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Controlling elements in the mouse

V. Linkage tests with X-linked genes

By B. M. CATTANACH and D. PAPWORTH

M.R.C. Radiobiology Unit, Harwell, Didcot, Oxon OX11 0RD

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SUMMARY

Previous studies have shown that the mouse *X* chromosomal locus, *Xce*, which causes non-random *X* chromosome inactivation, is closely linked to the Is(*X*; 7)Ct *X*-autosome translocation. This has placed it either near *Ta* on one side of the breakpoint or near *jp* on the other. Linkage tests with Mo^{vbr} and *Ta* now demonstrate that the locus in fact lies close to *Ta*. The data also provide genetic evidence which establish that the C3H/HeH *X* chromosome carries the Xce^a allele of this gene and the JU/FaCt and C57BL/GoH *X* chromosomes carry the Xce^b allele, and further suggest that the *X*-linked modification of the heterozygous phenotypes of *X*-linked genes observed by various other investigators are all attributable to differences at the *Xce* locus. Evidence of a maternal influence upon Mo^{vbr} phenotypes is also presented. This appears to operate independently of the *X*-inactivation process, probably through an effect of differing levels of copper in the milk in early life upon the mutant coat colour in the young.

1. INTRODUCTION

The *X* chromosome of the mouse carries a locus, designated *X chromosome controlling element* (*Xce*), which causes non-random *X* chromosome activity in the somatic cells of females (Cattanach, Perez & Pollard, 1970; Cattanach & Williams, 1972) and, from evidence presented in a recent paper (Johnston & Cattanach, 1981), it seems that this derives from a primary non-randomness in the *X*-inactivation process itself. Three alleles of this locus are known, Xce^a, Xce^b and Xce^c, and the non-randomness can be observed in the heterozygotes, e.g. Xce^a/Xce^b, Xce^b/Xce^c, with the use of appropriate *X*-chromosomal marker genes (Cattanach, Pollard & Perez, 1969; Johnston & Cattanach, 1981). It is then evident that cells with an active Xce^b *X* chromosome tend to predominate over those with an active Xce^a *X*, and those with an active Xce^c *X* tend to predominate over those with an active Xce^b *X*.

Xce^a and Xce^b were first identified in the rearranged *X* chromosomes of Is(*X*; 7)Ct (formerly T(7; *X*)Ct) heterozygotes by their influences upon the inactivation mosaicism associated with the translocation (Cattanach & Isaacson, 1965, 1967). Subsequent investigation revealed that they could also be recognized

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by their effects upon the heterozygous phenotypes of X-linked genes (Cattanach, Pollard & Perez, 1969) and, by this means, the X chromosomes of the A/H, CBA/H, C3H/HeH, 101/H and BALB/c (Searle Laboratories) inbred strains were typed as Xce^a and those of the JU/FaCt and C57BL/GoH inbred strains as Xce^b (Cattanach & Williams, 1972; Cattanach, unpublished). The third allele, Xce^c, was found in an X chromosome of feral mouse origin through its effect both upon the expression of PGK-1 allozymes in $Pgk\text{-}1^a/Pgk\text{-}1^b$ heterozygotes and *Ta* in *Ta* heterozygotes (Johnston & Cattanach, 1981). The non-random expression found when Xce^c was heterozygous with Xce^b and the greater non-randomness when heterozygous with Xce^a provided the first line of evidence that a new, more extreme *Xce* allele was involved.

Proof that the *Xce* genotypes of the various X chromosomes are as described has not yet been fully established. It is possible that the effects observed derive from changes at a number of different loci. However, in the case of the C3H/HeH, JU/FaCt, C57BL/GoH and the feral mouse chromosomes it has been shown that the mechanism of action is the same, i.e. they all bring about their effects by causing primary non-random X-inactivation (Johnston & Cattanach, 1981). In addition, all the available evidence on X-linked modification of X-linked expression in females recorded in different stocks by a number of laboratories implicates one region of the X-chromosome and, on this basis, it seems highly likely that only a single locus – *Xce* – is involved. Thus:

(1) The original studies with Is(X; 7)Ct and JU/FaCt chromosomes (Cattanach, Perez & Pollard, 1970) indicated close linkage between *Xce* and the translocation (2·78 %, lower and upper 95 % confidence limits 0·57 % and 8·54 %) and this has placed *Xce* either close to *jimpy* (*jp*) on one side of the breakpoint or close to *tabby* (*Ta*) on the other (Fig. 1). Here it is important to note that since the translocation suppresses crossing-over throughout most of the region between *Mo* and *jp* (Cattanach, 1966, and Fig. 1), the *Xce* locus is unlikely to be between them.

(2) Kindred (quoted in Cattanach, Perez & Pollard, 1970) has found X-linked modifiers of *Ta* which are closely linked to *Ta*.

(3) Falconer & Isaacson (1972), Krzanowka & Wabik (1971, 1973), Ohno & Drews (Ohno *et al.* 1973; Drews *et al.*, 1974) and Grahn, Lea & Hulesch (1970) have independently observed X-linked modification of various *Mottled* (*Mo*) alleles and in each case the responsible modifiers can be deduced to be closely linked to the marker locus – and this lies close to *Ta* (Fig. 1). Grahn *et al.* (1970), in fact attempted to map their modifier and deduced that it was located 8·2 cM proximal to *Greasy* (*Gs*) which is very tightly linked to *Ta* (Fig. 1). The latter study, however, used marker gene expression in individuals as an indicator of modifier genotype, a procedure which is highly suspect owing to the obscuring effect of the major variation in mosaicism.

