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Evidence of non-random X chromosome activity in the mouse

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SUMMARY

X-linked modification of the heterozygous phenotypes of X-linked genes has been detected in the X chromosomes of several inbred strains of mice. The effect is similar to that of the alternative 'states' or alleles, of the *X chromosome controlling element*, *Xce*, identified in T(1; X)Ct X chromosomes. Tests on two such differing X chromosomes have indicated that the phenotypic modification results either from non-random inactivation of the two X chromosomes or from selection operating on the two cell populations differentiated by X-inactivation. The data provide evidence of non-random X chromosome activity in the somatic cells of the female mouse.

INTRODUCTION

Female mice heterozygous for the *flecked* (*fd*) X-autosome translocation, T(1; X)Ct express variegation for autosomal coat colour genes that have been inserted into the X (Cattanach, 1961; Ohno & Cattanach, 1962). The primary cause has been attributed to the inactivation of the rearranged autosomal loci when the rearranged X is inactivated (Cattanach & Isaacson, 1965) in the course of the normal process by which one or other of the two X chromosomes becomes genetically inactivated in the somatic cells of the female (Lyon, 1961). A secondary cause is thought to be a variation in the degree of spread of the inactivating properties of the X into the attached autosomal regions (Cattanach, 1961, 1963; Russell, 1963). The amount of variegation expressed in T(1; X)Ct heterozygotes is also subject to genetic control by an element, designated the *X chromosome* controlling *element, Xce* which is located in the X chromosome itself (Cattanach & Isaacson, 1965; 1967; Cattanach, Perez & Pollard, 1970). Thus, two alternative 'states', or alleles, of the controlling element have been detected, these permitting different levels of autosomal gene variegation. In all tests carried out the control has rested exclusively upon the element located in the rearranged X (X^T), no influence of the normal X (X^N) or of the genetic background having been detected. Primarily for this reason *Xce* has been postulated to control the demonstrable spread of the inactivating properties of the X into the associated autosomal region (Cattanach, 1961). Lack of interaction between the X^T and the X^N in the heterozygous female appeared to rule out the possibility that *Xce* operates by biasing the randomness of X-inactivation or by creating a difference between X^T and X^N

chromosomes which might allow selection to operate upon the X^T active or X^N active cell populations differentiated by X inactivation (Cattanach & Isaacson, 1965, 1967; Cattanach *et al.* 1969).

Xce has also been found to modify the heterozygous phenotypes of at least two X-linked genes and from this it has been concluded that the control operates upon the inactivation of the whole X chromosome and only incidentally upon the rearranged autosomal loci (Cattanach *et al.* 1969). On the premise that an interaction between X chromosomes is not the responsible mechanism, it was postulated that *Xce* controls the degree of inactivation of the X chromosome in the same manner as it controls the inactivation of the attached autosomal loci, i.e. the alternative 'states' of *Xce*, Xce^c and Xce^i, permit complete and incomplete inactivation of the X, respectively, with a corresponding greater or lesser spread of the inactivation into the autosomal region (Cattanach *et al.* 1969).

The two alternative 'states' of *Xce* have so far only been detected in T (1; X) Ct X chromosomes but from a series of genetic data (Cattanach *et al.* 1969, 1970) the X chromosome of the inbred strain JU/FaCt has been deduced to carry Xce^i. This communication reports the results of experiments upon other normal X chromosomes. These have provided evidence of non-random X chromosome activity.

METHODS AND MATERIALS

The aim of the experiments to be described was to distinguish X-linked control of the heterozygous phenotypes of X-linked genes by normal mouse chromosomes and determine whether this operates by the mechanism postulated for the *Xce* system, i.e. incomplete inactivation. The method envisaged was to screen the X chromosomes of several inbred strains for such properties by subjecting them to tests proven to be capable of distinguishing Xce^c and Xce^i in X^T chromosomes (Cattanach *et al.* 1969), and subsequently determine whether or not the substitution of differing normal X chromosomes in T(1; X)Ct heterozygotes can modify the translocation-induced variegation.

