Controlling elements in the mouse X-chromosome

II. Location in the linkage map

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SUMMARY

The frequency and nature of the changes in 'state' of the mouse X-chromosome controlling element (inactivation centre) have been investigated on an inbred background. The results indicate with near-certainty that meiotic crossing over is the responsible mechanism and that the frequency of recombination between the $T(1; X)Ct$ breakpoint and the locus of the controlling element is approximately 3 %. Maize-type 'changes in state' may occur under other experimental conditions. The data do not distinguish on which side of the autosomal insertion the element lies but when combined with observations of other investigators suggest that the location must be on the *Mo-Ta* side and very close to *Ta*.

1. INTRODUCTION

The variegation associated with the mouse X-autosome translocations (Cattanach, 1961; Russell & Bangham, 1959; Russel, Bangham & Saylors, 1962) normally occurs only in the heterozygous female. The primary cause is considered to be the inactivation of the associated autosomal genes (Cattanach, 1963; Russell, 1964) in those cells in which the rearranged X is inactivated in the course of the normal process of X-inactivation (Lyon, 1961). Most of the observed variegation can be attributed to the randonmess with which the rearranged and normal X chromosomes are inactivated but a second source of variegation also exists. The associated autosomal loci do not always become inactivated when the rearranged X is inactivated and the probability of their inactivation is dependent upon their proximity to the breakpoint (Cattanach, 1961; Russell, 1963). This second source of variegation is considered to be analogous to the V-type position effect variegation described in *Dropsohila* (see reviews by Baker, 1968; Lewis, 1950).

The position effect variegation caused by the *flecked* X-autosome translocation, $T(1;X)Ct$, has been found to be under the control of an element located in the rearranged X chromosome (Cattanach & Isaacson, 1965, 1967) and evidence has recently been obtained which suggests that the controlling element is responsible for the inactivation of the X chromosome itself (Cattanach, 1968; Cattanach, Pollard & Perez, 1969). Different 'states' of the element exist, these differing by the levels of position effect variegation they permit and also by their influence on the heterozygous phenotypes of at least two X-linked genes.

Distinguishable 'states' of the element were first detected in crosses between two lines of mice that had been selected over several generations for high and low levels of translocation-induced variegation and although changes in the 'state' of the element were occasionally found to occur, pedigree analyses indicated that the frequency of change was low, i.e. the 'state' associated with any single rearranged X chromosome was transmitted unchanged from generation to generation. Somewhat at variance with this finding was the observation that when changes occurred they tended to appear in high frequencies in certain families and apparently independently of the normal X chromosome of the heterozygous female. For this reason it was thought that the changes might occur by a process analogous to the 'changes in state' of the controlling elements described in maize (McClintock, 1950, 1965). However, because of the many technical difficulties of the system, meiotic crossing over could not be ruled out as the responsible mechanism.

Over the past 2 years two distinguishable 'states' of the element carried in $T(1;X)Ct$ X chromosomes have been introduced into a common inbred background and established in two separate sublines by repeated backcrossing to the inbred. Under these more controlled conditions it has been possible to screen for changes in the two original 'states' of the element and determine the frequency. Moreover, now that the two 'states' can be recognized by their influence on the heterozygous phenotypes of X-linked genes (Cattanach *et al.* 1969), it has been possible to determine the 'state' carried in the X chromosome of the inbred stock. With this extra peice of information the nature of the observed changes in the 'states' of the mouse X-chromosome controlling element can be more readily deduced.

The present communication presents data on the occurrence and frequency of change in the two 'states' carried in the inbred background. They suggest that the observed changes occur by meiotic crossing over and the frequency of change indicates that the locus of the controlling element lies within three crossover units of the $T(1;X)Ct$ breakpoint.

