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A sex-linked coat-colour mutation in the mouse non-transmissible through the female

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SUMMARY

A sex-linked mutation with a phenotype similar to that of an allele of the Mottled series has been discovered in a mosaic male. This mutation can be normally transmitted through the sperm of the original male but not through the oocytes. Several interpretations of this abnormal transmission are discussed.

Several sex-linked mutations causing coat colour variegation in the female mouse have been reported previously (Searle, 1968). In some instances the mutant hemizygote dies *in utero* or shortly after birth. Here we report a new sex-linked mutation affecting coat colour which shows the additional pecularity of being an an embryonic lethal when transmitted by the female.

1. DISCOVERY OF THE MUTATION AND STUDIES OF ITS MODE OF TRANSMISSION

The mutation was discovered in a male belonging to a recombinant inbred line ($F = 14$) which had been established from the C57BL/6-JPas and 129 Sv-Pas strains. This animal had a coat consisting of a mixture of white hairs, lacking all trace of pigmentation, and of black non-agouti hairs (Plate 1, Fig. 1). When this male was mated to either C57BL/6, 129 or F1C57BL/6 × CBA females, all the male progeny were normal but the female progeny could be subdivided into two classes, about 80% having normal coat pigmentation and 20% having a striped coat similar to the male parent phenotype although darker and more intense.

The original male remained fertile for more than 2 years. During this entire period, all the female progeny consisted of these two phenotypes, suggesting that this abnormal male was mosaic (somatic and gonadic) for an X-linked mutation. Its karyotype, as established from fibroblast or kidney cell cultures, was apparently normal.

The phenotype of the striped females (Plate 1, Fig. 2, 3) is very similar to that shown by the sex-linked mutation Mottled (*Mo*) which exists in several allelic forms (Fraser, Sobey & Spicer, 1953; Dickie, 1954; Lyon, 1960; Phillips, 1961; Phillips, 1963; Searle, 1968). The striped females have highly curled vibrissae and grow somewhat more slowly than normal individuals, so that about 10% of the affected females are runted at weaning. While a large proportion of affected

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females recover, others die from generalized physiological breakdown. Intraperitoneal copper injection ($CuCl_2$, 5 $\mu g/g$ of body weight as described by D. Hunt, 1976), which increases the survival of animals mutant for certain alleles of the Mottled series, does not appear to correct these symptons. All striped females attaining 5 weeks of age survive and are fertile. However, when mated to wild-type males (129/Sv or F1C57BL/6 × CBA), they do not produce the proportion of mutant offspring expected if the anomaly is a simple X-linked trait (Table 1).

Table 1. *Offspring recovered from* X^MX^+ *mothers*

Number of normal males	168
Number of normal females	187
Number of mutant males	0
Number of mutant females	2*
Total	357

* Both died before weaning.

Of 357 offspring of both sexes, only two animals with the maternal phenotype were observed. Both were females: one died after 7 days and the other died on the 20th day after having exhibited important symptoms of emaciation. This mutation, which first appeared in a mosaic male, can therefore be transmitted to female offspring when introduced by the male gamete but cannot apparently be transmitted by the mother through the egg.

2. ANALYSIS OF UTERINE CONTENTS OF PREGNANT MUTANT FEMALES

Certain alleles of the Mottled series, especially Brindled (Mo^{Br}) and Dappled (Mo^{Dp}), cause the death of affected males *in utero*. In view of these observations, we have dissected 13-day pregnant females (the presence of the vaginal plug being taken as day 1) and have tried to find out why these females failed to transmit the anomaly.

Table 2 shows that by the 13th day of pregnancy, 50 % of the embryos are already dead. If one compares the size of the dead embryos to that of normal conceptus, it appears that they probably died well before day 13. According to Edwards & Fowler's (1959) histological descriptions of resorbed embryos, one can consider that most of the moles found in the uterine horns of the pregnant mutant females correspond to embryos which were dead before the establishment of the allantoic circulation.

Therefore, even the precise cause of death is presently not known, it is clear that the mutation described here, when transmitted by the female, is lethal *in utero* for both sexes between day 7 and day 9.