Previous experiments have shown that the alternative 'states' of *Xce* in X^T and JU X chromosomes can be distinguished in test-crosses utilizing the X-linked genes, *Tabby* (*Ta*) and *Viable-brindled* (Mo^{vbr}, hereafter denoted *Vbr*). The procedure carried out was as follows: Males carrying the X chromosomes to be tested were crossed with *Ta* and *Vbr* females and the influence of the tested X upon the $Ta/-$ and $Vbr/-$ phenotypes of the daughters was assessed. In the case of *Ta*, this was achieved by scoring the secondary vibrissa number which is reduced from the reportedly invariant number of 19 in non-*Ta* animals to an average of about 14–17 in the *Ta* heterozygote (Cattanach *et al.* 1969; Dun, 1959; Dun & Fraser, 1959). The vibrissa numbers of the *Ta* male progeny from this cross were also obtained to serve as a check on any non-X-chromosomal influences upon *Ta* that could invalidate the test. In the case of *Vbr*, the amount of white hair (*Vbr*) in the coats of individual females was estimated to the nearest 5%, scoring at least 30 females at a time and without knowledge of their origin. This method

has been employed successfully as a standard procedure in experiments with T(1; X)Ct variegation (Cattanach *et al.* 1969).

The expression of *Ta*, both in the heterozygote and hemizygote, has been found to be subject to modification by the genetic background (Dun & Fraser, 1959; Gruneberg, 1965; Sofaer, 1969), and this may well also be true for *Vbr*. Thus, in order to distinguish X chromosomal influences, it is necessary to standardize the genetic background both of the males, whose X chromosomes are being tested, and of the *Vbr* and *Ta* females employed in the test-crosses. In the case of the males this was achieved in the following way: the JU X, which is known to carry the Xce^i form of the *controlling element*, was taken as the standard for comparison. Reciprocal crosses were made between JU and each of the inbred strains to be investigated. Within each set of reciprocal crosses the F_1 males would then have the same uniform genetic background but those from one cross would possess the JU X while those from the reciprocal would possess the X of the inbred under test. The F_1 males from each set of reciprocal crosses were subjected to the *Vbr* and *Ta* test-crosses and the influence of each tested X upon the heterozygous phenotypes of the daughters was then compared with that of the JU X on the same genetic background. The *Vbr* and *Ta* females employed in the test-crosses were rotated among the males under test. It was hoped that this procedure would reduce any bias brought into the test-crosses from this source.

The X chromosomes of five inbred strains were subjected to comparative tests with JU. These included A/H, C57BL/H-a^t, 101/H, CBA/H, and C3H/HeH.

The *Vbr* and *Ta* females employed in the test-crosses were of a somewhat different genetic background from those used in previous experiments (predominantly C3HeB/FeJ) (Cattanach *et al.* 1969). This was necessitated by our move from the City of Hope Medical Center to the Harwell unit. In this laboratory, the *Vbr* stock is maintained on a C3H/HeH × 101/H hybrid background by crossing *Vbr*/+ females to +/F_1 hybrid males. In all crosses the males carry the C3H X. Hence as in the original stock, the *Vbr* X is carried heterozygously with a C3H X. All *Vbr* females, both from the stock and progeny of the test-crosses, showed the *Vbr* mosaicism on an *agouti* background coat colour. The *Ta* females employed in the test-crosses were heterozygotes derived from a 39-chromosome XO stock of mice maintained on the same C3H/HeH × 101/H hybrid background. Since the *Ta* gene is carried in the hemizygous condition (*Ta* O and *Ta* Y) in the XO stock, the use of these animals assured that all *Ta* chromosomes introduced into the test-crosses were identical.

X-linked modification of the heterozygous phenotypes of X-linked genes could be postulated to result from a number of mechanisms. It could result from incomplete inactivation, as postulated for the *Xce* system, or it could result from an X-X interaction operating at either the chromosomal or cellular level, i.e. from a bias to the X-inactivation process or from selection operating upon the two cell populations differentiated by X-inactivation.

The first of these cannot be distinguished from the other two in experiments with normal X chromosomes for the alleles of any gene studied are present on the

two chromosomes. However with an X-autosome translocation, such as T(1; X)Ct, a distinction can be made; the gene responsible for the variegation is carried on only one X, the X^T, and, hence, the substitution of normal X chromosomes known to differ in their effects upon the heterozygous phenotypes of X-linked genes should only influence T(1; X)Ct variegation if the mechanism responsible operates through some kind of differential interaction (chromosomal or cellular) between the X^N chromosome under test and the X^T chromosome. This test is therefore essential for the interpretation of all the data.

