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Bare-patches, a new sex-linked gene in the mouse, associated with a high production of *XO* females

I. A preliminary report of breeding experiments*

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SUMMARY

Bare-patches (*Bpa*), a semi-dominant, sex-linked, male-lethal gene in the mouse is described. It shows $14{\cdot}5 \pm 3{\cdot}0\,\%$ recombination with *Blo* and $5{\cdot}4 \pm 2{\cdot}2\,\%$ with *Ta* and may, therefore, be allelic with *Str*.

Only about half the expected number of heterozygous females is observed; the remainder appear to lose their mutant chromosome and become *XO* females as there is an excess of non-*Bpa* females, at least 24 % of which proved to be *XO*. It is not yet certain whether the lost *X* chromosome is always the mutant one nor is it certain whether the loss is a property of the *Bpa* gene or due to a closely linked factor: a separate factor seems more likely. Some possible mechanisms for *XO* production are discussed.

1. INTRODUCTION

A number of sex-linked genes affecting the coat are known in the mouse and these fall into two main categories: (*a*) those which affect the colour and texture of the hair, giving a variegated coat in heterozygous females, such as the *mottled* alleles and *tortoiseshell*, and (*b*) those where some alteration in the formation of the coat gives an effect of dark stripes in heterozygous females, e.g. *tabby*, *striated*, *greasy* and *harlequin* (Barber, 1971). The gene described in this paper falls into the second category but it differs from the others in the underlying cause of the striping.

A major interest of the stock is the association of the gene with the production of a high percentage of *XO* mice.

2. ORIGIN AND DESCRIPTION

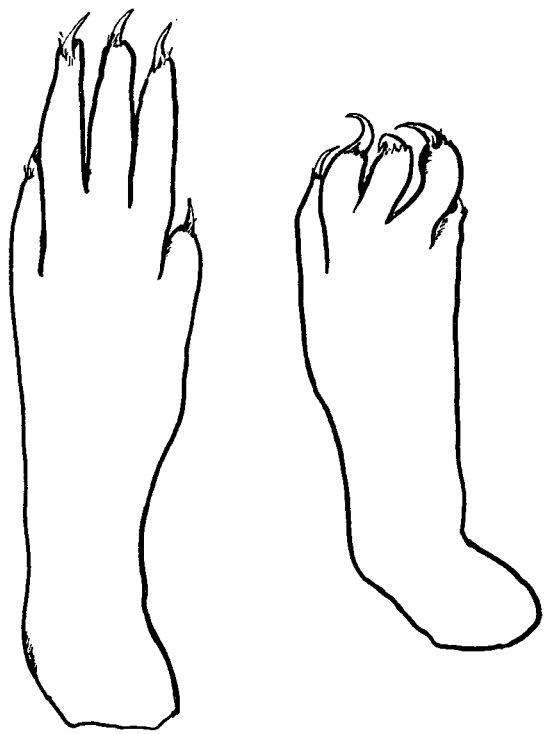
Bare-patches (*Bpa*) arose in the F_1 of a male who had received 12×50 rad X-rays in 12 consecutive weeks (Lyon, Phillips & Bailey, 1972).

The adult heterozygous female has dark stripes across the coat due, as in *striated*, to the mutant patches allowing the dark base of the immediately posterior hair to show (Lyon, 1963).

* This paper is dedicated to Professor H. Grüneberg, F.R.S., on his retirement from the Chair of Animal Genetics at University College London.

Bpa+ females are first identifiable at about 5 days, when patches of bare skin are visible amongst the first emerging hairs. A little later, at about 8 days, scurf flakes and occasionally scabs, which may last up to several weeks, form round these patches. By 12 days most of the patches are overlapped by the normal growing hair and animals look similar to *striated* or *tabby* heterozygotes. As adults, *Bpa*+ females tend to look more 'scruffy' than *Ta*+ due to some of the larger bare patches again becoming visible.

Many *Bpa*+ females are smaller and weigh less than their normal sibs and many have abnormal feet, one or more hind toes being bent and sometimes shortened