(4) Lyon (quoted in Cattanach, Perez & Pollard, 1970) has observed that the penetrance of the distal marker *Gyro* (*Gy*) may change as a result of crossing over between *Gy* and *Ta* (Fig. 1).

(5) From the history of the feral mouse X chromosome (Johnston & Cattanach, 1981) it is evident that Xce^c must be closely linked to *phosphoglycerate kinase* (*Pgk-1*) which lies between *Mo* and *Ta* (Fig. 1).

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The primary purpose of the experiments to be described was to provide further, direct evidence on the location of *Xce* in the linkage map. This was obtained by linkage testing, using two centrally located marker genes and *X* chromosomes of different origin which have previously been typed as Xce^a and Xce^b. Although the extensive progeny testing necessary to identify the *Xce* genotype of individual animals severely limited the amount of linkage test data obtained, and the major variation in the inactivation mosaicism made the detection of recombinants difficult even with this procedure, the results provide reasonable evidence that, at

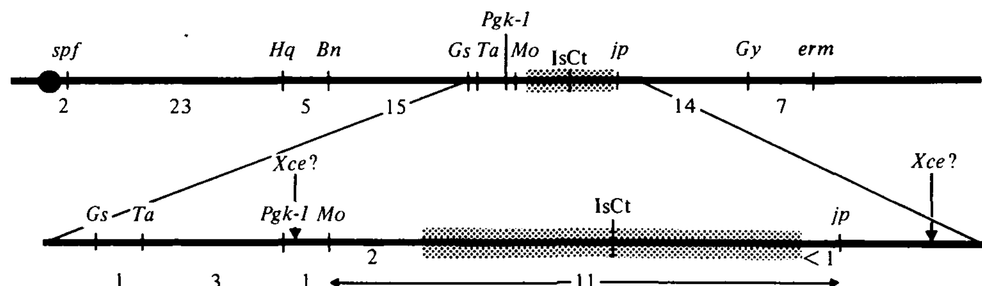

Fig. 1. Partial *X* chromosome linkage map of the mouse. The shaded area indicates the region of crossover suppression caused by the Is(*X*; 7)Ct translocation. Data taken from Cattanach (1966 and unpublished); Cattanach, Perez & Pollard (1970) and Davisson & Roderick's (1979) linkage map of the mouse.

least for the *X* chromosomes employed, the *Xce* typing does reflect differences at the one, *Xce* locus and that this locus lies centrally in the linkage group in the vicinity of *Ta*.

2. MATERIALS AND METHODS

Linkage testing was carried out with two coat structural genes, *viable brindled* (Mo^{vbr}, hereafter abbreviated to *Vbr*) and *Tabby* (*Ta*) which are situated 4 cM apart near the centre of the *X* chromosome linkage group. The genes served two functions in these tests; (1) as in any linkage test, to mark the loci being tested, and (2) to identify the *Xce* genotype by their degree of expression in family groups. The *X* chromosomes of three different inbred strains, C3H/HeH, JU/FaCt and C57BL/GoH (hereafter referred to as C3H, JU and C57) were also employed as sources of Xce^a (C3H) and Xce^b (JU and C57) (Cattanach & Williams, 1972). The *X* chromosome of the 101/H inbred strain (101) was also incidentally involved in some experiments through the use of 3H1 (C3H × 101 hybrid) females in certain test-crosses. Since 101 carries Xce^a as does C3H (Cattanach & Williams, 1972; Cattanach, unpublished), such 3H1 females are homozygous for this *Xce* allele.

(i) *Experiments with* Vbr

Vbr produces a variegated/striped phenotype in the coats of the heterozygous female, the bands alternatively being composed of wild type hair or whitish, structurally abnormal hair like that of the hemizygous *Vbr* male. *Vbr* expression

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in the heterozygous female was assessed as in previous studies by estimating the amount of mutant hair in the coat to the nearest 5%, scoring where possible at least 30 females at a time and without knowledge of their identity (Cattanach, Pollard & Perez, 1969). Since *Vbr* is usually sterile in the male all experimentation with the gene was limited to females.

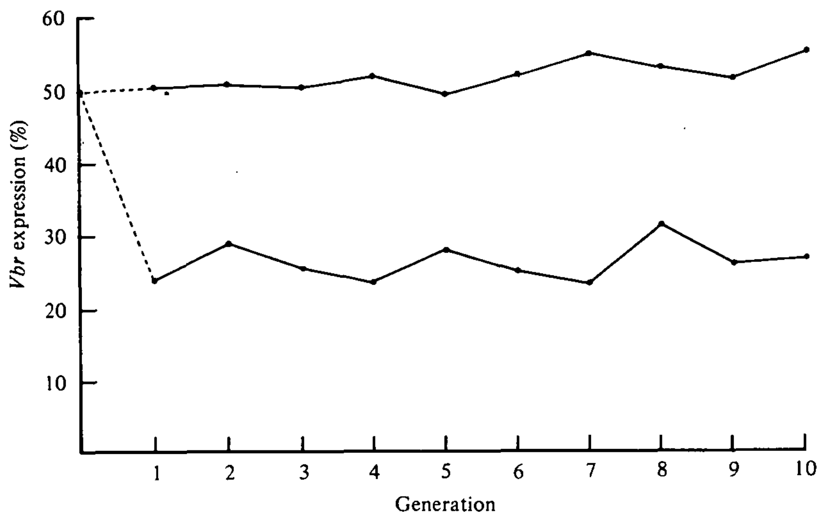

Fig. 2. Levels of *Vbr* expression in *Vbr*/+ females of the high and low lines observed over several generations.

