

' *Dappled* ', a new allele at the *Mottled* locus in the house mouse

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INTRODUCTION

There have been five reported occurrences of sex-linked mutants in the mouse that produce a variegated appearance of the coat in heterozygous females: *Mottled* and *Brindled* (Fraser, Sobey & Spicer, 1953), *Tortoiseshell* (Dickie, 1954), *26K* (Welshons & Russell, 1959), and a fifth recently described by Lyon (1959). All except *Brindled* are lethal before birth in hemizygous males, and therefore the usual breeding tests for genetic identity or allelism cannot be carried out. On the grounds of linkage relationships and phenotypic resemblances, however, it seems probable that *26K* and Lyon's mutant are recurrences of *Mottled Mo* (Lyon, 1960; Welshons & Russell, 1959) and that *Brindled* is a different allele at the same locus *Mo^{br}* (Falconer, quoted by Lyon, 1959). The status of *Tortoiseshell* is more doubtful. It resembles *Mottled* closely, the main difference in their descriptions being that *Tortoiseshell* is said to have a silky coat, whereas no such effect has been reported for *Mottled*. However, no direct comparison appears to have been made, and its linkage relationships have not been reported.

This paper describes a sixth sex-linked gene producing a variegated coat in heterozygous females. It is concluded from the data presented below that the hemizygous males die shortly before birth and that the new mutant is therefore different from *Mottled* and *Brindled*, though is probably at the same locus: it has been called *Dappled*, symbol *Mo^{dp}*.

ORIGIN

The mutation first appeared in an F₁ male from the low-dosage γ -irradiation experiment described by Carter, Lyon & Phillips (1958); the parents of this animal produced one *Dappled* male out of 64 classified offspring. On outcrossing to unrelated females, he sired 10 *Dappled* females, phenotypically similar to himself, 278 normal females and 290 normal males. The 10 *Dappled* females were all sired in the first 4 weeks of a total breeding life of 46 weeks (Table 1).

GENETICS

Single factor segregations (Table 2) indicated that *Dappled* was a sex-linked semi-dominant gene, lethal in the hemizygous male, but could not exclude the possibility of the phenomenon of sex limitation. Linkage tests were set up to confirm that *Dappled* was sex-linked and to find its position on the sex chromosome. Falconer (1953) found that *Mottled*, which *Dappled* closely resembles

Table 1. Breeding performance of original Dappled male

Litters in chronological order	Age of male (in days) when litter born	Females		Males	
		<i>Dappled</i>	Normal	<i>Dappled</i>	Normal
1	67	2	0	0	5
2	73	4	1	0	1
3	84	2	2	0	3
4	95	2	2	0	6
5-83	110-388	0	273	0	275
Total		10	278	0	290/578

Table 2. Single factor segregations from Dappled daughters of original male crossed to unrelated males

Sex of offspring at birth		Phenotypes of offspring at weaning				Total
		Females		Males		
Females	Males	<i>Dappled</i>	Normal	<i>Dappled</i>	Normal	
119	62	38	56	0	55	149

phenotypically, gave a recombination frequency of 4.1% (5% fiducial limits: 1.7 and 8.4) with *Tabby* and (Falconer, 1954) that the order of genes on the sex chromosome was *Bent-tail-Tabby-Mottled*: therefore *Dappled* was tested against both *Tabby* and *Bent-tail*. Two-point tests between *Tabby* and *Dappled* indicated that they also lay about 4 units apart (Table 3). Similar crosses made between *Bent-tail* and *Dappled* showed a recombination value of about 29% (Table 4), but the

Table 3. Linkage data between Dappled and Tabby

Genotype of parents	Phenotype of female offspring						Recombination and standard error (%)
	<i>Mo^{dp}Ta</i>	<i>Mo^{dp}+</i>	<i>+Ta</i>	<i>++</i>	OC*	RC*	
<i>Mo^{dp}+/+Ta</i> ♀ × <i>+Ta/-</i> ♂	15	331	367	16	698	31	4.80 ± 0.58
<i>Mo^{dp}Ta/++</i> ♀ × <i>+Ta/-</i> ♂	6	3	2	10	16	5	
					714	36	

* OC, RC = old combinants; recombinants.

Table 4. Linkage data between Dappled and Bent-tail

Genotype of parents	Phenotype of female offspring						Recombination and standard error (%)
	<i>Mo^{dp}Bn</i>	<i>Mo^{dp}+</i>	<i>+Bn</i>	<i>++</i>	OC*	RC*	
<i>Mo^{dp}+/+Bn</i> ♀ × <i>++/-</i> ♂	9	154	93	83	247	92	29.272 ± 2.32
<i>Mo^{dp}Bn/++</i> ♀ × <i>++/-</i> ♂	—	13	3	14	14	16	
					261	108	

* OC, RC = old combinants; recombinants.