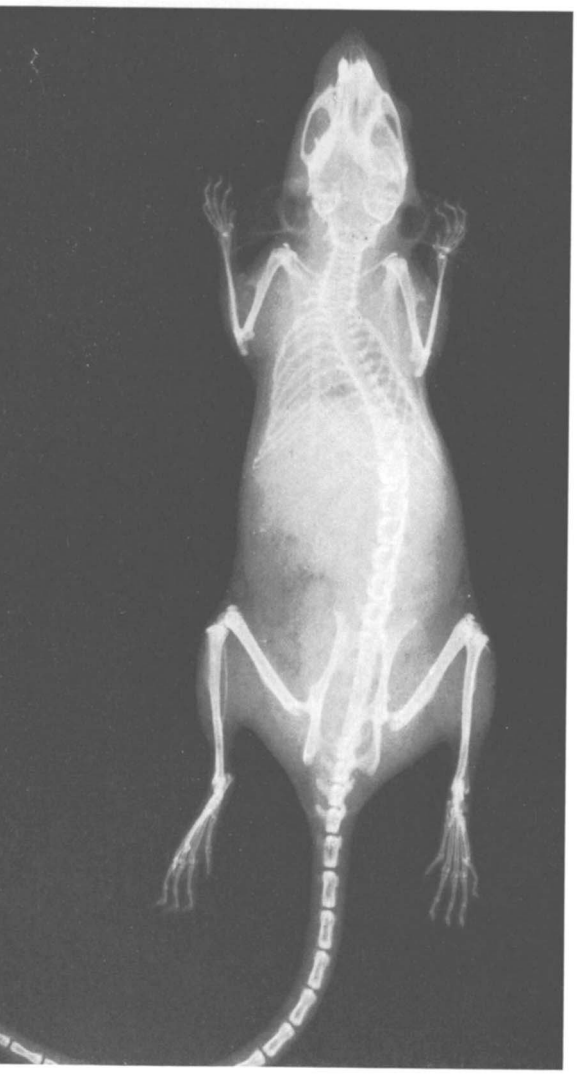


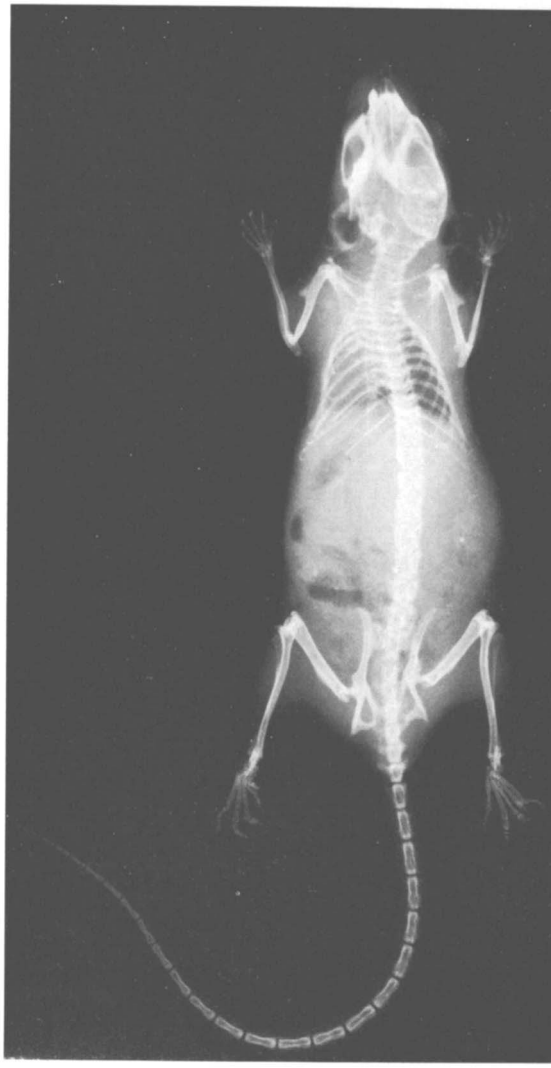
a. *b.*

Fig. 1. Drawing of left hind foot of (*a*) normal, (*b*) *Bpa*+ female.

(Text-fig. 1; Plate 1); in a few extreme animals the forefeet and possibly other parts of the skeleton may also be affected. Abnormal growth of the ears and tail, due to the formation of scabs, may also occur. These supplementary effects are more likely to be present, the greater the degree to which the coat is affected (Table 1).

Deaths between birth and weaning (Table 2) were much more frequent amongst the offspring of *Bpa*+ females than amongst those of *striated*, *Str*+ females, another similar sex-linked male-lethal gene (Phillips, 1963) crossed on to a similar background. However, the ratio of females to males dying is as expected if no





X-ray photographs of a *Bpa*+ female (right) and a normal litter-mate.