The *Vbr*/+ females employed derived from a stock maintained in two separate lines. In both lines the *Vbr*/+ females of each generation are crossed with 3H1 males; the Vbr^+ chromosome therefore always carries Xce^a. The *Vbr X* chromosome of one line, from its origin (JU) and history, is thought to carry Xce^b. The females of this line are therefore genotypically $Vbr\ Xce^b/+Xce^a$ and typically show high levels of *Vbr* expression. This distinguishes them from the *Vbr*/+ females of the second line which typically show much lower levels of *Vbr* expression. This low *Vbr* expression line (low line) was originally isolated as a family group from the high *Vbr* expression line (high line) because of its atypical *Vbr* expression and, under the standard breeding regime, has bred true for this character. The *Vbr* chromosome of this line can reasonably be interpreted to carry Xce^a as a result of a recombinational event between the *Vbr* and *Xce* loci, i.e. females of this line are genotypically $Vbr\ Xce^a/+Xce^a$.

Fig. 2 shows the mean assessed levels of *Vbr* expression in females of the two lines observed over several generations. The fact that the high line as well as the low line generally breeds true has provided the first indication that the *Vbr* and *Xce* loci are closely linked. However, since there was usually some limited selection of females on the basis of phenotype (*Vbr* expression) when setting up new matings in each generation and the individual matings were seldom kept long enough to provide large numbers of *Vbr*/+ progeny, the evidence of linkage is not strong.

Two experiments were carried out with *Vbr*. These comprised:

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(1) Screening tests for *Vbr–Xce* recombination in the *Vbr* $Xce^b/+Xce^a$ females of the high line, detectable by the appearance of *Vbr*/+ females breeding true for low *Vbr* expression. Equivalent tests upon the *Vbr* $Xce^a/+Xce^a$ females of the low line served as a control. The approach was limited to investigation of *Vbr*/+ females selected on the basis of individual *Vbr* expression atypical of their line, i.e. they were pre-screened on the basis of phenotype. Genotype was ultimately established by progeny test, i.e. by determining mean progeny score, and this was amplified when inadequate by equivalent testing of the progeny themselves. All crosses utilized 3H1 males.

(2) Similar screening tests for *Vbr–Xce* recombination after the specific introduction of JU(Xce^b) and C3H(Xce^a) X chromosome into the high and low *Vbr* lines. Preliminary investigation had revealed that genetic background can influence *Vbr*/+ phenotype in a way which creates scoring difficulties. Therefore, in this experiment the *JU* and *C3H* chromosomes were introduced by way of F_1 males derived from reciprocal crosses between the JU and C3H strains. However, because of the scale of the experiment, evidence of *Vbr–Xce* recombination was ultimately sought only from the low line (*Vbr* Xce^a)–JU(Xce^b) cross. Equivalent testing of animals derived from the low line (*Vbr* Xce^a)–C3H(Xce^a) cross served as a control.

(ii) *Experiments with* Ta

Ta heterozygotes, like *Vbr* heterozygotes, exhibit a striped coat, but a more effective way of assessing the expression of this gene is provided by the fact that it reduces the secondary vibrissa number from the invariant number of 19 in normal animals to a mean of about 14–17 in *Ta*/+ animals (Dun, 1959; Dun & Fraser, 1959). *Xce* allele substitutions on the Ta^+ chromosomes of such animals have previously been shown to shift the mean vibrissa score by one or more, a difference which, though small, becomes statistically significant when 20–30 animals are scored (Cattanach, Pollard & Perez, 1969). This means of assessing *Ta* expression was therefore employed in the present tests.

The *Xce* status of the *Ta* chromosomes introduced into the linkage tests was not known and hence the tests had to be carried out 'blind'. Two *Ta* chromosomes of different origin were employed in the hope that they might carry different *Xce* alleles. This subsequently proved not to be the case. One *Ta* chromosome derived from a Harwell *X* chromosome linkage testing stock (NZJ) and the other from the Harwell *X*O female stock (OX).

Only one investigation was carried out with *Ta*. This basically comprised a linkage test, like that with *Vbr*, but had to be carried out in duplicate, (*a*) with Xce^a on the non-*Ta* chromosome and (*b*) with Xce^b on the non-*Ta* chromosome, because the *Xce* status of the *Ta* chromosomes was not known. Recombination between *Ta* and *Xce* to give an inherited, altered *Ta* expression in females was only expected in one of the two crosses, the other serving as a technical control. The two *Ta X* chromosomes employed (OX and NZJ) were each introduced into the test by way of single *Ta*/Y males crossed to C3H (Xce^a) and C57 (Xce^b) females. The F_1 *Ta* $Xce^?/+Xce^a$ and *Ta* $Xce^?/+Xce^b$ daughters were then collected and mated when adult to 3H1 males to produce F_2 *Ta*/Y males. These males were then

