875
Recomb.	<i>T Low . +</i>	0.125	Br +!	0.000625	0.125
region 2	+ + . <i>tf</i>	0.875	+ tf!	0.004375	0.875
Totals				1.000000	1.000000

* Assuming that *Low* acts after recombination.

† Assuming that *Low* acts before recombination.

whether *Low* affects the transmission of that segment in which it is located after emergence from the exchange bivalent.

The alternative outcomes of the recombination experiment expected on the above alternative assumptions are shown in Table 4. It appears that the two assumptions lead to detectable differences only in the case of recombination between *Low* and the marker gene *T* (region 1).

In computing the expected proportions of recombinants in the two regions, it is assumed that the frequency of the phenotype normal-tailed tufted! is equal to the sum of the two genotypes + . *Low tf* and + + . *tf*, where the former is due to crossing over in the *T-Low* interval and the latter is due to crossing over in the *Low-tf* interval. If *Low* acts after recombination (Table 4), the sum of the nt tf! recombinants is

$$\begin{aligned}\text{region 1} &= .003125 = 41.7\% \text{ of total} \\ \text{region 2} &= .004375 = 58.3\% \text{ of total} \\ \text{sum} &= .007500\end{aligned}$$

When derived from *male* nt tf! recombinants mated with *T++/+++* (Brachy) females, the expected frequency of *T++/+ . Low tf* would be 0.417 (gamete frequency) times 0.125 (transmission ratio of *Low*) = .057. Thus about 5–6% of Brachy males derived from normal-tailed tufted! exceptional males should carry the + . *Low tf* chromosome.

When derived from normal-tailed tufted! exceptional *females*, the frequency of *T++/+ . Low tf* males should be 0.417 (gamete frequency) times 0.5 (transmission ratio of *Low* in females); that is, about 20% of *T++/+ . Low tf* males.

The corresponding expected proportions of + . *Low tf* in normal-tailed tufted! exceptions, if *Low* acts before recombination would be:

$$\begin{aligned}\text{from region 1} &= .021875 = 83.3\% \text{ of total} \\ \text{from region 2} &= .004375 = 16.6\% \text{ of total} \\ \text{sum} &= .026250\end{aligned}$$

TABLE 5

Proportions of observed recombinants between Low and tf compared with proportions expected on alternate assumptions concerning effect of Low

Brachy males tested	Expected (post recomb. effect)	Observed	Expected (pre recomb. effect)
$\frac{*T \quad + \quad +}{+.Low \quad tf!}$	0.057	0.025 (2/79)	0.10
$\frac{†T \quad + \quad +}{+.Low \quad tf!}$	0.20	0.048 (1/21)	0.42

* From ♂ recombinants.

† From ♀ recombinants.

When derived from *male* normal-tailed tufted! recombinants, the frequency of sons carrying $+ \cdot Low \quad tf$ would be $.833 \times .125 = .104$, i.e., about 10% of the Brachy sons of the exceptions would be $T \quad + \quad + / + \cdot Low \quad tf$; when derived from *female* normal-tailed tufted! recombinants, the frequency of $T \quad + \quad + / + \cdot Low \quad tf$ sons should be $.833 \times .5 = .416$, i.e., about 42% of the sons should carry the $+ \cdot Low \quad tf$ recombinant chromosome.

These expectations are compared with the results of breeding tests in Table 5. The results are clearly closer to those expected if *Low* affects transmission ratio after recombination.

Transmission ratio: Brachy sons of $\frac{+ \quad Low \quad tf!}{+ \quad + \quad tf}$ recombinants which gave low ratios of normal-tailed offspring were diagnosed as $\frac{T \quad + \quad +}{+ \quad Low \quad tf!}$. The normal-tailed sons $\frac{+ \quad Low \quad tf}{+ \quad + \quad +}$ of matings of such males with normal-tailed non-tufted females were tested by matings with tufted females in progenies in which the relative transmission of the $+ \quad Low \quad tf$ chromosome would be detected. The results showed that tufted was transmitted by five $\frac{+ \quad + \quad +}{+ \quad Low \quad tf}$ males mated with tufted females to 61/578 offspring or 10.55%. This confirmed the conclusion that *Low* when heterozygous in males has the same effect on the locus of *tf* as upon the locus of *T*.