<h2 style="text-align:center">2. METHODS AND MATERIALS</h2>

The translocation, $T(1;X)Ct$, is one in which a piece of linkage group I bearing the wild-type alleles of *pink-eye* (*p*) and *albino* (*c*) has been inserted into the X (Ohno & Cattanach, 1962). In the experiments to be described all the translocation-bearing animals carry the chromosomally unbalanced, duplication form, $Dp(1;X)Ct$ of the rearrangement and *albino* (*c*) is present in both normal linkage group I chromosomes. The heterozygous female thus exhibits a *c*-variegated or *flecked* phenotype and this is normally observed on a *non-agouti* (*a*), *black* (*b*+) background coat colour. The term Dp will be used to describe $Dp(1;X)Ct$ heterozygotes and hemizygotes.

The two 'states' of the controlling element under study are normally distinguished by the levels of translocation-induced *c*-variegation they permit in the heterozygous female. However, they can also be recognized by their influence on the heterozygous phenotypes of at least two X-linked genes, *Tabby* (*Ta*) and

Viable-brindled (Mo^{vbr} or *Vbr*) (Cattanach *et al.* 1969). The 'state' designated high permits a near-50% level of *c*-variegation or a high level of expression of *Ta* or *Vbr*, and that designated low, a near-30% level of *c*-variegation or a lower level of expression of *Ta* or *Vbr* (see Table 1). The difference is attributed to the frequency of cells in which the c^+, Ta^+ or Vbr^+ genes are inactivated when the rearranged X (X^T) is inactivated (Cattanach & Isaacson, 1967; Cattanach *et al.* 1969). Both 'states' were derived from a single line of mice that had been selected for low levels of *c*-variegation (Cattanach & Isaacson, 1967) and each had subsequently been introduced into a common inbred (JU/Fa) background by way of single Dp males and established in two sublines. The two sublines were then routinely maintained by backcrossing the Dp females of each generation to JU males.

Table 1. *Observations, scores and interpretations of the* c-*variegated,* Ta/+ *and* Vbr/+ *phenotypes attributable to the low and high 'states' of the controlling element*

State of controlling element	Genotype of females		Expected phenotype	Mean score	Expression of mutant
Low	Dp (c^+)/-	c c	Low amount of *c*	Low	Low
	Dp $(c^+)Ta^+$/-*Ta*	c^+c	High vibrissa no.	High	Low
	Dp $(c^+)Vbr^+$/-*Vbr*	c^+c	Low amount of *Vbr*	Low	Low
High	Dp (c^+)/-	c c	High amount of *c*	High	High
	Dp $(c^+)Ta^+$/-*Ta*	c^+c	Low vibrissa no.	Low	High
	Dp $(c^+)Vbr^+$/-*Vbr^+*	c^+c	High amount of *Vbr*	High	High

Changes in the 'state' of the controlling element can be recognized only in males, since as a result of the randomness of X-inactivation, individual females may exhibit a wide range of *c*-variegated, $Ta/+$ or $Vbr/+$ phenotypes no matter which 'state' is present in the rearranged X chromosome. The genotypes of Dp males with respect to the 'state' of the controlling element can, however, be determined by means of a progeny-test with JU females (Cattanach & Isaacson, 1967) or a test-cross with females carrying *Ta* or *Vbr* (Cattanach *et al.* 1969); the mean levels of *c*-variegation, *Ta* or *Vbr* among 15–20 daughters accurately identifies the 'state' of the element carried by the father, for the randomness of the X-inactivation process is balanced out among the daughters.

The original aim of the experiments was to screen for changes in the 'state' of the element carried in each subline by progeny-testing Dp males derived from each generation of backcrossing. This, however, proved to be impracticable. After the second generation of inbreeding the Dp males, which normally exhibit a low viability (Cattanach, 1961) became a lethal class, but they could be recovered by outcrossing the increasingly inbred Dp females of each generation to an unrelated stock of males. Since the level of *c*-variation has been found to be independent of the genetic background (Cattanach & Isaacson, 1967), the Dp males that were produced in the outcrosses could equally well be progeny-tested to determine whether a change had occurred in the 'state' of the element carried in the

rearranged X inherited from the near-inbred mother. Further details of the crosses may be found elsewhere (Cattanach *et al.* 1969).