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Table 1. *Test for* Vbr/+ *females showing altered, inherited levels of* Vbr *expression among both high- and low-line animals*

Test female score (%)	Progeny No.	Progeny Mean score (%)	Test female score (%)	Progeny No.	Progeny Mean score (%)
		High line (Group A)			
70	12	40·41 ± 4·67	80	16	52·50 ± 3·16
80	11	46·36 ± 5·64	85	1	55
75	16	48·12 ± 2·77	85	11	55·00 ± 4·71
80	14	48·21 ± 4·43	80	9	58·33 ± 5·27
70	9	49·44 ± 4·74	80	10	61·00 ± 5·20
Mean maternal score = 78·5			Overall progeny mean = 50·69 ± 1·51		
		High test-group (Group B)			
30	10	31·00 ± 6·94**	40	17	46·32 ± 4·01
15	11	32·73 ± 5·15*	40	6	46·67 ± 4·01
30	9	36·11 ± 3·71	45	13	47·69 ± 5·59
15	11	38·64 ± 4·37	40	11	47·73 ± 3·65
45	16	40·94 ± 3·93	40	11	47·73 ± 4·97
40	10	41·82 ± 5·10	25	13	48·08 ± 3·82
35	7	42·14 ± 4·61	20	7	49·29 ± 3·69
40	15	42·67 ± 4·97	45	18	50·00 ± 3·57
25	7	42·86 ± 6·06	10	6	50·00 ± 5·63
40	10	43·50 ± 4·09	45	7	50·71 ± 7·11
10	12	43·33 ± 3·45	35	9	51·11 ± 7·30
45	7	44·28 ± 3·69	40	17	51·47 ± 3·66
40	13	44·62 ± 3·78	45	10	52·50 ± 5·39
35	14	45·00 ± 4·39	35	13	53·08 ± 3·08
40	13	45·39 ± 3·37	30	13	53·85 ± 3·95
Mean maternal score = 34·0			Overall progeny mean (excluding ** and *) = 46·45 ± 0·85		
		Low test-group (Group C)			
60	6	27·00 ± 9·30	55	8	34·38 ± 8·10
60	10	27·50 ± 6·29	50	6	36·67 ± 8·53
55	12	28·33 ± 4·86	85	23	38·70 ± 3·45
80	13	32·31 ± 4·86	65	10	44·00 ± 4·93*
60	11	32·73 ± 6·04	55	2	45·00
Mean maternal score = 62·5			Overall progeny = 34·45 ± 1·83		
		Low line (Group D)			
5	9	15·00 ± 3·63	5	10	27·00 ± 6·71
5	15	20·00 ± 3·59	5	12	27·92 ± 5·95
5	14	21·43 ± 3·75	5	11	29·55 ± 5·15
5	12	22·08 ± 3·56	5	16	32·50 ± 3·50
5	4	23·75	5	12	33·33 ± 4·19
Mean maternal score = 5·0			Overall progeny mean = 25·61 ± 1·49		

* $P < 0·05$; ** $P = < 0·01$.

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screened for *Ta–Xce* recombination on the basis of mean vibrissa score of their F_3 *Ta*/+ daughters. This progeny test was conducted with the use of 3H1 females.

3. RESULTS

(i) Vbr *experiments*

Experiment 1

Table 1 presents the results of the first test for *Vbr–Xce* recombination. Before considering the individual results attention must be drawn to the general observation that mean progeny score does not appear to be independent of maternal score. Thus, the 30 high-line females which had been selected for low *Vbr*

Table 2. *Linear regressions of daughter scores on maternal scores*

			Goodness of fit			Significance of slope		
Group	Intercept	Slope	*F*	D.F.	*P*	*t*	D.F.	*P*
A	−10·2±25·6	0·78±0·33	1·08	8603	0·37	2·38	603	0·017
B	39·5±3·1	0·173±0·085	1·31	28603	0·13	2·04	603	0·042
A+B	40·6±0·8	0·138±0·016	1·30	38603	0·11	8·63	603	0·01653
C	23·9±8·6	0·16±0·13	1·25	8603	0·27	1·26	603	0·21
D	—	—	—	—	—	—	—	—
C+D	24·9±1·5	0·143±0·032	1·37	18603	0·14	4·53	603	0·0000071

Data based on a pooled residual variance over all groups.

expression (group B) gave lower mean progeny scores than the standard high-line females of the same generation (group A) and, similarly, the 10 low-line females which had been selected for high *Vbr* expression (group C) gave higher mean progeny scores than the standard low-line population (group D). Statistical analysis of the data from each group and from appropriate combinations of groups (Table 2) revealed a clear positive correlation between mean progeny score and maternal score. This finding could suggest the operation of either a maternal effect or of modifying genes but, whatever the cause, this factor had to be allowed for when assessing the linkage test data. The correlation has since been found to be a typical feature of both high- and low-line data.

Considering now the individual results, it may be seen that of the 30 high-line females screened for *Vbr–Xce* recombination to produce an inherited low *Vbr* expression (Table 1, group B), several gave much lower mean progeny scores than typical of the high line (group A). However, only two of these (shown with asterisks) gave scores which were significantly lower than the mean score of the high line after allowance had been made for the daughter–mother correlation. One of these was tested further by progeny-testing two of her daughters. The results suggested that the altered, low *Vbr* expression was indeed inherited.