Tests for other heterozygous effects.

1. *Litter size:* We have already drawn attention (DUNN and BENNETT 1968) to the absence, in our first tests, of any strong effects of *Low* in males upon the litter sizes of their progenies. We now present, in Table 6, distributions of litter sizes in progenies sired by $\frac{T \quad Low \quad tf}{+ \quad + \quad +}$ males of generations F_6 – F_8 . The normal-tailed females with which these males were mated were not uniform, most of them being either from our standard inbred stocks $+ \quad + \quad +$ or $+ \quad + \quad tf$ in which litter sizes at birth average about nine. A few $+ \quad + \quad +$ females from a stock (ICR) with a somewhat higher litter size were also used to test male ratios. The normal test females were rotated amongst the mating pens so that the males being tested had access to what was probably a random sample of test females. As controls we used Brachy

TABLE 6

Comparison of numbers of offspring at birth in litters sired by *T Low*/++ and *T*+/++ males

Generation	No. $\sigma\sigma$	From <i>T Low</i> /++ $\sigma\sigma$ Offspring born	No. litters	Average litter size	No. $\sigma\sigma$	From <i>T</i> +/++ $\sigma\sigma^*$ Offspring born	No. litters	Average litter size
F_6	8	1171	135	8.7	3	204	22	9.3
F_7	14	1668	188	8.7	2	216	20	10.8
F_8	10	1181	124	9.5	7	539	56	9.6
Totals	32	4020	447	8.9	12	959	99	9.7

* Recombinants from *T Low*/++.

males giving normal ratios which had arisen as recombinants and thus lacked *Low*. The comparisons of litter size records tabulated for 44 males from three test generations (Table 6) showed clearly that the sizes of litters from *T Low* males had not suffered depletion before birth of such a magnitude as would result if the reduction in the proportion of Brachy among the offspring from 0.5 to 0.125 had been brought about by the selective death before birth of Brachy *Low* embryos. This we believe may be excluded as a means by which the ratio effect of *Low* is achieved. The comparability of litter sizes from *T Low* males and those from the inbred parent stocks lacking *Low* also make it unlikely that the *Low* ratio effect is due to loss or inactivation of such a fraction of sperm as would reduce the proportion from .5 *T* to .125 *T* although in view of the numbers of sperm produced by males, large losses might not be reflected in effects on litter size. However, such effects are to be studied by direct observation on sperm.

2. *Sex ratio*: In Table 7 are shown the results of classifying for sex within 24 hr of birth, all progenies born to Brachy males during a 5-month period. Reobservation of the sex distribution in a sample of the newborns at four weeks of age revealed a tendency to underestimate the frequency of males at birth. This is reflected in the sex ratio of the control progenies (0.475 $\delta\delta$) since observations in other stocks of mice usually lead to estimates of 0.5 males or slightly over. However, the same bias operated in both sets of progenies. The essential feature of the results is that no influence of *Low* on the sex ratio of the progenies is evident. The

TABLE 7

Sex ratios among offspring* of *T Low*/++ $\delta\delta$ and of *T*+/++ $\delta\delta$

	$26\sigma\sigma$ $\frac{T\ Low}{++} \times \frac{++}{++}$			$49\sigma\sigma$ $\frac{T\ +}{++} \times \frac{++}{++}$		
	Brachy	Normal	Totals	Brachy	Normal	Totals
$\varnothing$	608	65	673	507	525	1032
δ	594	63	657	443	491	934
Total	1202	128	1330	950	1016	1966
Fraction of $\delta\delta$	0.494	0.492	0.494 ± 0.014	0.466	0.483	0.475 ± 0.011

* Progenies classified at birth November 1968–March 1969.

difference in proportions of males from *T Low* and *T +* fathers was 0.019 ± 0.018 . In at least one other case of ratio distortion (DENNELL, JUDD and RICHARDSON 1969) distorted sex ratios due to autosomal segregation distorter (*SD*) in *D. melanogaster* had an important bearing on hypotheses concerning the mechanism. Such is apparently not the case with *Low*.