The T(1; X)Ct animals employed in the present experiment possessed the chromosomally unbalanced, duplication (Dp) form of the rearrangement (Cattanach, 1961), i.e. they carried a piece of linkage group I bearing the wild type allele of *albino* (*c*) inserted into the X in addition to an otherwise normal autosomal complement. The Dp stock is currently maintained on a JU/FaCt × A/H hybrid background by crossing Dp females of each generation to F_1 hybrid males carrying the JU X. Both JU and A are homozygous for *albino* (*c*), hence all Dp females show a *c*-variegated phenotype. Both strains are homozygous for non-*agouti* (*a*) but the A strain is also homozygous for *brown* (*b*). *Brown* (*b*) Dp females were not used in any of the matings and, hence, most of the Dp progeny showed a *c*-variegated phenotype on a *black* background coat colour. Only the *black* progeny were considered in the present study.

Two lines are maintained within the stock; one (HX) carries the Xce^c form of the controlling element in its X^T and the other (LX) carries the Xce^i form. On a *a* background colour recent estimated levels of *c*-variegation in the two lines have been approximately 60% and 50% respectively (Cattanach & Perez, 1970). The true levels are believed to be somewhat lower (Cattanach & Isaacson, 1967).

One of the X chromosomes found to differ from JU in the *Ta* and *Vbr* test-crosses was subjected to the further test for interaction with the X^T chromosomes. F_1 hybrid reciprocal cross males carrying the JU X or the X to be tested were mated with Dp females of both HX and LX lines. In each set of tests Dp sibs, matched for the level of *c*-variegation they showed, were employed in order to reduce any variables as far as possible. The levels of *c*-variegation among the progeny of the four crosses were assessed as described earlier when the animals were 6 weeks old. The scoring was carried out without knowledge of the genotypes.

RESULTS

Vbr test-crosses: The results of the *Vbr* test-crosses (Table 1) demonstrated clear differences between the X chromosomes of some of the inbred strains. Thus, the A, 101 and C3H X chromosomes markedly enhanced the *Vbr* scores over those obtained with the JU X whereas the C57 X did not. An unexpected finding emerged from the CBA tests; one male (CBA 1) yielded a mean score indistinguishable from that with the JU X, whilst the other (CBA 2), a sib, clearly differed, yielding the highest score of any test. Differences between the X chromosomes from within an inbred strain were not anticipated. If the result does not stem

Table 1. *The influence of the X chromosomes of the inbred strains upon the expression of* Vbr *in the female*

X chromosome	Animal	No.	*Vbr*/+ progeny Score	Mean score	Significance
JU	1	33	$40{\cdot}30 \pm 2{\cdot}54$	$43{\cdot}19 \pm 1{\cdot}89$	$t_{153} = 2{\cdot}90;\ P < 0{\cdot}01$
	2	39	$45{\cdot}64 \pm 2{\cdot}70$		
A	1	49	$49{\cdot}59 \pm 2{\cdot}56$	$50{\cdot}96 \pm 1{\cdot}88$	
	2	34	$52{\cdot}94 \pm 2{\cdot}70$		
JU	1	25	$43{\cdot}80 \pm 2{\cdot}79$	$43{\cdot}30 \pm 1{\cdot}94$	$t_{89} = 1{\cdot}07;\ P > 0{\cdot}05$
	2	20	$42{\cdot}00 \pm 2{\cdot}70$		
C 57	1	19	$36{\cdot}84 \pm 3{\cdot}36$	$39{\cdot}78 \pm 2{\cdot}28$	
	2	27	$41{\cdot}85 \pm 3{\cdot}08$		
JU	1	29	$39{\cdot}48 \pm 2{\cdot}37$	$39{\cdot}14 \pm 1{\cdot}75$	$t_{130} = 4{\cdot}12;\ P < 0{\cdot}001$
	2	35	$38{\cdot}86 \pm 2{\cdot}54$		
101	1	31	$52{\cdot}10 \pm 2{\cdot}44$	$49{\cdot}33 \pm 1{\cdot}75$	
	2	37	$47{\cdot}03 \pm 2{\cdot}45$		
JU	1	16	$43{\cdot}13 \pm 3{\cdot}53$	$44{\cdot}11 \pm 2{\cdot}69$*‡§	*$t_{106} = 2{\cdot}09;\ P < 0{\cdot}05$ †$t_{61} = 4{\cdot}04;\ P < 0{\cdot}001$ ‡$t_{74} = 0{\cdot}18;\ P > 0{\cdot}05$ §$t_{75} = 3{\cdot}91;\ P < 0{\cdot}001$
	2	29	$44{\cdot}66 \pm 3{\cdot}73$		
CBA	1	31	$43{\cdot}39 \pm 3{\cdot}02$†‡	$51{\cdot}19 \pm 2{\cdot}12$*	
	2	32	$58{\cdot}75 \pm 2{\cdot}33$†§		
JU	1	11	$43{\cdot}18 \pm 4{\cdot}68$	$43{\cdot}08 \pm 2{\cdot}96$	$t_{60} = 4{\cdot}24;\ P < 0{\cdot}001$
	2	15	$43{\cdot}00 \pm 3{\cdot}96$		
C3H	1	20	$56{\cdot}00 \pm 2{\cdot}10$	$56{\cdot}11 \pm 1{\cdot}51$	
	2	16	$56{\cdot}25 \pm 2{\cdot}21$		
HX (X^T, *Xce*^{c})	×	69	—	$52{\cdot}50 \pm 1{\cdot}96$	$t_{123} = 3{\cdot}86;\ P < 0{\cdot}001$
LX (X^T, *Xce*^{i})	×	60	—	$41{\cdot}92 \pm 1{\cdot}91$	
HX (JU X, *Xce*^{i})	×	45	—	$43{\cdot}72 \pm 1{\cdot}96$	$t_{87} = 0{\cdot}59;\ P > 0{\cdot}05$
LX (JU X, *Xce*^{i})	×	43	—	$41{\cdot}82 \pm 2{\cdot}43$	