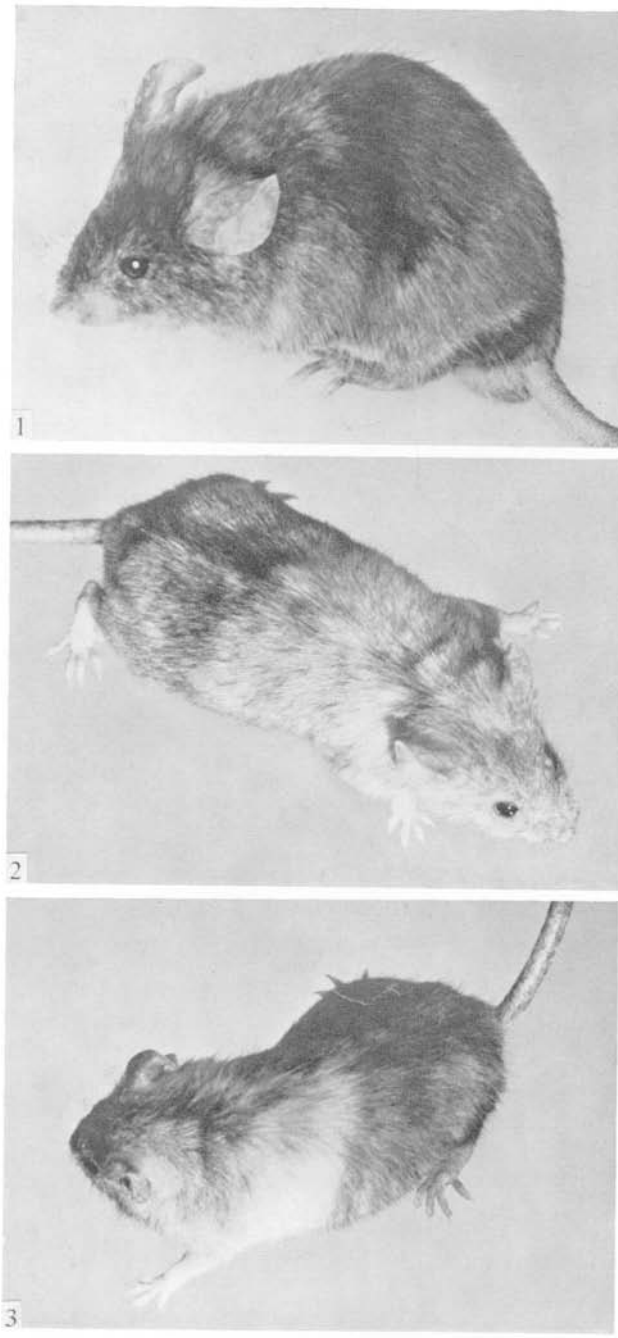

Fig. 1. Original male, mosaic for the sex-linked mutation.

Figs. 2 and 3. Presumed $X^{M}X^{+}$ daughters. All are adults photographed at the same magnifications.

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Table 2. *Uterine contents of six* X^MX^+ *pregnant females (13th day)*

Corpora lutea	Viable embryos	Dead implants
11	3	7
8	4	4
11	6	1
13	5	5
N.D.	3	2
11	6	5
Total	27	24

3. DISCUSSION

As we have not been able to prove that the anomaly is genetically related to any member of the Mottled series, we will designate it as *M* for discussion purposes. The mosaic male can thus be symbolized as $X^+Y \rightarrow X^MY$ and his mutant daughters X^MX^+. There are two possible explanations for the death of X^MX^+ embryos *in utero* when the X^M chromosome comes from the female parent: a maternal effect with partial complete dominance, or a non-random X chromosome inactivation. We will consider these two possibilities.

(i) *The lethality of the* X^MX^+ *embryos results from a maternal effect*

Studying the Hairpin tail (T^{Hp}) allele at the *T/t* locus, Johnson reported the first case of a maternal effect in the mouse in the sense that T^{Hp} is transmitted normally by males but cannot be transmitted through the egg by the female. In this case, the affected embryos die *in utero* with skeletal abnormalities (Johnson, 1974, 1975).

The absence of viable mutant offspring (X^MX^+ or X^MY) in the progeny of X^MX^+ mothers could be interpreted as a maternal effect, analogous in its consequences to the above-cited case although dependent on an X-linked mutation (and not autosomal like T^{Hp}) acting during the pregnancy. In this case, one must consider the two runted female offspring observed as two exceptions related to incomplete penetrance of *M*/+.

(ii) *A further example of non-random* X *inactivation*

According to the X-inactivation hypothesis (Lyon, 1961, 1972), one of the two *X* chromosomes is inactivated randomly in the somatic cells of normal female mammals. This inactivation occurs early in embryonic life, probably at the time of implantation or shortly thereafter. Although this theory is now widely accepted, some recent publications (Tagaki & Sasaki, 1972; Cattanach & Williams, 1972; West *et al.* 1977) have suggested that the paternal *X* chromosome is preferentially inactivated in the placental membranes. Thus, only the maternal *X* chromosome is active, and if the female is heterozygous for an *X*-linked mutation, no mosaicism occurs in the foetal membranes; the entire tissue being either of mutant type or

of normal type according to whether the mutant or wild-type chromosome is transmitted.