T*

disturbed ratio of *Bent-tail* to normal (due presumably to normal overlapping of the *Bent-tail* phenotype in females) makes these data suspect. However, as the recombination between *Tabby* and *Bent-tail* is approximately 15% (Phillips, 1954), it seemed probable that the order of genes was *Bent-tail-Tabby-Dappled*. This order was confirmed by three-point tests in which $Mo^{dp} + + / + Ta Bn$ females were crossed to *Tabby* males (Table 5); the putative double-crossover class of

Table 5. *Three-point linkage data between Dappled, Tabby and Bent-tail*

Genotype of parents	Phenotype of male offspring*				Mo^{dp} Lethal before birth— not classified
	+ Mo				
	$Ta Bn$	$Ta +$	+ Bn	+ +	
$Mo^{dp} + + / + Ta Bn \text{♀} \times + Ta + / - \text{♂}$	51	29	—	8	

* Data from female offspring not given, upset in $Bn/+$ ratio makes it impossible to be sure of the exact number of Mo^{dp} and Ta crossovers.

Bent-tail non-Tabby males was completely absent. (The data from male offspring only were used as Falconer (1954) found no evidence of normal overlapping in the hemizygous *Bent-tail* animals.) It seemed probable, therefore, that *Dappled* and *Mottled* were allelic.

Direct tests of *Dappled* with *Mottled* are not possible as both are lethal in hemizygous males, but further evidence in favour of allelism was obtained by crosses between three *Dappled* females and a viable *Brindled* (Mo^{br}) male, kindly sent by Dr D. S. Falconer. These yielded 22 classifiable offspring: 14 males, all normal, and 17 females. Of the females, 11 resembled normal heterozygous *Dappled* females, and 6, all of which died before weaning age, resembled hemizygous *Brindled* males as described by Falconer (1953); they presumably carried both *Dappled* and *Brindled*. The strong interaction, together with the phenotypic resemblance, and position on the chromosome, suggests that *Dappled* is another allele at the *Mottled* locus.

HETEROZYGOUS FEMALES

Most *Dappled* females are identifiable at birth by a curling of the vibrissae, but the degree of curling varies considerably, some having nearly straight whiskers. The adult coat has a variegated pattern similar to that of *Brindled*, *Mottled* and *Tortoiseshell* females. The coat is straight, though the whiskers remain slightly curled.

Some *Dappled* females show clubbing of one or both fore feet at birth, or, at weaning, have a tendency to walk on the dorsal surface of the hind feet; the presence of abnormal feet is associated with a greater degree of curling of the vibrissae (Table 6: $P = 0.0014$ by Fisher's exact test). The amount of curling of the whiskers is also correlated with the degree of lightness of the coat at weaning (Table 7: $r = -0.519$; $P < 0.001$); the greater the curling, the lighter the coat.

Table 6. *The association of curling of whiskers with foot abnormalities*

Description of feet	Description of whiskers at birth		Totals
	Very curly to curly	Slightly curly to straight	
Fore feet clubbed at birth Hind feet twisted at weaning } Normal	14	0	14
	28	21	49
Totals	42	21	63

Table 7. *The association of curling of whiskers with coat colour*

Description of coat colour at weaning	Description of whiskers at birth				
	Very curly	Curly	Slightly curly	Bent	Straight
Very, very dark	—	—	2	—	—
Very dark	—	—	1	—	—
Dark	—	—	3	2	—
Darkish	1	2	3	—	—
Medium dark	2	—	5	—	1
Medium	2	11	2	—	—
Medium light	5	7	2	—	—
Lightish	2	4	—	—	—
Light	1	5	—	—	—

Assigning arbitrary scores as follows:

y (curliness of whiskers): -2 (very curly) to $+2$ (straight)

x (colour of coat): -4 (very, very dark) to $+4$ (light),

then $r = -0.519$, $P < 0.001$.

There is no significant correlation between the colour of the mother's coat and that of the offspring ($P > 0.1$), and attempts to select for lighter or darker strains have met with no success.

Another condition which is found in some *Dappleds* is the development, with age, of calcified lumps in the region of the periosteum especially on the vertebral column, the thoracic and lumber regions of which are particularly affected. Whether these bodies are outgrowths from the bone is not yet known. None has been found in cleared *in toto* preparations of mice under 8 weeks old. This phenomenon was also reported by Lyon (1960) as occurring in her mutant.