× Data of Cattanach *et al.* (1969); X^T and JU X chromosomes carried on a JU genetic background. *, †, ‡, §: one of these symbols after two scores or mean scores identifies the values compared in the *t*-test preceded by the same symbol.

from some malfunction of the test, it must be concluded that either one or the other of the two CBA X chromosomes is a variant. The mutation responsible could have occurred in a maternal germ cell or it is possible that the mother was a heterozygote.

It may be noted that the scores obtained with the JU X differed little in each set of tests. This suggests that the genetic background does not play an important part in influencing the *Vbr* contribution as determined by the scoring procedure employed. This also appears true when a comparison is made with the results of earlier experiments (also shown in Table 1) upon the X^T chromosome carrying *Xce*^{i} and upon the JU X, which also carries *Xce*^{i}. If this comparison is a valid one, then the A, and 101 X chromosomes yielded *Vbr* scores which were very similar to that characteristic of the X^T chromosome carrying *Xce*^{c} (Table 1). The C3H and CBA-2 scores were somewhat higher.

Ta test-crosses: The results of the *Ta* test-crosses are shown in Table 2. It can

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Table 2. *The influence of the X chromosomes of the inbred strains upon the expression of* Ta *in the female*

X chromosome	Animal	No.	Vibrissa scores	Mean vibrissa score	Significance
JU	1	37	$15{\cdot}89 \pm 0{\cdot}16$	$15{\cdot}92 \pm 0{\cdot}14$	$t_{142} = 2{\cdot}85; P < 0{\cdot}01$
	2	48	$15{\cdot}94 \pm 0{\cdot}22$		
A	1	28	$15{\cdot}14 \pm 0{\cdot}37$	$15{\cdot}20 \pm 0{\cdot}22$	
	2	31	$15{\cdot}23 \pm 0{\cdot}24$		
JU	1	36	$15{\cdot}83 \pm 0{\cdot}32$	$15{\cdot}98 \pm 0{\cdot}21$	$t_{109} = 0{\cdot}36; P > 0\ 05$
	2	31	$16{\cdot}16 \pm 0{\cdot}27$		
C57	1	28	$15{\cdot}96 \pm 0{\cdot}19$	$16{\cdot}09 \pm 0{\cdot}18$	
	2	16	$16{\cdot}31 \pm 0{\cdot}38$		
JU	1	18	$16{\cdot}56 \pm 0{\cdot}27$	$15{\cdot}91 \pm 0{\cdot}20$	$t_{90} = 1{\cdot}68; P > 0{\cdot}05$
	2	28	$15{\cdot}50 \pm 0{\cdot}25$		
101	1	23	$15{\cdot}52 \pm 0{\cdot}33$	$15{\cdot}28 \pm 0{\cdot}32$	
	2	23	$15{\cdot}04 \pm 0{\cdot}54$		
JU	1	22	$16{\cdot}41 \pm 0{\cdot}24$	$16{\cdot}38 \pm 0{\cdot}16$	$t_{67} = 4{\cdot}48; P < 0{\cdot}001$
	2	25	$16{\cdot}36 \pm 0{\cdot}23$		
CBA	1	—	—	—	
	2	22	$14{\cdot}95 \pm 0{\cdot}31$		
JU	1	27	$16{\cdot}59 \pm 0{\cdot}26$	$16{\cdot}49 \pm 0{\cdot}17$	$t_{109} = 3{\cdot}96; P < 0{\cdot}001$
	2	24	$16{\cdot}37 \pm 0{\cdot}20$		
C3H	1	31	$15{\cdot}13 \pm 0{\cdot}37$	$15{\cdot}25 \pm 0{\cdot}25$	
	2	29	$15{\cdot}38 \pm 0{\cdot}34$		
HX (X^T, Xce^c)*	3 ♂♂	29	—	$14{\cdot}83 \pm 0{\cdot}38$	$t_{75} = 2{\cdot}69; P < 0{\cdot}01$
LX (X^T, Xce^t)*	3 ♂♂	48	—	$15{\cdot}90 \pm 0{\cdot}20$	