The lines carrying the high and low 'states' of the controlling element are designated H and L, respectively, and their outcross derivatives HX and LX. Numbers associated with these symbols, e.g. L_2, indicate the generation of inbreeding of the animals or, in the case of the outcross animals, e.g. L_2X, the generation of inbreeding of the mothers. The progeny-test matings are indicated by the letter T; thus H_4XT designates the test of a Dp male derived from H_4X mating.

The levels of *c*-variegation were determined in the standard manner (Cattanach & Isaacson, 1967; Cattanach *et al.* 1969); the amounts of white (*c*) areas in the coats of the individual females were estimated to the nearest 5 % and the scoring was carried out on groups of at least 50 animals, without knowledge of their identity. The scoring procedure overestimates the true levels of *c*-variegation by about 10–15%, but the data to be presented have not been corrected for this scoring bias.

Each altered 'state' detected by the progeny-test was further investigated to see whether its influence upon the heterozygous phenotype of *Ta* had also changed. This was achieved by subjecting Dp males, carrying the changed 'states', to the standard *Ta* test-cross (Cattanach *et al.* 1969). Each male was mated to a series of *Ta/Ta* females and the vibrissa number of the offspring scored. *Ta* reduces the vibrissa number (Dun, 1959; Dun & Fraser, 1959) and hence this character can be used as a measure of the level of expression of *Ta* in the heterozygous female (see Table 1).

3. RESULTS

The results of 53 L_2XT, L_3XT and L_4XT progeny-tests are summarized in Table 2. With two exceptions, each score was obtained from the classification of a minimum of 15 daughters and most were based on 20 or more. It can be seen that the values obtained ranged from 38 to 50% with most lying within the 41–47 % level (Fig. 1). On the grading system of classification originally used as a measure of the level of *c*-variegation (Cattanach & Isaacson, 1965, 1967) the scores ranged from 2·28 to 2·96 and thus all fell within the range typical of the low 'state' of the controlling element (Cattanach & Isaacson, 1967). Seven partially inbred L_2 males were progeny-tested in addition to the outcross Dp males, and all yielded similar scores (see Cattanach & Perez, 1970). No change in the low 'state' of the element was thus detected among 60 tested males.

Table 3 summarizes the equivalent results of 70 H_2XT, H_3XT and H_4XT progeny-tests and in only seven cases was a score based on less than 15 classified daughters. Here it can be seen that two distinct groups of animals were present and this is illustrated in Fig. 1. In one group the scores ranged from 54 to 62% with most lying within the 57–60% level, and on the grading system the scores ranged from 3·19 to 3·80. All of these thus fell within the range typical of the high 'state' of the controlling element (Cattanach & Isaacson, 1967). The second group of Dp males yielded scores representative of animals carrying the low 'state' of the

element; they produced scores ranging from 42 to 49% and the mean score of the group did not differ significantly from that of the LXT males ($t_{1146} = 0\cdot62$; $P > 0\cdot05$). Several changes in the high 'state' of the element had thus occurred. In all, 15 exceptional males were found among 70 outcross animals tested. None were found among eight partially inbred Dp males that had been tested earlier (see Cattanach & Perez, 1970).

Table 2. *Progeny-test c-variegation scores of Dp males carrying an X^T derived from the low line*

Scores in brackets based on less than 15 classified daughters.