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Table 1. *Correlation between increasingly affected coat, body weight at one week and abnormal feet in* Bpa+ *females.*

Coat affected (%)	Feet			Average weights
	Normal	Abnormal	Extremely abnormal	
1–9	3 (0·80)	1 (0·67)	0	0·78
10–19	3 (0·81)	4 (0·80)	0	0·81
20–29	1 (0·58)	5 (0·73)	2 (0·61)	0·67
30–39	0	0	3	0·67
40–49	0	0	1 (0·60)	0·60
Average weights	0·78	0·76	0·63	

$$\text{Figures in parentheses} = \frac{\text{weight } Bpa+}{\text{av. wt. of } ♂♂ \text{ (or of + } ♀♀ \text{ if no } ♂♂ \text{ in litter)}}.$$

preferential death of *Bpa*+ offspring is occurring, suggesting that some *Bpa*+ females are poor mothers, not that the mutant offspring have a reduced viability. Hemizygous males die before birth, probably at the small mole stage.

3. GENETICS

(i) *Single factor segregations, allelism and linkage tests*

The single factor segregations are given in Table 2. They indicate that *bare-patches* is a sex-linked, semi-dominant, male-lethal gene. To confirm sex-linkage and to test for allelism, four *Bpa*+ females were crossed to *tabby* males, and gave results suggesting allelism with *tabby* (1st four females, Table 3). Ten females, eight

Table 2. *Single factor segregations of* Bpa+ *and* Str+

Parents	Offspring: Females: *Bpa*+ or *Str*+	Females: Wild-type	Females: Died prior to classification	Males: Wild-type	Males: Died prior to classification	Mean-litter size	♀♀/♂♂	Mutant ♀♀/♂♂
Bpa+ ♀♀ × + ♂♂	565	1388	125	1151	58	4·5	1·7	0·49
Str+ ♀♀ × + ♂♂	208	264	6	240	3	5·7	2·0	0·87

from the '*TaTa-like*' class and the two viable '*TaTa-like*?' animals, were outcrossed. Only the two '*TaTa-like*?' proved to carry both *Bpa* and *Ta* (line 1 of Table 4): of the eight '*TaTa-like*', three were proved definitely *TaO* by chromosome counts and the other five probably so. We therefore crossed two more *Bpa*+ females (♀♀ 364 and 365 Table 3) to *tabby* males. It had been realized by this stage that the offspring could be classified at 5 days for *Bpa*+, and this allowed eight known

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Table 3. *Classification of the offspring produced by* Bpa+ ♀♀ × Ta♂♂

Mated female	Female offspring			Male offspring
	TaTa-like	*TaTa-like*?*	*Bpa*+ *or Ta*+†	wild-type
♀ 356	1	0	11	5
♀ 567	10	0	14	11
♀ 1589	1	0	0	4
♀ 1618	2	2	21	10
♀ 364	6	0	14 (none *Bpa*+)	9
♀ 365	0	1‡	24 (9 *Bpa*+)	13

* Very extreme but some indications of hair on tail.
† Only with ♀♀ 364 and 365 were the classes *Ta*+ and *Bpa*+ distinguished.
‡ Died in nest.

Bpa +/+*Ta* ♀♀ to be selected for the remaining linkage tests (lines 2 and 3 of Table 4). A small amount of data from *Bpa* +/+*Blo* females is also given in Table 4. The results are analysed in Table 5.

More wild-type females than expected were found. Besides being crossovers, this class could also include *XO* mice and, in view of the number of probable *XO* mice in the *Bpa*+ × *Ta* crosses, all were tested. Only 20 of the 36 from the *Bpa*+/+*Ta* crosses gave a definite answer; 15 were proved crossovers and 5 proved to be XO. All 7 wild-type females in the *Bpa*+/+*Blo* crosses proved to be non-*XO*. No evidence of normal overlapping was found, but tests were not conclusive.

Because the genetic make-up of all the wild-type females was not known, the estimate of the linkage value from the *tabby* data is based on male offspring only (Table 5). *Bare-patches* appears to lie nearer to *tabby* than to *blotchy*, probably in the region of *striated* (Lyon *et al.* 1964), with which it may be allelic: direct allelism tests are not possible as both are male-lethal.

Bpa may also be allelic with *harlequin*, *Hq*, which is also possibly in the region of *striated* (Falconer & Isaacson, 1972). Although as adults they are similar, *Hq* also showing patches of bare skin, in the nest *Bpa* is classifiable earlier than *Hq*. The latter seem to have a normal coat until about 6 days. *Hq*+/+*Bpa* is not completely hairless at weaning age like *HqHq*. We have not as yet had any litters from *Hq*+/+*Bpa* mice.

The single-factor segregations (Table 2) show a marked shortage of *Bpa*+ females as well as an absence of *Bpa* males and again there are more wild-type females than males. Taking the number of males observed as equalling one class of 1:1:1:1 segregation, the data indicate a 50% shortage of *Bpa*+ females and something like 21% excess of normal females. The litter size and sex ratio data (Table 2) indicate an overall reduction in females when compared with data on *striated* (Table 2).