* Pooled results of T(1; X)Ct males of a JU × A genetic background.

be seen that the same kinds of influence were exerted by the various tested chromosomes upon the *Ta*/+ phenotype as upon the *Vbr*/+ phenotype although the differences were generally less clear cut. Thus the A and C3H X chromosome again differed from that of JU and the shift in phenotype was in the same direction as in the *Vbr* test-crosses (greater mutant effect). Similar differences were found when the 101 and JU X chromosomes were compared but, in contrast to the results of the *Vbr* test-crosses, these did not prove to be statistically significant. With the *Ta* test, the C57 and JU X chromosomes again could not be distinguished. Reciprocal cross *Ta* male differences were not apparent in any of the tests (Table 3). This confirms that the *Ta*/+ female scores were not biased by genetic background differences.

The results of the CBA *Vbr* test-crosses could only be partially confirmed with *Ta* because of the death of one of the males (CBA 1). However, the CBA X of the surviving male (CBA 2) again proved to be different from that of JU; the shift in phenotype was in the same direction as in the *Vbr* test-crosses and the magnitude was greater than that of most other tests. The scores of the *Ta* males were also somewhat lower, however. It may be noted that in both the *Vbr* and *Ta* test-crosses the C3H and CBA-2 X chromosomes appeared to shift the phenotypes to a greater extent than either the A or 101 X chromosomes. Three types of X chromosome

Table 3. *Vibrissa scores of* Ta *male progeny of the* Ta *test-crosses*

X chromosome strain	Animal	No.	Vibrissa scores	Mean vibrissa score	Significance
JU	1	35	$7{\cdot}06 \pm 0{\cdot}21$	$7{\cdot}12 \pm 0{\cdot}15$	$t_{162} = 0{\cdot}99; P > 0{\cdot}05$
	2	62	$7{\cdot}16 \pm 0{\cdot}19$		
A	1	36	$7{\cdot}39 \pm 0{\cdot}24$	$7{\cdot}34 \pm 0{\cdot}16$	
	2	31	$7{\cdot}29 \pm 0{\cdot}22$		
JU	1	34	$7{\cdot}09 \pm 0{\cdot}26$	$7{\cdot}25 \pm 0{\cdot}22$	$t_{106} = 0{\cdot}22; P > 0{\cdot}05$
	2	27	$7{\cdot}44 \pm 0{\cdot}37$		
C57	1	29	$7{\cdot}34 \pm 0{\cdot}37$	$7{\cdot}32 \pm 0{\cdot}25$	
	2	18	$7{\cdot}28 \pm 0{\cdot}27$		
JU	1	26	$7{\cdot}85 \pm 0{\cdot}28$	$7{\cdot}66 \pm 0{\cdot}20$	$t_{100} = 0{\cdot}09; P > 0{\cdot}05$
	2	24	$7{\cdot}46 \pm 0{\cdot}30$		
101	1	19	$7{\cdot}89 \pm 0{\cdot}35$	$7{\cdot}64 \pm 0{\cdot}20$	
	2	33	$7{\cdot}49 \pm 0{\cdot}24$		
JU	1	24	$7{\cdot}42 \pm 0{\cdot}28$	$7{\cdot}32 \pm 0{\cdot}18$	$t_{64} = 1{\cdot}35; P > 0{\cdot}05$
	2	29	$7{\cdot}24 \pm 0{\cdot}25$		
CBA	1	—	—	—	
	2	13	$6{\cdot}77 \pm 0{\cdot}36$		
JU	1	21	$7{\cdot}81 \pm 0{\cdot}39$	$7{\cdot}35 \pm 0{\cdot}25$	$t_{95} = 0{\cdot}21; P > 0{\cdot}05$
	2	25	$6{\cdot}96 \pm 0{\cdot}30$		
C3H	1	29	$7{\cdot}35 \pm 0{\cdot}21$	$7{\cdot}41 \pm 0{\cdot}19$	
	2	22	$7{\cdot}50 \pm 0{\cdot}33$		
HX (X^T, Xce^c)*	3 ♂♂	50	—	$6{\cdot}96 \pm 0{\cdot}22$	$t_{105} = 2{\cdot}33; P < 0{\cdot}05$
LX (X^T, Xce^i)*	3 ♂♂	57	—	$7{\cdot}61 \pm 0{\cdot}18$	

* Pooled results of T(1; X)Ct males of a JU × A genetic background.

may thus be represented in these tests; that of JU or C57, that of A or 101 and that of C3H or CBA 2.