Line and generation	Family no.	Scores of individual males		
L_2XT	1	$45\cdot33 \pm 2\cdot69$		
	2	$44\cdot04 \pm 2\cdot60$	$41\cdot88 \pm 3\cdot76$	$42\cdot41 \pm 2\cdot09$
		$45\cdot77 \pm 2\cdot66$		
	3	$42\cdot81 \pm 2\cdot14$		
	6	$41\cdot94 \pm 3\cdot26$	$(43\cdot57 \pm 3\cdot73)$	
L_3XT	10	$43\cdot00 \pm 2\cdot33$	$42\cdot22 \pm 2\cdot54$	$45\cdot91 \pm 2\cdot69$
	14	$47\cdot50 \pm 2\cdot61$	$50\cdot26 \pm 2\cdot65$	$46\cdot67 \pm 2\cdot66$
	16	$42\cdot17 \pm 2\cdot13$	$44\cdot09 \pm 3\cdot33$	$46\cdot67 \pm 1\cdot97$
		$40\cdot95 \pm 2\cdot64$	$(41\cdot50 \pm 4\cdot28)$	
L_4XT	1	$41\cdot75 \pm 2\cdot55$	$48\cdot20 \pm 2\cdot14$	$47\cdot50 \pm 3\cdot21$
		$42\cdot00 \pm 2\cdot22$	$44\cdot37 \pm 2\cdot59$	
	3	$44\cdot50 \pm 2\cdot25$	$44\cdot21 \pm 2\cdot89$	$40\cdot48 \pm 3\cdot06$
		$45\cdot00 \pm 2\cdot42$	$47\cdot67 \pm 2\cdot92$	$43\cdot91 \pm 2\cdot29$
	4	$39\cdot44 \pm 2\cdot97$	$42\cdot80 \pm 2\cdot36$	$40\cdot26 \pm 3\cdot56$
		$45\cdot88 \pm 2\cdot58$	$45\cdot65 \pm 2\cdot50$	
	6	$43\cdot10 \pm 2\cdot81$		
	7	$40\cdot00 \pm 2\cdot70$	$45\cdot00 \pm 2\cdot20$	$45\cdot83 \pm 3\cdot27$
	9	$40\cdot28 \pm 2\cdot93$	$41\cdot00 \pm 2\cdot64$	
	10	$38\cdot43 \pm 3\cdot10$	$43\cdot54 \pm 1\cdot91$	$44\cdot29 \pm 1\cdot93$
		$42\cdot32 \pm 2\cdot91$		
	11	$43\cdot61 \pm 1\cdot93$	$48\cdot25 \pm 2\cdot70$	$43\cdot53 \pm 2\cdot26$
	13	$37\cdot95 \pm 1\cdot36$	$40\cdot00 \pm 3\cdot09$	$42\cdot00 \pm 1\cdot69$
	15	$42\cdot62 \pm 3\cdot21$	$40\cdot47 \pm 2\cdot88$	

In seeking an interpretation of the data, an additional piece of information can usefully be included. The heterozygous Dp females of both H and L lines carry the same normal X chromosome of the inbred (JU/Fa) in addition to their rearranged X (X^T), and it is known that the normal X carries the low 'state' of the controlling element (Cattanach *et al.* 1969). This was deduced from its influence upon the heterozygous phenotypes of *Ta* and *Vbr* in comparison with that of the high and low 'states' carried in X^T chromosomes. Of 6 animals tested, all produced scores in the test-crosses that were indistinguishable from those of Dp males carrying the low 'state' in their X^T chromosome and this result has since been confirmed in test-crosses of over 20 other males carrying the same normal X chromosome. The Dp females of the L line must therefore, be homozygous for the low 'state', i.e. $X^T - L/X - L$, whereas the equivalent H line females must be heterozygous, i.e.

$X^T - H/X - L$. It would therefore follow that changes in the high 'state' as detected by the appearance of Dp males giving low progeny-test scores, most likely occur by crossing over between the $T(1;X)Ct$ breakpoint and the locus of the controlling element. Changes in the low 'state' would not be expected, and none were in fact found. The crossover model thus provides an adequate explanation for the changes in the 'state' of the element detected in the present experiments.

Table 3. *Progeny-test c-variegation scores of Dp males carrying an X^T derived from the high line*

Scores in brackets based on less than 15 classified daughters. Those of exceptional animals are shown in italics.