Table 4. *Linkage of* Bpa *with* Ta *and* Blo

Genotype of parents	Nos. ♀♀	Female offspring				Male offspring		Results of *XX*/*XO* test		
		BpaTa/++	*Bpa*+/++	+*Ta*/++	Wild-type*	*Ta*	+	*XX*	*XO*	?†
Bpa+/+ *Ta*×+/*Y*	2	29	32	20		30	0	1	5	14
Bpa+/+ *Ta*×+/*Y*	4	28	69	9		52	7	7	0	2
Bpa+/+ *Ta*× *Blo*/*Y*	4	19	47	7 (Blo+)		41	0	(7)‡	0	0
		76	148	36		123	7	15	5	17
		BpaBlo/++	*Bpa*+/++	+*Blo*/++	Wild-type*	*Blo*	+	*XX*	*XO*	?†
Bpa+/+ *Blo*×+/*Y*	4	2	23	36	5	26	7	5	0	0
Bpa+/+ *Blo*×*Ta*/*Y*	1	0	13	9	2 (*Ta*+)	11	4	(2)‡	0	0
		2§	36	45	7	37	11	7	0	0

* Wild-type unless otherwise stated.
† Inconclusive tests, includes some possible mosaics.
‡ Assumed *XX* by phenotype.
§ Slight doubt about classification, died in nest.

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Table 5. *Estimates of linkage values*

Mating type	Old combinants	Recombinants	Linkage value (%)
Bpa + / + *Ta**	123	7	5·4 ± 2·2
Bpa + / + *Blo*	118	18	14·5 ± 3·0

* Data from male offspring only (see text).

(ii) XO *production*

In consequence of the high percentage of *XO* mice produced in the linkage matings and the excess of normal females in the single factor segregations, we started routine testing of wild-type females from some *Bpa* + × + *Y* matings by scanning mitotic metaphases from corneal preparations (Fredga, 1964). Of 339 females tested, 260 proved to be *XX*, 79 *XO* and 2 doubtful. That is, 23·3 % of the wild-type females from *Bpa* + × + matings proved to be *XO* whereas the usual figure for spontaneous *XO* production in females is less than 1 % (Russell, 1966). This is in reasonable agreement with the 21 % excess of wild-type females estimated from the single-factor segregation. The *XO* production could be due to a high frequency of non-disjunction, in which case *XXX* and *XXY* mice would be expected, but none of the 1154 males listed in Table 2 looked striped and 5- to 6-week testes weights of 44 males from known *XO*-producing matings plus another 73 ♂♂ from untested matings have ranged from 67 to 120 mg with a median of around 88 mg, suggesting that none were sterile. Corneal preparations of eighteen *Bpa* + females have been examined and none have been *XXX*, also none of the wild-type females looked at have been *XXX*. On the other hand, no *XXX* female has been reported in the mouse, so this condition may be lethal.

It is not yet known for certain whether *XO* production is a function of *Bpa* itself or is due to a closely linked factor. The latter explanation might seem unlikely as it would have involved two independent events in the originally mutant animal. However, heterogeneity between matings does exist, and one line ($P_1 + 2F_1 + 2F_2$) has been established in which no *XO* females have been found out of 63 wild-type animals so far tested. Investigations are continuing and will be published later.

(iii) *Dissections of pregnant females*

Dissections of *Bpa* + and wild-type females done to discover the age of death of the mutant male class indicated a prenatal mortality of 36–45 % rather than the 25 % expected (Tables 6A, B).

Even these mortality estimates could be too low because the death rate in the control (wild-type) females might be inflated if (i) some were by chance *XO*; these would show a high pre-implantation death rate (Morris, 1968); and (ii) the *XX* wild-type females suffered from more post-implantation death than the nearly semi-sterile mutant females, due to accidental overcrowding of genetically viable embryos.