In the case of the mutation studied here, if the effect of the mutation is expressed in the embryonic membranes, the X^MX^+ embryos receiving the mutant chromosome from their mother will die *in utero* like the X^MY counterparts (and for the same reasons) the X^MX^+ animals receiving the mutant chromosome from the father surviving normally.

4. CONCLUSION

At present, we have no way of choosing between the two possible explanations mentioned above since, in spite of repeated chemical treatments (such as *in utero* copper supplementation) or reproductive manipulations (such as ovary grafts), we have not been able to by-pass the embryonic lethality and have now lost this mutation.

We thought however that it might be interesting to report this particular mutation which could be related with unsuccessful attempts aimed at the isolation of sex-linked recessive lethals in the mouse (Auerbach, Falconer & Isaacson, 1968; Schröder, 1971; Grahn *et al.* 1972; Searle, 1973).

If, as appears, the maternal X is always the functional chromosome in extra-embryonic tissues, a maternal X which coded a non-functional variant of an essential enzyme would impair the functional integrity of the extra embryonic tissues and could never be transmitted by the female.

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REFERENCES

AUERBACH, C., FALCONER, D. S. & ISAACSON, J. H. (1962). Test for sex-linked lethals in irradiated mice. *Genetical Research* **3**, 444–447.

CATTANACH, B. M. & WILLIAMS, C. E. (1972). Evidence of non-random X chromosome activity in the mouse. *Genetical Research* **19**, 229–240.

DICKIE, M. M. (1954). The tortoise shell house mouse. *Journal of Heredity* **45**, 158 and 190.

EDWARDS, R. G. & FOWLER, R. E. (1959). Fetal mortality in adult mice after superovulation with gonadotrophins. *Journal of Experimental Zoology* **141**, 299–322.

FRASER, A. S., SOBEY, S. & SPICER, C. C. (1953). Mottled a sex modified lethal in the house mouse. *Journal of Genetics*. **51**, 217–221.

GRAHN, D., LESLIE, W. P., VERLEY, F. A. & LEA, R. A. (1972). Determination of the radiation-induced mutation rate for sex-linked lethals and detrimentals in the mouse. *Mutation Research* **15**, 331–347.

HUNT, D. M. (1976). A study of copper treatment and tissue copper levels in the murine congenital copper deficiency mottled. *Life Sciences* **19**, 1913–1920.

JOHNSON, D. R. (1974). Hairpin tail: a case of post reduction gene action in the mouse egg? *Genetics*, **76**, 795–805.

JOHNSON, D. R. (1975). Further observations on the hairpin-tail (T^{Hp}) mutation in the mouse. *Genetical Research* **24**, 207–213.

LYON, M. F. (1960). A further mutation of the mottled type in the house mouse. *Journal of Heredity* **51**, 116–121.

LYON, M. F. (1961). Gene action in the X chromosome of the mouse (*Mus musculus* L). *Nature* **190**, 372–373.

LYON, M. F. (1972). X-chromosome inactivation and developmental patterns in mammals. *Biological Reviews* **47**, 1–35.

PHILLIPS, R. J. S. (1961). 'Dappled', a new allele at the mottled locus in the house mouse. *Genetical Research* **2**, 290–295.

PHILLIPS, R. J. S. (1963). Striated, a new sex linked gene in the house mouse. *Genet. Res.* **4**, 151–153.

SCHRÖDER, J. H. (1971). A search for radiation induced recessive sex-linked lethal and detrimental mutation in immature germ cells of the house mouse. (*Mus musculus*). *Genetics* **68**, 35–57.

SEARLE, A. G. (1968). *Comparative Genetics of Coat Colour in Mammals*. London and New York: Academic Press Ltd.

SEARLE, A. G. (1973). Mutation induction in mice. *Advances in Radiation Biology* **4**, 131–207.

TAGAKI, N. & SASAKI, M. (1972). Preferential inactivation of the paternally derived X chromosome in the extra embryonic membranes of the mouse. *Nature* **256**, 640–642.

WEST, J. D., FRELS, W. I., CHAPMAN, V. M. & PAPAIOANNOU, V. E. (1977). Preferential expression of the maternally derived X chromosome in the mouse yolk sac. *Cell* **12**, 873–882.