Line and generation	Family no.	Scores of individual males		
H$_2$XT	1	57·71 ± 2·00	55·19 ± 2·24	
	2	60·21 ± 2·34		
	3	63·00 ± 2·04		
	5	55·63 ± 2·05	56·59 ± 2·82	
	7	58·95 ± 2·49	62·11 ± 2·49	59·32 ± 2·53
H$_3$XT	1	56·92 ± 2·66	57·00 ± 2·35	
	2	60·83 ± 3·46	59·05 ± 2·66	
	2A	59·93 ± 2·26	60·50 ± 2·99	55·40 ± 2·31
		56·36 ± 1·90	(61·00 ± 2·87)	
	3A	58·33 ± 3·03	57·04 ± 2·34	60·45 ± 2·15
		55·88 ± 3·13		
	4	(56·79 ± 3·87)	(60·45 ± 2·82)	60·88 ± 2·20
		58·33 ± 2·61	60·83 ± 2·74	57·89 ± 2·92
	5A	61·94 ± 2·33		
	6	57·25 ± 2·47	58·26 ± 2·51	55·22 ± 2·28
	6A	59·62 ± 2·09	59·13 ± 2·05	
	7	60·83 ± 2·65		
	7A	60·56 ± 1·85		
	9	(*46·67 ± 5·53*)	*48·00 ± 2·87*	*46·39 ± 3·66*
		45·40 ± 2·20		
	9A	*45·29 ± 1·93*		
	10A	57·83 ± 2·68	55·95 ± 2·00	
H$_4$XT	2	59·12 ± 2·65	(55·36 ± 2·25)	56·82 ± 2·34
	3	53·10 ± 3·28	59·55 ± 2·92	*44·17 ± 2·36*
		58·57 ± 2·72		
	6	59·78 ± 2·10	56·74 ± 2·55	52·50 ± 2·60
	11	54·00 ± 2·22		
	12	55·24 ± 2·68	59·71 ± 2·21	
	15	*43·50 ± 1·99*	*42·11 ± 3·18*	*42·50 ± 2·89*
	18	(57·73 ± 3.59)		
	20	*43·95 ± 3·12*		
	21	*42·39 ± 2·84*	*48·81 ± 2·26*	
	22	*45·29 ± 3·11*	*44·40 ± 2·74*	(*44·23 ± 2·65*)
	24	55·63 ± 2·36	60·00 ± 2·78	56·74 ± 2·32
		57·54 ± 2·35		

If the crossover model is the correct one, then it is clear that the number of changes that occured must be far fewer than the number of exceptional males detected. From the data presented in Table 3 is it apparent that most of the exceptional males occurred in whole family groups and this suggests that

changes in the 'state' had occurred in earlier generations. The validity of this conclusion is demonstrated by the pedigree analysis of families in which exceptional males were detected. Fig. 2, Case A, illustrates one such pedigree. Here, it can be seen that H_3XT families Nos. 9 and 9A and H_4XT families Nos. 20, 21, and 22 are all related. Since all the tested males in these families appeared to possess the changed 'state' it is unlikely that each represents an independent change. It is far more probable that they all trace back to a single change or crossover event in the H_1 female, and that the H_2 female was the original excep-
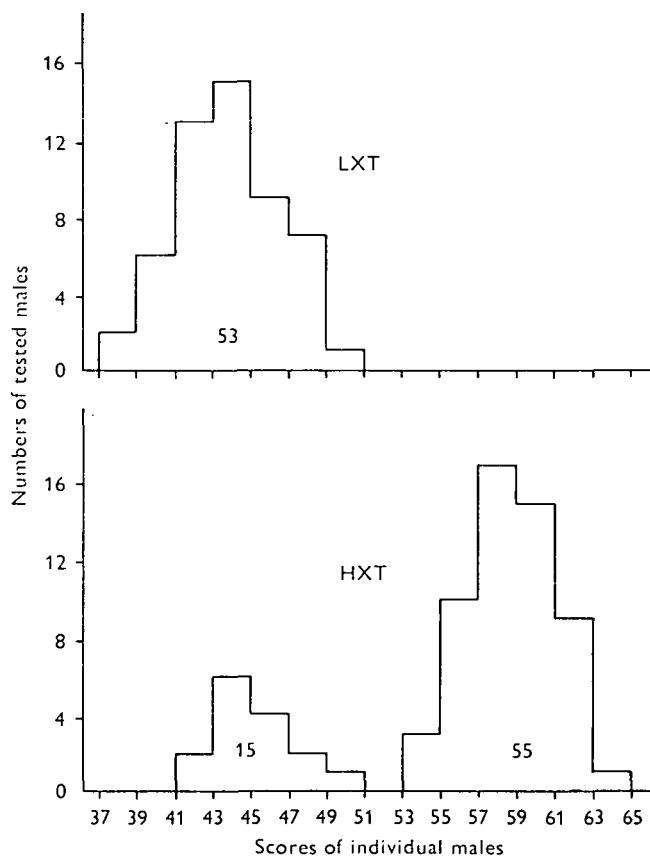