Equivalent testing 10 low-line females exhibiting high *Vbr* expression (Table 1, group C) indicated that one (shown with asterisk) gave a significantly higher progeny score than the mean score of the low line (Table 1, group D). However, further testing of the progeny revealed that this was transient, i.e. they bred as

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normal low-line females. Amplification of group B and C data for the less adequately tested females (giving less than 9 progeny) by progeny testing some of the daughters did not alter any of the above findings.

Although the scale of this experiment was large, as a linkage test it provided relatively little data. Despite this, two conclusions can reasonably be drawn. (1) The data are consistent with that expected if the *Xce* genotypes of the two *Vbr* lines are as proposed. Thus, *Vbr–Xce* recombination to give altered, inherited levels of *Vbr* expression would only be expected from the *Xce* heterozygotes of high line, i.e. $Vbr\ Xce^b/+Xce^a$. None could be expected from the Xce^a homozygotes of the low line. (2) The estimated frequency of recombination (2/30 or 6·7 %; lower and upper 95 % confidence limits, 0·8 % and 22·1 %) supports the view that the *Xce* locus may lie close to *Ta* rather than close to *jp* (Fig. 1). It should be noted, however, that the R.F. may be an over-estimate owing to the pre-screening of the tested animals by phenotype and the fact that confirmation of a genetic change was only obtained for one of the two possible recombinants.

Experiment 2

Table 3 presents the results of progeny testing F_2 $Vbr/+$ progeny derived from F_1 $Vbr\ Xce^a/+Xce^b$ (experimental) and $Vbr\ Xce^a/+Xce^a$ (control) females. As in the previous experiment these had been pre-selected for *Vbr–Xce* recombination on the basis of phenotype. In contrast to the findings made within the high and low *Vbr* lines no significant correlation between progeny and maternal scores was found in either set of data, and this may perhaps be attributed to the differing genetic background of the two generations. In the absence of the correlation the mean progeny score was computed for the control series and the mean progeny scores of individual experimental F_2 females were then directly compared with this overall mean. On this basis, only one (shown with asterisk) of the 22 females of the experimental series was distinguished as having a higher *Vbr* expression than that typical of the control series. Amplification of both experimental and control data by similarly testing members of the F_3 generation (Table 3) revealed that there were significant differences within family groups, and when all the data in the experimental series were considered a correlation between progeny score and maternal score was found. An overall mean score was therefore computed for all family groups of the control series and the mean scores per family group in the experimental series were then compared with this overall mean. By this analysis two more F_2 females of the experimental series (shown with asterisks) could be deduced to have an altered genotype which resulted in an inherited, high *Vbr* expression. No such change within the control group was detected when they were compared in a similar fashion with the mean scores of the low *Vbr* line of the equivalent generation.

The data thus support the earlier conclusion that the *Xce* genotypes of the high and low *Vbr* lines are as proposed and that *Xce* allele substitutions in the *Vbr* chromosomes can be achieved by recombination. The observed *Vbr–Xce* R.F. was 13·6 % or 3/22 (lower and upper 95 % confidence limits 2·9 % and 35·0 %) which, as in the previous experiment, may be an overestimate owing to the pre-selection

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Table 3. *Tests for recombinant* Vbr $Xce^b/+Xce^a$ F_2 *females from* Vbr Xce^a/Xce^b *mothers and control data from* Xce^a *homozygotes*

Experimental series (% *Vbr*)

Test F_2 ♀ progeny score (*n*)	Re-test with F_3 progeny (*n*)	Total score (*n*)
13·57 (7)	—	13·57 (7)
21·59 (22)	—	21·59 (22)
18·57 (7)	26·67 (6) 22·14 (14)	22·22 (22)
29·29 (7)	19·00 (10)	23·24 (17)
23·85 (13)	—	23·85 (13)
25·00 (10)	20·00 (5) 28·33 (3)	24·17 (18)
25·21 (24)	—	25·21 (24)
26·11 (9)	—	26·11 (9)
26·67 (21)	—	26·67 (21)
27·00 (10)	24·44 (18) 30·71 (14)	27·14 (42)
35·00 (13)	22·00 (20) 35·00 (6)	28·59 (39)
24·44 (9)	20·93 (16) 32·19 (16) 36·94 (18)	29·41 (59)
32·38 (21)	—	32·38 (21)
42·50 (8)	30·36 (14) 23·76 (4)	33·08 (14)
35·00 (7)	28·53 (17) 40·83 (12)	33·89 (36)
38·93 (14)	25·00 (2) 33·89 (9)	36·00 (25)
37·31 (13)	42·66 (15) 30·26 (19)	36·17 (47)
38·08 (13)	—	38·08 (13)
47·66 (15)	35·00 (11) 39·58 (12)	41·45 (38)
45·00 (10)	53·21 (14)	49·79 (24)*
49·38 (8)	50·63 (8) 43·46 (13)	47·07 (29)*
53·00 (13)*	51·43 (7)	52·50 (20)*

22 F_2 ♀♀ tested, 3 recombinant. R.F. = 13·6%.

Control series (% *Vbr*)