Also included in Table 2 are the mean vibrissa scores of the progeny of three HX and three LX males carrying Xce^c and Xce^i, respectively, in their X^T chromosomes. The genetic background of these males was similar to that of the JU × A reciprocal cross males, and, hence, may justifiably be compared. It can be seen that while the LX and JU scores do not differ, that for HX is somewhat lower than that for A, and the difference is statistically significant at the 5 % level ($t_{105} = 2{\cdot}30$). However, since the *Ta* male scores were also rather low in the HX group, it is not clear whether the HX – A *Ta*/+ difference is meaningful.

The scoring of non-*Ta* animals in these tests yielded an additional piece of information. Both male and female non-*Ta* animals were regularly found which possessed 18, rather than 19, vibrissae. The frequency was highest in the test-crosses involving the JU × A hybrid males (25 %) but considerably lower (8 %) in all other crosses. There was no indication of any difference in the frequencies found in reciprocal crosses or between male and female progeny. The data thus suggest that the secondary vibrissa number is modified by the genetic background and that it is most markedly influenced by that of the A strain.

Tests for interaction. Of the four strains (A, 101, CBA and C3H) whose X chromosomes were found to differ from that of JU in the *Vbr* and *Ta* test-crosses,

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Table 4. *The influence of the A and JU X chromosomes upon T (1; X) Ct variegation*

Line	Generation	X^T, Xce	X^N	No.	Mean level of c-variegation	Significance
HX	8	Xce^c	JU	35	$59{\cdot}29 \pm 1{\cdot}42$	
HX	9	Xce^c	JU	76	$56{\cdot}84 \pm 1{\cdot}07$	
HX	10	Xce^c	JU	31	$57{\cdot}74 \pm 1{\cdot}85$	$t_{67} = 2{\cdot}56; P < 0{\cdot}02$
HX	10	Xce^c	A	38	$51{\cdot}45 \pm 1{\cdot}63$	
LX	10	Xce^i	JU	39	$50{\cdot}64 \pm 1{\cdot}40$	$t_{71} = 2{\cdot}37; P < 0{\cdot}05$
LX	10	Xce^i	A	34	$45{\cdot}29 \pm 1{\cdot}80$	
LX	9	Xce^i	JU	76	$48{\cdot}82 + 1{\cdot}18$	
LX	8	Xce^i	JU	83	$48{\cdot}80 \pm 0{\cdot}96$	

only the A strain carries *albino* (*c*). Since *c* is required for the appearance of a *c*-variegated phenotype in Dp females, the A X was the obvious first choice to test with the JU X for interaction with the X^T chromosomes in Dp females. The results are shown in Table 4 which also presents the data obtained with X^T chromosomes carried heterozygously with the JU X in earlier generations. It can be seen that Dp females carrying Xce^c in their X^T chromosomes (HX line) exhibited significantly lower mean scores when the A X was present rather than the JU. Suppression of *c*-variegation by the A X was also indicated in the tests with X^T chromosomes carrying Xce^i (LX line); the latter difference between the JU and A X crosses was statistically significant (at the 5% level) but this was not true when the results of the A X cross was compared with those of earlier generations, e.g. with LX, generation 9, $t_{118} = 1{\cdot}86$; $P < 0{\cdot}05$. The lesser suppression obtained with Xce^i may indicate that an opposing selective force is operating; the hemizygous Dp male genotype has a low viability and it may be that selection is taking place either at the cell or organism level in the heterozygous female, enhancing the mean levels of *c*-variegation. On the other hand, the data suggest that the effect of the JU–A substitution is less than an X^T, $Xce^c - X^T$, Xce^i substitution and this was also suggested by the results of the *Ta* test-crosses (Table 2). It may be that the A–JU difference is so small that it is hard to establish in experiments of this magnitude.