Fig. 1. Distribution of progeny test scores.

tional animal. The pedigree analysis of a second family in which several exceptional males were detected is also shown in Fig. 2, Case B. Here it can be seen that 3 H_4XT family No. 15 males all appeared to possess the changed 'state' but that no other exceptional relatives were found. It is therefore probable that all three males inherited the changed 'state' from the mother, either the mother or maternal grand-mother being the original exceptional animal. The pedigrees shown in Fig. 1 account for 14 of the 15 exceptional males; the remaining animal occurred in a family of 4 (H_4XT family No. 3) and therefore must represent a new and independent change. If the changes in the 'state' arose by crossing over as proposed, then

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the 16 exceptional animals detected indicate the occurrence of only three cross-over events.

On the crossover model, two of the three changes in the high 'state' of the element must have first appeared in Dp females rather than in Dp males. Therefore, in order to estimate the frequency of change it is necessary to include all animals tested, females as well as males, and for this purpose a female will be considered tested if she produced at least one progeny-tested son. For example, in the
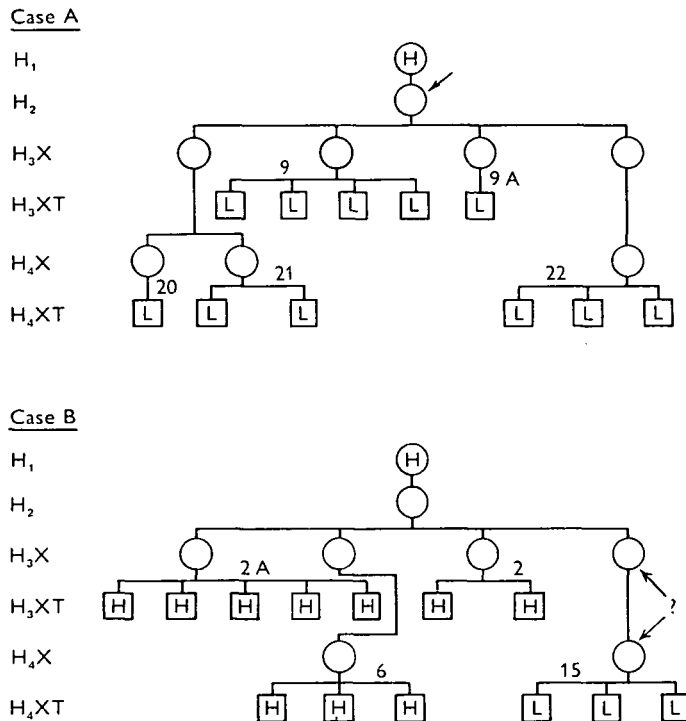

Fig. 2. Pedigree analysis of families in which changes in the 'state' of the element were detected. Circles represent females, squares represent males. Arrows indicate original exceptional animals.

pedigree shown in Fig. 2, Case B, there are 16 (or 17) tested animals, ten males and six (or seven) females; the three exceptional H_4XT males are not included since they only indicated a change in the mother (or grandmother). Similarly, in the pedigree shown in Fig. 2, Case A, there is only one animal, the H_2 female that has been tested for a high to low change. When all the data are evaluated in this manner, 108 animals can be said to be tested and among these three changes were found. The frequency of change is, therefore, 2·78% (lower and upper 95 % confidence limits, 0·57 and 8·54, respectively) and if the changes result from crossing over in the region between the $T(1;X)Ct$ breakpoint and the locus of the controlling element, this figure provides an estimate of the distance between the two. Similar treatment of the L line data showed that 94 animals had been tested. To

this can be added the number of changed H line animals tested for a further change, i.e. for the reconversion of the low 'state' back to the high (Fig. 2); the total is then 115 with no changes detected.

