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Location of the *Mup-a* locus on mouse linkage group VIII

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1. INTRODUCTION

In a previous communication we reported that two codominant alleles, *Mup-a*^{1} and *Mup-a*^{2}, controlling electrophoretic variation of one of the components of the major urinary protein (MUP) complex of the mouse (*Mus musculus*) were linked to the black–brown coat colour locus, and were thus present on linkage group VIII (Hudson, Finlayson & Potter, 1967). Since then, tryptic peptide mapping carried out on isolated MUP components 1 and 2, the genetic variant under control of the *Mup-a* locus, indicated that these components differed by a single peptide (Finlayson, Mushinski, Hudson & Potter, 1968). The present work was undertaken to determine the precise location of this locus on linkage group VIII.

2. MATERIALS AND METHODS

Mice of the C57BL/6J-*Pt*/+ stock were obtained from The Jackson Laboratory. These mice are homozygous with respect to the black (*B*) coat colour gene and heterozygous with respect to pintail (*Pt*), a dominant gene causing tail abnormalities. Urine was collected from individual animals, and the urinary non-dialysable fraction was subjected to agar-gel electrophoresis as described previously (Finlayson, Potter & Runner, 1963). MUP components 2 and 3 were observed,* meaning that this stock, like the C57BL/6N strain (Hudson *et al.* 1967), carries the *Mup-a*^{2} allele. Its genotype is therefore *Pt*/+ *B*/*B* *Mup-a*^{2}/*Mup-a*^{2}.

These mice were crossed with DBA/2N mice, which do not exhibit the pintail trait but carry the brown (*b*) and *Mup-a*^{1} alleles (i.e. genotype +/+ *b*/*b* *Mup-a*^{1}/*Mup-a*^{1}). Members of the F_1 generation bearing the pintail trait were then backcrossed with DBA/2N, thus:

$$\frac{Pt\,B\,Mup\text{-}a^2}{+\,b\,Mup\text{-}a^1} \times \frac{+\,b\,Mup\text{-}a^1}{+\,b\,Mup\text{-}a^1}.$$

Maintenance of the animals, urine collection by bladder massage, dialysis of individual urines against distilled water, and freeze-drying were done as described by Hudson *et al.* (1967). The dried non-dialysable fraction was stored at −20 °C until analysed. For phenotyping it was dissolved in distilled water, usually at a concentration of 1·5 % in samples from male mice and at 2·0 % in those from females. The higher concentration was used for female mice since their non-dialysable fraction contains more non-MUP material than does that of male mice (Finlayson *et al.* 1968). Agar-gel electrophoresis was carried out at pH 5·5 with tris(hydroxymethyl)aminomethane acetate buffer, ionic strength 0·05, as described previously (Hudson *et al.* 1967).

* Components are numbered in order of increasing mobility toward the anode. Component 3 has been found in all strains tested and is thus not used in determining the genotype.

3. RESULTS AND DISCUSSION

A total of 121 offspring was examined. The following distribution was observed:

Pt B Mup-a^1 *Mup-a*^{2}	48	*Pt b Mup-a*^{1} *Mup-a*^{2}	0
+ *b Mup-a*^{1}	60	+ *B Mup-a*^{1}	0
Pt B Mup-a^1	3	*Pt b Mup-a*^{1}	2
+ *b Mup-a*^{1} *Mup-a*^{2}	5	+ *B Mup-a*^{1} *Mup-a*^{2}	3

The following recombination proportions could therefore be computed:

$$Pt\text{–}b \qquad \frac{0+0+2+3}{121} = 0{\cdot}041$$

$$b\text{–}Mup\text{-}a \qquad \frac{3+5+0+0}{121} = 0{\cdot}066$$

$$Pt\text{–}Mup\text{-}a \qquad \frac{3+5+2+3}{121} = 0{\cdot}107$$

Thus the order of these loci on linkage group VIII is *Pt–b–Mup-a*, and the intervals between successive loci determined from this study are 4·1 and 6·6, respectively. The former (*Pt–b*) agrees well with the accepted value of 5·0 (Green, 1968), and the latter (*b–Mup-a*) is consistent with the figure of $4{\cdot}1 \pm 2{\cdot}0$ obtained from a study in which the F_2 generation was used (Hudson *et al.* 1967).

