Genet. Res., Camb. (1969), **14**, *pp.* 163–166
Printed in Great Britain

Muted, a new mutant affecting coat colour and otoliths of the mouse, and its position in linkage group XIV

By MARY F. LYON and R. MEREDITH*
M.R.C. Radiobiology Unit, Harwell, Berkshire

(*Received* 19 *May* 1969)

1. INTRODUCTION

The name and symbol *muted*, *mu*, have been given to a new autosomal recessive mutant of the mouse, *Mus musculus* L., which arose spontaneously in a stock segregating for *t*-alleles (Lyon & Meredith, 1965). Its locus is in linkage group XIV and studies with it have helped to elucidate the order of loci in this group (Lyon, 1966).

2. DESCRIPTION

The coat colour of muted homozygotes is diluted slightly to a muted version of the colour that it would otherwise have been. Black becomes dark grey, yellow and brown become a somewhat lighter shade, and many animals have white underfur. The eye colour also is diluted, so that the iris ring looks very light at birth, and the eye appears a dark red colour in the adult. In combination with other genes affecting eye colour, the eye may be colourless at birth and pink in the adult. In general, the colour change produced by the muted gene resembles that produced by ruby-eye, *ru*, and beige, *bg*, and is much less drastic than that produced by pallid, *pa*.

However, as well as affecting coat colour the gene causes changes in posture and postural reflexes very similar to those seen in pallid homozygotes (Lyon, 1951). Some muted homozygotes hold the head tilted to one side; some flex the spine and tuck the head under when held up by the tail, and give no normal landing reaction when dropped head downwards towards a table. Whole mounts of the inner ears (by the method of Lyon, 1954) showed that many, but not all, muteds lack otoliths from the sacculus and utriculus of one or both ears. Of 17 such animals examined, 12 lacked all otoliths, 3 had one otolith in each ear, 1 had two otoliths in the left ear and 1 in the right, and the remaining one had otoliths in both ears. The bony labyrinth appeared completely normal in all the animals, and none was deaf or showed circling behaviour.

3. GENETICS

The single factor segregations given in Table 1 show that the gene is inherited as an autosomal recessive with good viability and penetrance, when classified on the basis of coat colour.

Allelism tests with pallid, *pa*, proved negative as did those with *a*, *b*, *bg*, *c*, *ep*, *ln*, Mi^{wh}, *p*, *pe*, *ru* and W^{v}.

Linkage relations. Tests indicated linkage with pearl (*pe*) and extra-toes (*Xt*) in linkage group XIV. Further tests were then carried out to determine the order of loci in the linkage group.

* Present address: Middlesex Hospital Medical School, London, W.1.

Balanced three-point linkage backcrosses with *Xt*, *mu* and *pe* showed that the *mu* locus was the middle one of the three (Table 2). Other loci lying between *Xt* and *pe* include those for congenital hydrocephalus, *ch*, and flexed-tail, *f* (Lyon, Morris, Searle & Butler, 1967). Linkage intercrosses for *mu*, *f* and *pe* were made (Table 3) and the genotypes of the phenotypic crossovers among the offspring were investigated. All of six phenotypic $+f+$ animals were proved not to carry *mu*, all of eight phenotypic $++pe$

Table 1. *Single factor segregation of* muted

Genotype of parents		Offspring: Normal	Offspring: Muted
$++ \times mumu$		120	.
$+mu \times +mu$	Obs.	197	57
	Exp.	190·5	63·5
$+mu \times mumu$	Obs.	95	74
	Exp.	84·5	84·5
$mumu \times mumu$		.	159

Table 2. *Results of three-point linkage backcrosses with* muted

	Genotype and sex of heterozygote: $\frac{Xt+pe}{+mu+}$ ♀	$\frac{Xt+pe}{+mu+}$ ♂	$\frac{Xt++}{+mu\,pe}$ ♀	$\frac{Xt++}{+mu\,pe}$ ♂	$\frac{Xt\,mu+}{++pe}$ ♂
Offspring					
$Xt++$	6	8	41	15	.
$Xt\,mu+$	1	4	2	.	47
$Xt+pe$	26	12	18	3	2
$Xt\,mu\,pe$	.	.	4	1	8
$+++$	6	2	16	2	21
$+mu+$	22	19	14	4	4
$++pe$	8	6	4	.	29
$+mu\,pe$	15	1	38	11	.
Total	84	52	137	36	111

Recombination			%	S.E.
Xt–mu	♀	41/221	18·6	2·6
	♂	21/199	10·6	2·2
mu–pe	♀	65/221	29·4	3·1
	♂	47/199	23·6	3·0
Xt–pe	♀	82/221	37·1	3·3
	♂	64/199	32·2	3·3

did carry *mu*, and the single $mu+pe$ animal tested was shown to be genotypically $mu+pe/mufpe$. Thus the types of crossover chromosomes discovered were $+f+$, $mu+pe$, and $mufpe$. If the order of loci is taken to be *mu–f–pe* then all these types are single crossovers, but if the order were *f–mu–pe* then the $mufpe$ chromosome would represent a double crossover and one would have expected some single crossovers of the types $muf+$ and $++pe$. It is therefore concluded that the order of loci is *Xt–mu–f–pe*.

This is consistent with the observed recombination values, as the *Xt–mu* recombination in our backcrosses was lower than the *Xt–f* recombination found by Lyon *et al.* (1967).