Test F_2 ♀ progeny score (*n*)	Re-test with F_3 progeny (*n*)	Total score (*n*)
20·00 (13)	—	20·00 (13)
33·57 (7)	25·91 (11) 18·21 (14)	22·22 (32)
22·83 (23)	—	22·83 (23)
31·25 (8)	27·67 (15) 16·00 (10)	25·00 (33)
31·54 (13)	37·00 (5) 20·00 (6)	29·79 (24)
36·43 (14)	20·00 (7) 30·00 (16)	30·54 (27)
31·43 (21)	30·83 (6) 30·31 (16)	30·93 (43)
37·72 (11)	27·50 (12)	31·57 (35)
—	30·00 (12)	
43·13 (8)	15·00 (13) 26·67 (6) 43·21 (14)	32·83 (41)
38·86 (1)	30·11 (11) 31·11 (18)	33·21 (42)
38·75 (4)	32·50 (8)	34·58 (12)
39·54 (22)	29·17 (12) 33·93 (14)	35·31 (48)
39·06 (16)	34·16 (6) 30·36 (14) 35·58 (11) 38·46 (13)	35·76 (66)
48·33 (6)	32·37 (19)	36·20 (25)
39·00 (20)	37·50 (12) 28·75 (8)	36·50 (40)
—	—	—
—	—	—
—	—	—
—	—	—
—	—	—

15 F_2 ♀♀ tested.

* Significantly different from mean score of controls.

3-2

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Table 4. *Results of tests upon F_2* Ta/Y *males for recombination between* Ta *and* Xce

C57 series		C3H series	
No. F_3 daughters	Mean vibrissa score	No. F_3 daughters	Mean vibrissa score
29	12·55	23	12·48
31	12·87	31	12·94
34	12·88	15	13·07
36	13·06	22	13·23
30	13·07	33	13·24
30	13·17	21	13·38
31	13·23	25	13·44
34	13·24	24	13·54
30	13·27	17	13·65
30	13·33	35	13·66
31	13·39	19	13·68
37	13·59	29	13·69
32	13·66	30	13·83
31	13·68	24	13·83
36	13·94	14	13·86
30	14·03	15	13·87
35	14·03	19	13·95
33	14·03	18	14·06
35	14·11	20	14·10
32	14·12	20	14·10
31	14·16	32	14·22
30	14·23	25	14·36
30	14·30	8	14·37
33	14·36	21	14·43
29	14·41	15	14·47
41	14·59	21	14·48
32	14·66	16	14·62
27	14·67	Overall mean score ($n = 27$)	13·65
16	14·75		
32	14·75		
33	14·76		
26	14·81		
28	14·96		
31	15·10		
Overall mean score ($n = 34$)	13·92		

* Significantly lower than mean score of the C3H series.

of F_2 females on the basis of phenotype. The combined results for both experiments give an R.F. of 9·62 % (lower and upper 95 % confidence limits, 3·2 % and 21·0 %).

(ii) *Experiments with* Ta

The expected effects of the C57(*Xce*^{b}) and C3H(*Xce*^{a}) upon *Ta*/+ vibrissa score were observed in the first cross preceding the linkage, but neither in this cross nor that to produce the F_2 *Ta*/Y males for progeny testing was any significant difference found between the mean vibrissa scores obtained with the two *Ta X* chromosomes. It may therefore be concluded that the *Ta* chromosomes carry the

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same *Xce* allele. In assessing the results of the progeny tests on the F_2 *Ta*/Y progeny the data derived from males carrying the different *Ta* chromosomes have therefore been pooled. The individual progeny test results on 34 *Ta*/Y males derived from the C57 crosses and 27 males from the C3H crosses are shown in Table 4.

Statistical analyses were computed in two ways. One way assumed that the *Xce* allele present on the *Ta* chromosome was Xce^a and, hence, that recombination would only be found in the C57 series and be detectable by a greater reduction in mean vibrissa score than had been found in earlier crosses. The other assumed the converse, and that recombination might only be found in the C3H series and be detectable by a higher mean vibrissa score than previously found. With the first approach, no significant differences in the mean scores were found within the C3H series, so that the overall mean score for the series could be calculated with good precision. Comparisons of the individual scores obtained with males from the C57 series were then made with this score. Only one animal gave a mean progeny score which was significantly lower than the mean of the C3H series and was therefore suspected of being a recombinant. With the second approach, there were significant differences between the mean scores of the C57 series, so that the overall mean could be calculated with only poor precision. However, when comparisons of the individual mean scores for the C3H series of males were made with the overall C57 mean and the above heterogeneity was allowed for, no male could be shown to have a significantly higher mean score.

The one possible recombinant *Ta* X chromosome was then tested for its effect upon *Vbr* expression and data from five other *Ta*/Y males for the C57 series, similarly tested, served as a control. The mean *Vbr* score of the $Vbr + Xce^b/ + Ta$ $Xce^?$ daughters of each of the 6 males was found to be in excess of 50%. It can be concluded that no genetic change had in fact occurred in the one suspect recombinant – all had the same *Xce* genotype – and from the high level of *Vbr* expression for all 6 males it can be concluded that the *Ta* chromosomes carried Xce^a. On this basis it can be concluded that the valid linkage test was that with C57. No recombination between *Ta* and *Xce* was detected, but because only 34 animals were tested the upper 95% confidence limit is quite high, 10·3%.