3. *Effects of late mating on the expression of Low in heterozygous males*: Since it appears that *Low* exerts its effect on the male transmission ratio after recombination has taken place, an experiment was made to test whether any differences in the relative fertilizing capacities of two categories (*Low* and *+*) could be detected. There is some evidence (BRADEN 1958; YANAGISAWA, DUNN and BENNETT 1961) that the male transmission ratio of certain *t* alleles characterized by moderately high ratios when tested under normal mating conditions may be shifted toward a normal ratio when test matings are artificially restricted to a period nearer the time of ovulation (late mating). Alleles in which the transmission ratio is lowered by reduction of the interval between copulation and ovulation were assumed to affect sperm behavior after ejaculation. The methods of controlling time of ovulation in the present experiments were the same as those described by YANAGISAWA, DUNN and BENNETT (1961).

A sample of Brachy males from Generations F_6 – F_8 were ratio-tested under normal conditions to ensure that they carried both *T* and *Low*; then they were mated (a) with *++* females under a standard day length (10 hr light, 14 hr dark) and (b) with *++* females kept in the light except for a dark period of 4 hr (midnight to 4 AM). Since females under the (b) regime usually completed ovulation at about midday, males to be tested by late mating were placed in the (b) pens only from 10 AM to 12 noon, near the time of ovulation.

The results in Table 8 gave no indication of an effect of the late mating conditions on the transmission ratio of *T Low*. Only five of the six males yielded offspring in sufficient numbers to justify application of variance estimates. These yielded no systematically significant differences.

The best judgement is obtained by comparing the transmission ratios observed in the first and second control periods with those in the late mating tests as shown in the matrix below:

Test	1	2	3	4	5
First control	.09	.10	.14	.07	.16
Second control	.05	.15	.10	.13	.18
L.M. test	.07	.11	.09	.07	.09

The two control values show the fluctuations normally encountered; the values under late mating tend to be slightly lower than the average of the controls but not significantly so.

If the effect of *Low* were due to the competitive disadvantage of *Low*-containing sperm in the period spent in the female reproductive tract between copulation and fertilization, then shortening that period by the late mating restriction should decrease the disadvantage, that is, the transmission of *Low* should tend to rise. There is no indication of such a response in the tests reported here.

TABLE 8

Progenies produced by six T Low/++ males mated to normal females (++) to test effect of late mating (LM) on the transmission ratio of Low

Brachy male No.	<i>T Low</i>	nt†	Br‡	Offspring at birth Total	Br/total	S.E.*
	++					
1	Ratio test	209	21	230	0.091	.019
	LM control	63	3	66	0.045	.025
	LM test	97	7	104	0.067	.025
2	Ratio test	73	8	81	0.099	.011
	LM control	117	20	137	0.146	.030
	LM test	150	19	169	0.112	.024
3	Ratio test	81	13	94	0.138	.035
	LM control	91	10	101	0.099	.030
	LM test	82	8	90	0.089	.030
4	Ratio test	113	9	122	0.074	.024
	LM control	74	11	85	0.129	.036
	LM test	110	8	118	0.068	.023
5	Ratio test	109	21	130	0.162	.031
	LM control	64	14	78	0.179	.043
	LM test	59	6	65	0.092	.030
6	Ratio test	40	1	41	0.024	...
	LM control	9	3	12	0.250	...
	LM test	37	5	42	0.119	...

* Standard error = $\pm \frac{\sqrt{pq}}{n}$

† nt = normal tail.

‡ Br = Brachy (short) tail.

4. *Other parameters:* We have examined one other peculiarity of *Low* when transmitted by heterozygous males. This is the occasional occurrence of significant heterogeneity (as estimated by χ^2) in the distributions of the Brachy and normal phenotypes in successive litters where $\frac{T \text{ Low}}{++}$ males are tested by ++ females. Such intramale heterogeneities reach P values of 0.05 or less in about the proportions expected if χ^2 values are randomly distributed. However, in one case a single male produced two distinctly different types of litters: fourteen litters with nearly normal undistorted ratios of Brachy and normal (.42) and 25 litters with low ratios (.11) of Brachy. Analysis of this case and comparison with litter heterogeneities from other males is incomplete and is reserved for later publication. A search for other causes of variability amongst males heterozygous for *Low* such as those due to genes at other loci has begun with selection for high and low transmission ratios of *Low*. Similar statements may be made about effects of advancing age of males on changes in transmission ratio of *Low*. No striking effects of this sort have been noted in the records routinely kept but no statistical search for small trends has yet been made.