HEMIZYGOUS MALES

A study of the offspring of the *Dappled* daughters of the original *Dappled* male showed that there were about half as many males as females at birth (Table 2), suggesting that hemizygous males died before birth, as had been found for *Mottled* (Falconer, 1953). To study this matter further, heterozygous females and their normal sibs were mated to unrelated males, killed 14 to 18 days after a vaginal plug had been observed and their embryos classified. The data are given in Table 8. As there is no significant difference between the two series in the proportion of death prior to 15 days' gestation (Table 9: $\chi^2 = 0.02$), it was assumed that *Dappled*

Table 8. *Classification of embryos from (I) Dappled ♀♀, (II) their normal sisters*

Age of embryos (in days)	Number of uteri classified	Classification of embryos*								Other implants dead prior to 15 days	Total no. of implants	Numbers of corpora lutea
		Females		Males		Unsexed		Moles				
		N	A	N	A	N	A					
(I)												
14	15	—	—	—	—	103 ⁽⁵⁾	—	10	5	118	135-6	
15	12	—	—	—	—	62	14	8	4	88	101	
16	3	—	—	—	—	13	7	—	—	20	28-9	
16½ to 18	16	65	0	22	18 ⁽¹⁾	—	—	8	6	125 ⁽²⁾	141-6	
(II)												
14	10	—	—	—	—	88 ⁽³⁾	0	0	7	95	110-13	
15	7	—	—	—	—	53	0	5	3	61	74	
16	3	—	—	—	—	20	0	2	1	23	33	
16½ to 18	9	43 ⁽⁴⁾	0	27	0	—	—	3	8	81	86-9	

* N = Normal ribs. A = Abnormal ribs.

(1) Including 1 with doubtful sex classification.

(2) Including 6 embryos which died later than 15 days, but whose ribs were not classifiable.

(3) Including 1 with blood clot at base of spine.

(4) Including 1 small embryo, sex doubtful.

(5) Includes 21 animals with ribs difficult to see.

Table 9. *A comparison of data on deaths prior to 15 days' gestation (from table 8)*

Genotype of mother	Classification of implants			Preimplantation loss*
	Not dead prior to 15 days	Moles	Other deaths prior to 15 days	
<i>Mo</i> ^{dp} +	310	26	15	61
+ +	231	19	10	49

* Figure obtained by subtracting 'Total implants' from 'Corpora lutea'.

males survived past this stage. Amongst embryos of 15 days or more some were found with ribs which were very white, thickened and bent. Most of these abnormal embryos were dead or dying at 17-18 days of gestation: death at this stage is most unusual in the mouse, where embryonic death usually occurs prior to the 15-day stage. These abnormal embryos are assumed to be the hemizygous males but were not as frequent as expected on a three-to-one basis: a study of the 17-18 day data, in which the embryos were sexed, showed that half the males and none of the females were affected (Table 8), so the shortage of abnormal embryos is presumably due not to the *Dappled* gene but to the upset ratio observed in both series. Males with abnormal ribs have occasionally been found at birth, but all were dead when found and they appeared to have died at about the time of birth. The cause of death is unknown. A preliminary study of stained and cleared

in toto preparations of presumed hemizygous males has shown that there is distortion of the pectoral and pelvic girdles and the limb bones as well as of the ribs: a full investigation of the skeletal abnormalities has not yet been completed.

As *Dappled* has proved to be lethal in the hemizygous condition, the original male, the phenotype of which resembled that of a heterozygous female, presumably carried a sectorial mutation, the coat and part of the testes at least being affected. Only a small proportion of the testes were mutant unless there was heavy selection against the mutant gametes. The data (Table 1) on the breeding performance of the male also indicate the possibility of there being a time sequence in the use of the tubules of the testis such as is found in insects. It is surprising that the coat of this animal should be similar to that of a heterozygous female rather than a *Brindled* male, the only known hemizygote in this allelic series.

SUMMARY

Dappled, a new completely sex-linked semi-dominant lethal mutation, is described. It is probably a new allele at the *Mottled* locus and has been given the symbol Mo^{dr} .

Heterozygous adult females have a variegated coat very similar to that of *Brindled* and *Mottled* females; they have curly vibrissae at birth and some also have clubbed feet. All the characteristics are variable.

Hemizygous males die at about 17 days' gestation; they show a characteristic bending and thickening of the ribs and other skeletal defects. The cause of death is unknown.

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