4. DISCUSSION

The *Xce* locus was first identified in the rearranged and normal (JU derived) *X* chromosomes of Is(*X*; 7)Ct heterozygotes (Cattanach & Isaacson, 1967; Cattanach, Perez & Pollard, 1970; Cattanach, Pollard & Perez, 1969). Inherited changes in the levels of variegation associated with the translocation were observed among some of the progeny of X^T Xce^a/X^N Xce^b females, and this was interpreted to mean that crossing over had occurred between the *Xce* locus and the translocation to place Xce^b on the rearranged chromosome. The low frequency of this occurrence (2·78%) suggested that *Xce* may lie either close to *jp* on one side of the breakpoint or close to *Ta* on the other (Fig. 1). No such alterations were recovered from the Xce^b homozygotes. The basic question posed in the present experiments concerned the identity and location of the controlling factors distinguished in normal

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chromosomes other than that of the JU inbred strain, namely those of the C3H and C57 strains.

The data obtained in the *Vbr* experiments permit the comparison of the JU and C3H *X* chromosomes. They showed that inherited, altered levels of *Vbr* expression can be recovered from presumptive *Xce* heterozygotes – *Vbr* $Xce^b/+Xce^a$ (Vbr *X* of JU origin and Vbr^+ *X* of C3H origin) and Vbr $Xce^a/+Xce^b$ (recombinant *Vbr* *X* and Vbr^+ *X* of JU origin) – but not from the corresponding presumptive *Xce* homozygotes – *Vbr* $Xce^a/+Xce^a$ and *Vbr* $Xce^b/+Xce^b$. This finding strongly supports the premiss that the different controlling factors present in the C3H and JU chromosomes represent different alleles of the *Xce* locus. If this is correct then the data provide some indication of the position of the *Xce* locus in the linkage map. When the results of the two *Vbr* experiments are combined the overall R.F. is 9·6 %. On the basis of this estimate *Xce* could lie either between *Mo* and *jp* or more proximally towards *Bn* (Fig. 1) but, given that recombination occurs between *Xce* and Is(*X*; 7)Ct (Cattanach, Perez & Pollard, 1970) and that the translocation suppresses recombination in the *Mo–jp* region (Cattanach, 1966), it would seem the more likely that *Xce* is located in the latter position, nearer *Ta*. If it could be shown that the pre-selection of females on the basis of phenotype prior to testing in fact caused the R.F. to be overestimated, this conclusion would be further strengthened. However, because of the many such practical difficulties with these experiments and because the 95 % confidence limits on the available estimates of the R.F. are so wide (lower 3·2 %, upper 21·0 %), an *Xce* location close to *jp* cannot entirely be ruled out.

In the *Ta* experiment C3H and C57 *X* chromosomes were compared. Here, no inherited alterations in *Ta* expression were found among the progeny of presumptive *Ta* $Xce^a/+Xce^b$ or *Ta* $Xce^a/+Xce^a$ females (Ta^+ chromosomes of C57 and C3H origin, respectively). Again, this supports the premiss that the difference between the *X* chromosomes compared resides at the *Xce* locus, and the data now more definitely suggest that the *Xce* locus must lie close to *Ta*. Thus, the upper 95 % confidence limit of the R.F. with *Ta* is 10·3 % which, in the distal direction, would place *Xce* between *Mo* and *jp* (Fig. 1), a location which is near-untenable because of Is(*X*; 7)Ct data (Cattanach, 1966; Cattanach, Perez & Pollard, 1970). A position either close to *Ta* and *Mo* or more proximally towards *Bn* (Fig. 1) would seem more likely. In total, therefore, all the data obtained with Is(*X*; 7)Ct, JU, C3H and C57 *X* chromosomes suggest that only a single locus is responsible for the control of *X*-linked gene expression studied, and this accords well with the finding that the C3H, JU and C57 chromosomes all bring about their effects by the same rather special mechanism, i.e. by causing primary non-random *X* inactivation (Johnston & Cattanach, 1981). Furthermore, the proposed position of the *Xce* locus in the linkage map supports the view that the controlling factors identified by other investigators in other *X* chromosomes (Drews *et al.* 1974; Falconer & Isaacson, 1972; Grahn, Lea & Hulesch, 1970; Kindred, quoted in Cattanach, Perez & Pollard, 1970; Lyon, quoted in Cattanach, Perez & Pollard, 1970, Ohno *et al.* 1973) also represent differences at the *Xce* locus.

One observation which may appear to complicate this interpretation is that described as a maternal effect and seen in the *Vbr* studies. The key finding was

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that in all crosses on a constant C3H-101 hybrid genetic background a significant positive correlation between mean progeny score and maternal score could be found. The genetic data rule out the possibility that this could be due to autosomal modifiers, the presence of different more extreme *Xce* alleles or controlling factors other than at the *Xce* locus. It would therefore seem that the maternal effect is brought about either by (1) some further mechanism which operates upon the inactivation process or the derived cell populations to influence patch size, etc. or (2) some artifact caused by the gene itself which in a secondary way influences the scoring system. The latter interpretation may be favoured for the following reasons.

(1) The primary defect of the *Mo* locus mutations is that of a copper transport deficiency (Hunt & Johnston, 1972). Final phenotype is therefore liable to environmental influence. Thus, it is known that the feeding of copper in the first few weeks after birth will enhance the survival of the hemizygous male and, more significantly, darken its coat (Hunt, personal communication). A similar effect can be obtained by fostering (Styrna, 1975; cf. also Falconer & Isaacson, 1972). Any such darkening of the often diffuse mutant patches in the coat of heterozygous females might therefore be expected to reduce the *Vbr* scoring.