Homozygous effect: In order to produce a homozygous stock of $\frac{+ \text{ Low } tf}{+ \text{ Low } tf}$, a few matings were made between $\frac{+ \quad + \quad +}{+ \text{ Low } tf}$ males and females from the testcross just cited above. If *Low* is transmitted by 12.5% of sperm and 50% of eggs, the expected frequency of tufted offspring from above (neglecting recombination between *Low* and *tf*) is about 6%. The actual result was 3 tufted out of 59 offspring (expectation: $3.5 = 59 \times 0.06$). Descendants of the three tufted mice assumed to be $\frac{\text{Low } tf}{\text{Low } tf}$ have produced a viable tufted stock giving litters of normal size (avr. 10). But a small probability exists (about 0.004) of recombination having occurred between *Low* and *tf* so that the tufted phenotype, by which the assumed homozygotes were selected, does not guarantee the presence of *Low*. The male ancestor of this stock was tested for *Low* by mating him with $\frac{T \quad + \quad +}{+ \quad + \quad +}$ Brachy females from our standard stock. Nine of his Brachy sons were tested in matings with normal-tailed females and all gave low ratios of normal-tailed offspring, showing that all were $\frac{T \quad + \quad +}{+ \text{ Low } tf}$. This indicates that one ancestor at least of the assumed $\frac{\text{Low}}{\text{Low}}$ stock was homozygous.

In a more rigorous test of the relative viability of *Low* homozygotes, a balanced stock $\frac{t^{105} \quad + \quad +}{+ \text{ Low } tf}$ has been set up. Since *t*¹⁰⁵ is lethal and known to suppress recombination in the *T-tf* region, the balanced matings should produce offspring as follows:

$\frac{t^{105} \quad + \quad +}{+ \text{ Low } tf}$	×	$\frac{t^{105} \quad + \quad +}{+ \text{ Low } tf}$
$\frac{t^{105}}{t^{105}}$	$\frac{t^{105} \quad + \quad +}{+ \text{ Low } tf}$	$\frac{+ \text{ Low } tf}{+ \text{ Low } tf}$
lethal	normal-tailed non-tufted	normal-tailed tufted
Observed	113	44
Expected	104.7	52.3

The ratio of non-tufted to tufted, if of equal viability, should be $\frac{2}{3} : \frac{1}{3}$. Departures from this serve as a measure of viability of $\frac{\text{Low } tf}{\text{Low } tf}$ relative to $\frac{t^{105} \quad +}{\text{Low } tf}$ in the period from conception to 4 weeks of age when the diagnosis of tufted is made. The first results from breeding this balanced stock do not indicate any great difference in viability. It should be noted that the above expectation depends on the proof (DUNN and BENNETT 1968) that males heterozygous for both *Low* and a high ratio *t* allele transmit each in equal proportions. This rule has now been extended

TABLE 9

Results of reciprocal matings of $\frac{T \text{ Low } +}{+ \text{ Low } tf} \times + \text{ Low } tf$ to test effects of *Low*, when homozygous, on transmission ratio and recombination

Brachy parents	nt	Offspring at birth		Total/Br	nt tf	Br	At 28 days		Recomb.
		Br	Total				Br tf!	nt!	
9 ♂ $\frac{T \text{ Low } +}{+ \text{ Low } tf}$	392	369	761	0.485 ± .018	226	223	10	19	29/478 (.061 ± .011)
25 ♀ $\frac{T \text{ Low } +}{+ \text{ Low } tf}$	169	123	292	0.421 ± .029	92	79	7	13	20/191 (.105 ± .022)

to t^{tes} , a high ratio t allele. A $\frac{t^{tes}}{Low}$ male when mated with Brachy $\frac{T}{+}$ females produced equal proportions of tailless and Brachy offspring (49 and 40, respectively), whereas $\frac{t^{tes}}{+}$ males produce a proportion of .9 tailless to .1 Brachy when so tested.