It was of interest also to locate *muted* with respect to the loci of satin (*sa*) and beige (*bg*), which are also in linkage group XIV (Lane, 1965), since beige is phenotypically similar to muted. Three-point linkage backcrosses with *bg*, *Xt* and *sa* (Table 4) gave no

Table 3. *Results of linkage intercrosses with* muted

Offspring	Heterozygote $\frac{mu++}{+f\ pe}$	Offspring*	Heterozygote $\frac{Xt\ sa++}{++mu\ pe}$
+++	107	*Xt*+++	70
mu++	36	*Xt sa*++†	5
+*f*+	10	*Xt*+*mu*+	4
++*pe*	16	*Xt*++*pe*	10
mu f+	.	*Xt*+*mu pe*‡	5
mu+*pe*	4	++++§	10
+*f pe*	16	++*mu*+	12
mu f pe	.	++*mu pe*	16

* Other types of offspring were possible, but not found.
† Genotypes: 3 *Xt sa*++/+*sa*++.
‡ Genotypes: 2 *Xt*+*mu pe*/++*mu pe*, 1 *Xt sa mupe*/++*mu pe*.
§ Genotypes: 4++*sa*+/++*mu pe*, 3++++/++*mu pe*.

Table 4. *Results of three-point linkage tests with* +Xt+/bg+sa *heterozygotes*

Offspring		Heterozygote ♀	Heterozygote ♂	
+*Xt*+		107	73	
+*Xt sa*		8	1	
bg Xt+		1	1	
bg Xt sa		.	.	
+++		.	.	
++*sa*		.	.	
bg++		10	3	
bg+*sa*		99	69	
Total		225	147	
Recombination			%	S.E.
bg–Xt	♀	1/225	0·44	0·44
	♂	1/147	0·68	0·68
Xt–sa	♀	18/225	8·0	1·8
	♂	4/147	3·7	1·3
bg–sa	♀	19/225	8·4	1·9
	♂	5/147	3·4	1·5

crossovers of the types *bg Xt sa* or +++. If one assumes the least frequent types to be the double crossovers, then the order of loci is as shown. In order to find whether *satin* and *muted* lay on the same or on opposite sides of *Xt* four-point intercrosses with *Xt*, *sa*, *mu* and *pe* were set up (Table 3), and the genotypes of phenotypic crossovers were tested. Four types of crossover chromosome were found: *Xt*+*mupe*, +*sa*++, *Xtsamupe*, and ++++. If the order of loci were *sa–Xt–mu–pe* the first two would be

https://doi.org/10.1017/S0016672300002007 Published online by Cambridge University Press

double crossovers, and if the order were *Xt–mu–sa–pe* the second two would be triple crossovers, but if the order is *Xt–sa–mu–pe* then all four are single crossovers. It is therefore concluded that this is the correct order.

The complete order and approximate map distances known at present in linkage group XIV are therefore

	bg	–	*Xt*	–	*sa*	–	*mu*	–	*f*	–	*pe*
♀		0·5		8		11		4		25	
♂		0·5		3		8		8		16	

with crinkled (*cr*) very close to *bg* (Lane, 1965) and *ch* close to *sa* and *mu*.

4. DISCUSSION

Muted is interesting as another example of a gene like *pallid* which affects both coat and eye colour and otoliths, and hence it may provide information about otolith formation. Pallid is thought to interfere with otolith development by affecting the formation of the sulphated mucopolysaccharides of the otolith matrix (Erway, Shrader & Hurley, 1968). A third mutant, unbalanced, which is allelic with tilted-head, *th*, apparently acts in a different way, since the otolith is reduced, to large crystals, rather than absent (Erway, Hurley & Fraser, 1966), and, whereas supplementation of the mother's diet during pregnancy with manganese would prevent otolith defect in pallid offspring, it would not do so in unbalanced mice. Further studies with muted might reveal whether it acts in the same way as pallid, or whether yet a third mode of action is involved. In this way further insight into otolith formation could be obtained. The stock is available to interested workers.

SUMMARY

The autosomal recessive gene muted, *mu*, which arose spontaneously, dilutes coat and eye colour and causes absence of otoliths in some but not all homozygotes. Its locus is in linkage group XIV of the mouse, and the order of loci was shown to be *bg–Xt–sa–mu–f–pe*.

We are grateful for technical assistance at different times to Miss J. M. Butler, Mrs M. Daglish, Mr R. Grinter and Miss S. G. Hawkes.

REFERENCES

ERWAY, L., HURLEY, L. S. & FRASER, A. (1966). Neurological defect: manganese in phenocopy and prevention of a genetic abnormality of inner ear. *Science, N.Y.* **152**, 1766–1768.

ERWAY, L., SHRADER, R. & HURLEY, L. S. (1968). Private communication. *Mouse News Letter* **39**, 20.

LANE, P. W. (1965). Private communication. *Mouse News Letter* **32**, 47.

LYON, M. F. (1951). Hereditary absence of otoliths in the house mouse. *J. Physiol., Lond.* **114**, 110–118.

LYON, M. F. (1954). Stage of action of the litter-size effect on absence of otoliths in mice. *Z. indukt. Abstamm.- u. VererbLehre* **86**, 289–292.

LYON, M. F. (1966). Private communication. *Mouse News Letter* **34**, 28.

LYON, M. F. & MEREDITH, R. (1965). Private communication. *Mouse News Letter* **32**, 38.

LYON, M. F., MORRIS, T., SEARLE, A. G. & BUTLER, J. (1967). Occurrences and linkage relations of the mutant 'extra-toes' in the mouse. *Genet. Res.* **9**, 383–385.