(2) Conversely, recent investigations have shown that the *Vbr*/Y male, which typically is a light grey colour and near-fully viable, becomes much lighter in colour and effectively a lethal class when produced by hemizygous *Vbr*/O females (Cattanach, unpublished). This is also true for both *Vbr*/Y and *Blo*/Y males produced by *Vbr*/*Blo* females. Any lightening of the mutant patches in the coat of the heterozygous *Vbr* daughter of such females would therefore be expected to increase the *Vbr* scoring and this, in fact has been found (Cattanach, unpublished). The $Vbr\,Xce^a/+Xce^a$ and $Vbr\,Xce^b/+Xce^a$ daughters of $Vbr\,Xce^a/O$ and $Vbr\,Xce^b/O$ females generally have higher mean scores than genotypically equivalent mice derived from *Vbr*/+ mothers.

(3) Re-investigation of data obtained from studies using the Is(*X*; 7)Ct translocation as a coat colour marker (Cattanach & Isaacson, 1967; Cattanach, Perez & Pollard, 1970; Cattanach, Pollard & Perez, 1969) has failed to reveal any indication of a correlation between progeny score and maternal score.

On balance, therefore, it would seem unlikely that the maternal effect observed in the *Vbr* experiments represents a chromosomal phenomenon which manifests itself by changing patch size. It is much more likely that it derives from a scoring error as a result of differing levels of copper feeding based upon the lightening/darkening of the mutant hair of the diffusely 'variegated' coats of *Vbr*/+ progeny of *Vbr*/+ females which have differing proportions of cells with the *Vbr* allele active. This, of course, implies a positive correlation between the proportion of such cells active in the coat with that active in the blood/mammary tissues.

Further investigation of both the *Vbr* maternal effect and the map position of the *Xce* locus is currently being conducted using the new, more extreme Xce^c allele.

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REFERENCES

CATTANACH, B. M. (1966). The location of Cattanach's translocation with X-chromosome linkage maps of the mouse. *Genetical Research* **8**, 253–256.

CATTANACH, B. M. & ISAACSON, J. H. (1965). Genetic control over the inactivation of autosomal genes attached to the X-chromosome. *Zeitschrift für Vererbungslehre* **96**, 313–323.

CATTANACH, B. M. & ISAACSON, J. H. (1967). Controlling elements in the mouse X-chromosome. *Genetics*, **57**, 331–346.

CATTANACH, B. M., PEREZ, J. N. & POLLARD, C. E. (1970). Controlling elements in the mouse X-chromosome. II. Location in the linkage map. *Genetical Research* **15**, 189–195.

CATTANACH, B. M., POLLARD, C. E. & PEREZ, J. N. (1969). Controlling elements in the mouse X-chromosome. I. Interaction with X-linked genes. *Genetical Research* **14**, 223–235.

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

DAVISSON, M. & RODERICK, T. (1979). Linkage map of the mouse. *Mouse New Letter* **61**, 19.

DREWS, U., BLECHER, S. R., OWEN, D. A. & OHNO, S. (1974). Genetically directed preferential X-inactivation seen in mice. *Cell* **1**, 3–8.

DUNN, R. B. (1959). The development and growth of vibrissae in the house mouse with particular reference to the time of action of the Tabby (*Ta*) and ragged (*Ra*) genes. *Australian Journal of Biological Sciences* **12**, 312–330.

DUNN, R. B. & FRASER, A. S. (1959). Selection for an invariant character, vibrissa number, in the house mouse. *Australian Journal of Biological Sciences* **12**, 505–523.

FALCONER, D. S. & ISAACSON, J. H. (1972). Sex-linked variegation modified by selection in brindled mice. *Genetical Research* **20**, 291–316.

GRAHN, D., LEA, R. A. & HULESCH, U. (1970). Location of an X-inactivation controller gene on the normal X-chromosome of the mouse. *Genetics* **64**, s25.

HUNT, D. M. & JOHNSON, D. R. (1972). Aromatic amino acid metabolism in brindled (Mo^{br}) and viable brindled (Mo^{vbr}), two alleles at the mottled locus in the mouse. *Biochemical Genetics* **6**, 31–40.

JOHNSTON, P. & CATTANACH, B. M. (1981). Controlling elements in the mouse X-chromosomes. IV. Evidence of non-random X-inactivation. *Genetical Research* **37**, **151–160.**

KRAZANOWSKA, H. & WABIK, B. (1971). Selection for expression of sex-linked gene *Ms* (*Mosaic*) in heterozygous mice. *Genetica Polonica* **12**, 537–544.

KRAZANOWSKA, H. & WABIK, B. (1973). Expression of the sex-linked gene *Ms* (*Mosaic*) in mice selected for different levels of variegation. *Genetics* **74**, s146.

OHNO, S., CHRISTIAN, L., ATTARDI, B. J. & KAN, J. (1973). Modified expression of the *Testicular feminization* (*Tfm*) gene of the mouse by a 'Controlling Element' gene. *Nature New Biology* **245**, 92–93.

STYRNA, J. (1975). Survival of *Ms/*— males in two lines of mice selected for a different expression of the gene *Mosaic* (*Ms*) in heterozygous females. *Genetica Polonica* **16**, 213–219.

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