Additional data bearing on the homozygous effects of *Low* are shown in Table 9. Testcrosses of Brachy males homozygous for *Low* ($\frac{T \text{ Low } +}{+ \text{ Low } tf}$) showed that *T* is transmitted normally, i.e., to half the offspring. The deviation from equality in the proportion of Brachys is within the sampling error. It appears more markedly in the offspring of $\frac{T \text{ Low } +}{+ \text{ Low } tf}$ ♀♀ and may indicate a reduced viability of Brachy before birth. The ratio effect of *Low* thus appears only in heterozygous *T Low*/++ males. It may be noted also that the litters produced by males homozygous for *Low* do not differ markedly in size (average 8.7) from those produced by males which are heterozygous for *Low* but otherwise similar. This provides further evidence against the assumption that *Low* renders inactive most of the sperm which transmit it, since from homozygotes all sperm transmit it, yet no impairment of fertility is evident.

In Table 9 are shown also the frequencies of recombination in the *T-tf* region in both males and females when these are homozygous for *Low*. Recombination in *Low* homozygotes occurs at about the normal rate and indicates that *Low*, when homozygous, has no effect on recombination. The frequencies differ significantly from those observed when the parents tested are heterozygous for *Low*. In *Low*/+ males recombination in this region is about 0.03; in *Low*/*Low* males it is 0.06; in *Low*/+ females it is about 0.06; in *Low*/*Low* females it is 0.11 (Table 2).

DISCUSSION

The *Low* allele has some novel features when compared with the few cases of ratio distortion which have been studied in other animals. In the most studied case, that of male segregation distortion in *Drosophila melanogaster*, a mutation,

SD, near the centromere of chromosome 2 is transmitted by *SD/SD*⁺ males to more offspring than its normal allele. In the case of certain *SD* chromosomes, the ratio of *SD* offspring approaches 1.0. Such males have about half as many total offspring as *SD*⁺/*SD*⁺ (or *SD* suppressed) control males. This was independently proved by NICOLLETTI, TRIPPA and DEMARCO (1967) and by HARTL, HIRAIZUMI and CROW (1967). In addition, NICOLLETTI (1968) has examined by electron microscopy the testes of males carrying a high ratio (*SD*^{Roma}) allele, and has found evidence of zones of degenerating spermatids and of abnormal sperm heads. It thus appears likely that the ratio distortion of *SD* is caused by failure in formation or function of sperms with the *SD*⁺ chromosome. The effects of temperature on the degree of ratio distortion (MANGE 1968) and of ageing (HIRAIZUMI and WATANABE 1969) also suggest action of *SD* in early meiotic stages of the male. DENNELL and JUDD (1969), who found that *SD* affects the sex ratio of *SD*⁺ offspring, suggested that *SD* produces a gene product before meiosis which differentially affects the functioning of *SD*⁺ cells or sperm. In effect, the above interpretations invoke what is equivalent to a paternal effect of the *SD/SD*⁺ zygote genotype on the fertility of sperm without *SD*. No evidence at present points to an effect of the *SD* mutation on the sperm which transmit it.

Another type of interpretation, applied to the ratio distortion associated with certain alleles in the house mouse (*t* alleles), assumed that the effect of an allele transmitted to about 80% of offspring was postmeiotic. The effect which gave sperm carrying it an advantage in fertilization was assumed to be a direct one upon the function of the sperm which transmit it (BRADEN 1958; YANAGISAWA, DUNN and BENNETT 1961).

This hypothesis is further discussed below. At present it should be pointed out that the *t* allele ratio distorters all show one essential difference from *Low*. The effect of *Low* is exerted upon a chromosome segment between *T* and *tf* and is separable from each of these loci, whereas *t* distorters are strictly allelic with *T*. The ratio effects of each of the different *t* alleles have not been found to be separable by regular recombination from the phenotypic effect upon the tail by which each is recognized. Although most lethal and semilethal *t* alleles suppress crossing over in the region between *T* and *tf*, others which do not suppress recombination have ratio effects likewise not separable from the *t* allele. The locus of *Low* is included in the region of recombination suppression by *t* lethals with high ratios. The question is thus raised whether *Low* is allelic with another ratio-controlling locus in this area (one associated with *t* alleles). This can best be discussed in connection with studies of interaction between two different *t* alleles, and between *Low* and various *t* alleles. This is treated in a separate paper. (BENNETT and DUNN 1971). The point should be made here however that the type of interaction found between two different *t* alleles differs sharply in several ways from the interaction noted between *t* distorters and *Low*. It does not seem likely, therefore, that *Low* represents a ratio factor in some way isolated from a pre-existing *t* allele.