The complexity of expression of the genetic information regarding the MUP type (see Finlayson *et al.* 1963) was again demonstrated by the effect of sex on the electrophoretic pattern of a given phenotype. For example, in the urine of backcross males of genotype *Mup-a*^{1}/*Mup-a*^{2}, and hence exhibiting MUP components 1, 2 and 3, component 1 was present in greatest amount (Plate 1). The electrophoretic pattern was thus very similar to that reported for *Mup-a*^{1}/*Mup-a*^{2} males of the F_1 generation, but quite different from that of *Mup-a*^{1}/*Mup-a*^{2} males of F_2 (Hudson *et al.* 1967). In the urine of backcross females of genotype *Mup-a*^{1}/*Mup-a*^{2}, however, component 2 was present in greatest quantity (Plate 1), and in a few samples component 1 was not discernible. These differences presented no difficulty in determining the phenotype but indicate the need for caution in ascribing the 'absence' of a component to a deletion. Thus in the case of the females described above, component 1 could be demonstrated when new samples were collected and analysed at a higher protein concentration.

The location of the *Mup-a* locus on linkage group VIII is of particular interest in view of its proximity to other biochemical markers such as diabetes (*db*), levulinate (*Lv*), and autosomal glucose-6-phosphate dehydrogenase (*Gpd-1*) (D. L. Coleman, personal communication). The availability of such markers may offer even greater precision in the placement of these loci.

SUMMARY

By the use of pintail (*Pt*) and brown (*b*) as markers, the location of *Mup-a*, a locus controlling electrophoretic variation of one of the components of the major urinary protein (MUP) complex, on mouse linkage group VIII has been determined. The order and intervals determined from recombination frequencies in 121 offspring from a backcross were *Pt* 4·1 *b* 6·6 *Mup-a*.

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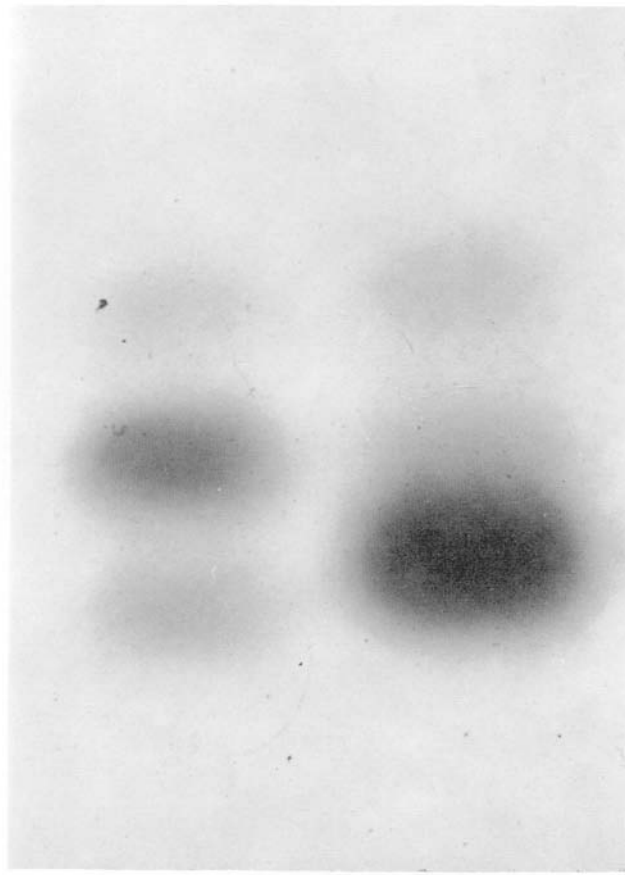

Agar-gel electrophoresis of urine samples from individual mice exhibiting MUP components 1, 2 and 3. Left, sample from female mouse; right, sample from male. Anode is at top. Components are numbered in order of increasing mobility toward the anode.

REFERENCES

FINLAYSON, J. S., MUSHINSKI, J. F., HUDSON, D. M. & POTTER, M. (1968). Components of the major urinary protein complex of inbred mice: separation and peptide mapping. *Biochem. Genet.* **2**, 127–140.

FINLAYSON, J. S., POTTER, M. & RUNNER, C. C. (1963). Electrophoretic variation and sex dimorphism of the major urinary protein complex in inbred mice: a new genetic marker. *J. natn. Cancer Inst.* **31**, 91–107.

GREEN, M. C. (1968). Linkage map of the mouse (*Mus musculus*). In *Handbook of Biochemistry*, pp. I-81 to I-86. Ed. H. A. Sober. Cleveland: Chemical Rubber Co.

HUDSON, D. M., FINLAYSON, J. S. & POTTER, M. (1967). Linkage of one component of the major urinary protein complex of mice to the brown coat colour locus. *Genet. Res., Camb.* **10**, 195–198.