Whatever the degree of effect, the data indicate that the substitution of one X^N for another can modify T(1; X)Ct variegation and this implies that some kind of interaction operating at either the chromosomal or cellular level is involved. It should be noted that in these tests a lower score means a higher effect by the c^+ allele carried in the X^T chromosome. The direction of the effect caused by the A–JU substitution is thus the same as in the *Vbr* and *Ta* test-crosses. This would indicate that an X chromosomal or cellular interaction, operating in the somatic cells of the female, is the mechanism responsible for the differences in the heterozygous phenotypes observed in the various crosses.

DISCUSSION

The results of the *Vbr* and *Ta* test-crosses clearly show that the substitution of one X chromosome for another, each carrying the wild-type alleles of *Vbr* and *Ta*, can markedly influence the phenotypes of *Vbr* and *Ta* heterozygotes. On this basis, two or more probably three different types of X chromosomes have been identified. One type is that of JU and C57, another is that of A and 101, and a possible third is that of C3H and the single male carrying a CBA X (CBA 2) subjected to both the *Vbr* and *Ta* tests. It is interesting to note that the effect of the X chromosome substitutions parallels observations of other investigators concerned with the influences of the whole genetic background upon the heterozygous *Ta* phenotypes. Thus, Sofaer (1969) has noted that the influence of *Ta* on the teeth, coat and secondary vibrissa number is enhanced in crosses to the A strain and reduced in crosses to JU. Likewise, data obtained by Kindred (1961) suggest that the vibrissa scores of *Ta*/+ females derived from crosses to the A and CBA strains tend to be lower (greater *Ta* effect) than those derived from crosses to C 57. 101 appeared to have an intermediate effect. It is also noteworthy that Russell (1971) in attempting to distinguish the *controlling element* in the X chromosomes of several inbred strains found that the tail-kinking caused by the X-linked gene, *Bent-tail* (*Bn*), in the heterozygote is lower in crosses introducing a BL/10 (a subline of C57) X than in equivalent crosses introducing the X chromosomes of other inbreds. Unfortunately, her experiment did not permit the distinction of X chromosomal and genetic backgrounds effects. The data reported in the present communication suggest that a great part, but not all, of the genetic background influences upon *Ta* are due to the X chromosomes and that the mechanism operates not only upon the *Ta* locus but probably on all X-linked loci, i.e. on the X chromosome as a whole. It is of interest that Kindred's (1961) data suggest that the CBA X modifies *Ta*/+ vibrissa scores in a similar manner to the A X. If this does not stem exclusively from the genetic background differences that existed, her data suggest that the CBA 2 X of the present study is that typical of the strain.

The data presented demonstrate X-linked modification of the heterozygous *Vbr* and *Ta* phenotypes. They do not demonstrate whether or not the hemizygous phenotypes are also affected for the simple reason that the X chromosomal differences were only detected in chromosomes carrying the wild-type alleles of the genes investigated. However, since the probability is so remote that X-linked modifers exist which operate on more than one locus it will not be considered further in this paper. With the knowledge that one or other of the two X chromosomes is genetically inactivated in the somatic cells of the female (Lyon, 1961) it is much more probable that the control in some way relates to the X-inactivation process.

The finding that the substitution of an A X for a JU X modifies the levels of Dp female *c*-variegation in the same way as it influences the heterozygous *Vbr* and *Ta* phenotypes demonstrates that the mechanism involved operates upon some kind of interaction between the X chromosomes in female cells or between the two

cell populations differentiated by X-inactivation. The consequence, as observed at the phenotypic level in at least two different cell types, melanoblasts (i.e. *c*) and hair follicle cells (i.e. *Vbr* and *Ta*), is non-randam X chromosome activity. The data do not distinguish how the non-random X activity is brought about but the two most probable mechanisms are cell selection and non-random X-inactivation and of the two, the former must initially be favoured. The X-inactivation process creates two genetically different cell populations and it would not therefore be unexpected if selection should favour one or the other during growth and development. Evidence that cell selection can modify the mosaic phenotypes resulting from the presence of two differing populations has been obtained by Mintz (1971) in her studies on chimaeric (allophenic) mice but more pertinent evidence is available from studies on the reciprocal horse × donkey hybrids, the mule and hinney. X-inactivation in these animals creates cells with the horse X or donkey X genetically active and Hamerton *et al.* (1971) and Giannelli & Hamerton (1971) have obtained extensive evidence of *in vitro* selection favouring cells with the horse X active. Further, Hook & Brustman's (1971) study of a large group of female mules has provided evidence that cells with the horse X active also tend to predominate *in vivo*. If the mouse data are interpreted on the basis of cell selection, it may be concluded that when in competition with the cells with the *Vbr* or *Ta* X active, or with cells with the Xce^c or Xce^i X^T active, in the female progeny of the respective test-crosses, cells with an A X active are less successful than are cells with a JU X active.