Concerning the manner in which *Low* may cause its effect in males, the following possibilities may be considered.

1. *Low* may act locally to reduce the probability that the chromosome segment containing *Low* will enter a spermatid (selective segregation). We have no evidence bearing on this.

2. *Low*, having been distributed normally to half of the spermatids may interfere with the differentiation of some fraction of them. Thus if, in a tubule section containing 128 spermatids, 64 with + and 64 with *Low*, all of the former differentiated into spermatozoa and only 8 of the latter, then the proportion of *Low* would be reduced from $\frac{1}{2}$ to $\frac{1}{8}$ (12.5%). The proportion is purely arbitrary and chosen to conform to the average proportion of functional *Low* spermatozoa at fertilization. This has the merit however of predicting that a proportion of abnormal spermatids should be found in the tubules. It should be noted that this possibility envisages effects of a gene on the spermatid or secondary spermatocyte which contains it, thereby implying transcription after segregation. It would have to be assumed also that loss of cells at an early stage would not cause significant reduction in fertility.

3. *Low* may act upon sperm behavior as expressed in such attributes as length of functional life or motility in such a way as to reduce the proportion of sperm containing *Low* which penetrate the egg (selective fertilization or gametic selection). This mode of action would also require transcription after segregation. One test (late mating) which if positive might have given support to such a mechanism was negative and therefore throws no light on the question. It should be noted that if $\frac{7}{8}$ of the *Low* spermatozoa failed in fertilization, a loss of $\frac{7}{16}$ of all sperm might be expected to measurably reduce fertility of *Low*/+ males. No reduction in fertility is found, however.

4. *Low*, having entered the zygote might have a dominant lethal effect before birth causing a loss of $\frac{7}{8}$ of the zygotes with *Low* or $\frac{7}{16}$ of the embryos, an assumption opposed by our observations on litter size.

Against these modes of operation is the generally positive evidence, recently summarized by BEATTY (1970), against the activity of genes in animal gametes after segregation. Lethals, chromosomal deficiencies, and genes with other deleterious effects are normally transmitted by sperm (in *Drosophila*, amphibia, mouse, and man). Effects of genes on plant gametes have long been known. Genes have been shown to produce active products in both male and female gametophyte generations, in which nuclear activity is evident and this occasions no surprise. BEATTY refers to "one apparently indisputable instance" of postsegregational gene action in animals, namely, the apparently differing response of sperm carrying certain high ratio *t* alleles and normal alleles to conditions in the female reproductive tract (late-mating effect). This however seems to us to rest on rather slender and not always consistent evidence. In the few cases studied by BRADEN (1958) and by YANAGISAWA, DUNN and BENNETT (1961) certain high ratio alleles did not respond at all to late-mating conditions; in subsequent tests (unpublished) neither have low ratio alleles responded nor have those moderately high ratio alleles responded which initially appeared to yield positive results in earlier tests. In the absence of other explanations of the above exceptions and failures,

we tend to suspend judgment and seek for alternative explanations, paying special attention to cells immediately following meiosis before differentiation into spermatozoa has occurred. Interactions may occur between genes within gametes and the zygotic constitution of the male. This is suggested by effects on male fertility of certain combinations of *t* alleles (DUNN and BENNETT 1969).

It should be recalled that in animals, transmission ratio distortion effects have been noted and studied only in males. Studies of male ratio distorters have not revealed the mode of action of such genes either in *Drosophila* or the mouse. They may, however, point to features peculiar to male gametes which are worth studying for their own sake.

SUMMARY

A mutation *Low* is described with locus near that of *tufted*, linkage group IX. When heterozygous, *Low* controls the transmission through sperm of loci at that end of the 9th chromosome which is toward the centromere. The transmission ratio of *Low* has a mean value of about 12.5 percent. *Low* also reduces recombination in that region when heterozygous. No influence of homozygous *Low* has been detected on transmission ratio, on viability, or on recombination.—The mode of action of *Low* has not been established but it is thought to produce its ratio effect after the time of recombination. It appears not to act by lethal effect on zygotes or by gametic selection on sperm. Action on the spermatid stage has not been eliminated as a possible means of transmission ratio distortion.

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