Table 6. *Dissections of* Bpa+ ♀♀ *and their wild-type* (W.T.) *sisters, plus some data on* Str+ ♀♀ (*Phillips*, 1963)

Genotype of parents	Number of females (N)	Corpora lutea (C.L.)	Number ovulated (C.L.)/(N)	Classification of embryos: Implants (I)	Live (L.E.)	Dead	Moles	Pre-implantation death (%) $1-\left[\frac{I/\text{C.L.}\ Bpa}{I/\text{C.L.}\ C^*}\right]$	Post-implantation death (%) $1-\left[\frac{\text{L.E.}/I\ Bpa}{\text{L.E.}/I\ C^*}\right]$	Total death (%) $1-\left[\frac{\text{L.E.}/\text{C.L.}\ Bpa}{\text{L.E.}/\text{C.L.}\ C^*}\right]$
(A) *Bpa*+ × +	16	—	—	117	66	4	47	—	34·4	—
W.T. × +	8	—	—	64	55	1	8			
(B) *Bpa*+ × +	5	41	8·3	31	16	0	15	None	44·5	44·0
W.T. × +	5	56	11·1	42	39	0	3			
(C) *Str*+ × +	8	69	8·6	67	44	19	4	None	26·3	25·1
W.T. × +	7	67	9·6	64	57	1	6			
(D) *Bpa*+ × +	From group B							22·1	21·5	38·9†
Str+ × +	From group C									
(E) *Bpa*+ *XX* × +	15	126	8·4	96	47	3	46	10·9	37·2	44·1
Bpa+ *XO* × +	8	80	10·0	69	53	3	13			
	7	87	12·4	57	44	5	8			

* C = control; for comparisons ABC wild-type sibs, for comparison D unrelated *Str*+ ♀♀, for comparison E *XX* wild-type sibs.

† As the divisor here is *striated* in which 1/2 ♂♂ die, the actual total death indicated is in the region of 64%.

To try to eliminate the effect of these two variables the data were compared with some previous data on *striated* (Table 6D), where the background (repeated crosses on to F_1 (C3H × 101)) was similar, the number of eggs ovulated also similar and the litter size reduced by 25% due to the post-implantation death of *Str* ♂♂ (Table 6C). This comparison seemed to indicate that in *Bpa*+ females there were nearly 40% deaths additional to the death of mutant males. In a further experiment therefore the wild-type were classified for *XX* or *XO* by corneal preparations when they were killed. The results (Table 6E) are almost exactly as had been found previously except that 11% of the deaths were now shown to occur at the pre-implantation stage. Either the *striated* comparison (Table 6C) was spurious or the accidental embryonic overcrowding in wild-type controls masks something like 10% post-implantation death.

4. DISCUSSION

This is the first time that a genetic factor affecting *X*-chromosome loss has been reported in the house mouse. The overall *XO*/*XX* ratio is approximately 0·17, and in some matings even higher – very high compared with the normal control level of less than 0·01 (Russell, 1966) and about half that found by Cattanach (1962) and Morris (1968) from known *XO* mothers. Yong (1971) found 3/90 (a ratio of 0·033) *XO* females in a sample population of black rats and Bianchi & Contreras (1967) report the presence of one *XO* out of six females in a small polymorphic population of field mice (*Akodon azarae*), together with some animals showing a grossly deleted *X*. They interpreted this as possibly due to a dosage compensation mechanism ranging from the inactivation of one *X* chromosome, as occurs normally in mammals (Lyon, 1961) to its total elimination.

The stage or stages at which the chromosome is being lost in *Bpa* mice is not yet known but is under investigation. There are various possible mechanisms, the most obvious being non-disjunction, possibly due to some structural change in the *Bpa* chromosome. Although this cannot be excluded, no genetical evidence of non-disjunction has yet been found (see section 3*b* above). A search for evidence of a structural change, such as an inversion, is being made by various linkage tests.

As half the *Bpa*+ females are missing and *X*-inactivation is a random process (Lyon, 1961), it is tempting to consider that an inactivation centre may be involved. Primordial germ cells are thought to contain only one active *X*-chromosome, but by the time of differentiation into oogonia prior to first meiotic metaphase, both *X* chromosomes are normally euchromatic (Ohno, 1963). If the inactive *Bpa* chromosome was not reactivated then it might be lost at the first meiotic anaphase or fail to disjoin with the active *X* so that half the putative *Bpa*-carrying eggs would be nullo-*X* and the expected segregation ratios in the offspring would be 1 *Bpa*+ ♀:3 (2 *XX* + 1 *XO*) wild-type ♀♀:2 ♂♂. In fact this is in agreement with the data in Table 2 except that *XO* females are fewer than expected, the ratio among the wild-type females being nearer 3 *XX*:1 *XO*. However, in some matings the proportion of *XO* females may be as high as 2*XX*:1 *XO*. In any case a shortage of *XO*

females would not be surprising since *XO* mice themselves produce fewer than expected *XO* offspring (Cattanach, 1962; Morris, 1968).

Cytologically Dr M. H. Kaufman (personal communication) has found some indication of abnormal chromosome numbers at 2nd meiotic metaphase in eggs of *Bpa+* mice. This part of the work is as yet incomplete and will be published later.

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