Before considering the possibility that non-random X-inactivation, rather than cell selection, may be the responsible mechanism, it is necessary to evaluate the implications of the new data for the interpretation of the *Xce* system. The *Xce* locus has been found to lie close to the T(1; X)Ct breakpoint in the X chromosome and probably very close to *Ta* (Cattanach, 1970). The two alternative 'states', or alleles, of *Xce* detected in T(1; X)Ct X chromosomes permit different levels of variegation for all of the three autosomal coat colour genes (*c*, *p* and *ru*-2) inserted in the X (Cattanach, 1971) and also modify the heterozygous phenotypes of at least two X-linked genes, *Ta* and *Vbr*. The *Xce* system has been thought to operate upon the inactivation process, controlling the degree of inactivation of the X and inserted autosomal material, primarily because the effect had been found to be produced entirely independently of the normal X chromosomes present in the Dp females (Cattanach & Isaacson, 1967; Cattanach *et al.* 1969). In now demonstrating that an A X–JU X substitution modifies the levels of *c*-variegation, the results of the T(1; X)Ct test, here described, seriously weakens this hypothesis. The fact that the consequences of A X–JU X substitutions are much smaller than those of X^T, $Xce^c - X^T$, Xce^i substitutions does not alter this conclusion. Possibly a C3H X–JU X substitution would have produced a greater effect. Further, since the X^T and normal X chromosomes produced the same type of influence upon the *Vbr* and *Ta* heterozygous phenotypes and since, at least for the A–JU difference, the effect has been shown to operate by an interaction mechanism, it is perhaps unwarranted to consider that two different systems modifying the heterozygous

phenotypes of X-linked genes may exist. At least until it can be proven otherwise it should be taken that the *Xce* system and that governing the differences between X^N chromosomes, illustrated in the present experiments, all operate by the same mechanism, i.e. by some kind of interaction between the X chromosomes or between the two cell populations differentiated by X-inactivation. This conclusion should also be applied to those examples of X-linked modification of the heterozygous phenotypes of X-linked genes observed by other investigators. These include X-linked modification of *Ta* (Kindred, quoted in Cattanach *et al.* 1969), *Brindled* (Falconer & Isaacson 1969), an allele of *Vbr*, *Gyro* (Lyon, quoted in Cattanach *et al.* 1969) and *Brindled* and *Greasy* (Grahn, Lea & Hulesch, 1970). The possibility that non-random X-inactivation, rather than cell selection, is the mechanism responsible may now be considered.

It has been pointed out that in each of the above-mentioned cases, the modifying factor appears to be located in the vicinity of the *Ta* locus and that this is probably also true for *Xce* (Cattanach *et al.* 1970), i.e. it is possible that only one locus is involved. If this proves to be so, then this might constitute evidence that the mode of action is upon the X-inactivation process. On the other hand, should the modifications of the heterozygous phenotypes caused by the individual X chromosomes prove to result from differences at several loci, cell selection would be the more likely to be the correct explanation; it would seem highly improbable that several different loci could affect X-inactivation. In theory, at least, it should be possible to distinguish between the two mechanisms on the basis of the distribution among a large number of animals of the two cell types differentiated by X-inactivation. Non-random inactivation should produce a binomial distribution around the mean of the population. Random inactivation followed by cell selection should show a departure from a binomial distribution. None of the *Vbr* data (pooled A, 101; pooled C3H, CBA-2; JU; X^T, Xce^c or X^T, Xce^t) indicate a departure from a binomial distribution, but it must be stressed the scoring procedure is probably too inaccurate to allow any firm conclusion to be drawn from this. A cytological study of the HX and LX X^T lines might resolve the problem.

In concluding, it should be noted that, since the presence of different X^N chromosomes can modify translocation-induced variegation, the amount of variegation expressed for the separate autosomal loci concerned in any one rearrangement can only be validly compared when the X^N chromosomes are the same. For the T(1; X)Ct rearrangement *albino* (*c*)-, pink-eye (*p*)- and ruby-eye-2 (*ru*-2)- variegation have been compared when the X^N has been of a C57 or JU origin (Cattanach, 1970). Since these chromosomes have not been found to be detectably different, the levels of variegation characteristic of each gene and taken to indicate their relative distances from the autosomal breakpoints, do not need to be modified.

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