If, as postulated (Cattanach *et al.* 1969), one controlling element system is responsible for the modification of the level of *c*-variegation and also the levels of expression of the X-linked genes in heterozygous females, it would be expected that the three altered 'states' would be typed as low, not only in the progeny tests, but also in tests with X-linked genes. To investigate this, Dp males carrying the altered 'states' were subjected to the *Ta* test-cross (Cattanach *et al.* 1969), i.e. they

Table 4. *Influence of altered 'states' upon the* Ta *locus*

Each score represents the results of test-crosses upon changed Dp males.

| | Vibrissa score of progeny | | | |
| | $Ta/+$ ♀♀ | | Ta ♂♂ | |
Family and identity no.	No.	Score	No.	Score
Control H*	99	$15 \cdot 44 \pm 0 \cdot 18$	129	$7 \cdot 46 \pm 0 \cdot 14$
9. 1*e*	19	$16 \cdot 47 \pm 0 \cdot 30$	34	$7 \cdot 53 \pm 0 \cdot 25$
9. 4*f*	25	$16 \cdot 84 \pm 0 \cdot 18$	39	$6 \cdot 97 \pm 0 \cdot 19$
21.1*d*	25	$16 \cdot 44 \pm 0 \cdot 26$	39	$7 \cdot 26 \pm 0 \cdot 26$
Control L*	87	$16 \cdot 82 \pm 0 \cdot 14$	121	$7 \cdot 77 \pm 0 \cdot 15$
3. 2*h*†	20	$17 \cdot 65 \pm 0 \cdot 26$	34	$9 \cdot 09 \pm 0 \cdot 21$
15. 1*e*†	25	$17 \cdot 24 \pm 0 \cdot 18$	4	$8 \cdot 66 \pm 0 \cdot 44$
Control (1) L†	32	$17 \cdot 63 \pm 0 \cdot 12$	35	$8 \cdot 69 \pm 0 \cdot 26$
Control (2) L†	31	$17 \cdot 35 \pm 0 \cdot 24$	31	$8 \cdot 45 \pm 0 \cdot 33$

* Mean scores of males of the same generation, with Ta/Ta females of the same genetic background. (Data of Cattanach *et al.* 1969.)

† Scores obtained in test-crosses with Ta/Ta females of a different genetic background. Controls (1) and (2) are scores of individual males.

were mated to a series of Ta/Ta females and the mean vibrissa scores of their Dp $Ta^+/-Ta$ daughters determined. According to the hypothesis, when the low 'state' is present in the X^T the Ta^+ gene would be less frequently, or incompletely inactivated and hence the vibrissa number, reduced by Ta, would tend to be higher; when the high 'state' is present, inactivation would be more complete and the reduction in vibrissa number caused by the Ta allele would be more effective (see Table 1).

The results of the Ta test-crosses are presented in Table 4. They have been divided into two groups because they were obtained at different times and utilized Ta/Ta females of somewhat different genetic backgrounds. The second group of females had a higher proportion of the C_3H stock in their genome and perhaps for this reason the vibrissa scores of both the $Ta/+$ daughters and the Ta sons were higher. Controls for the first group of data are provided by the mean scores of several Dp males of similar genetic backgrounds and known to carry either the high or low 'state' (Cattanach *et al.* 1969). Controls for the second group are

provided by the mean scores of two males mated to the same group of Ta/Ta females and known to carry the low 'state' of the element.

The same conclusions can be drawn from the results of the test-crosses as from the progeny tests; the mean vibrissa scores of the $DpTa^+/-Ta$ daughters indicate that the exceptional males possessed the low 'state' of the controlling element. The scores of Ta sons were unaltered demonstrating that the female difference was not simply due to genetic background influences.

4. DISCUSSION

At the time the mouse X-chromosome controlling element system was first discovered (Cattanach & Isaacson, 1967), it was observed that the changed Dp males tended to occur in clusters within family groups and apparently independently of the normal X-chromosome present in the heterozygous female. For this reason it was thought that the changes in 'state' might occur by a process analogous to that of the 'changes in state' of the maize controlling elements (McClintock, 1950, 1965) but crossing over could not absolutely be ruled out as the responsible mechanism.
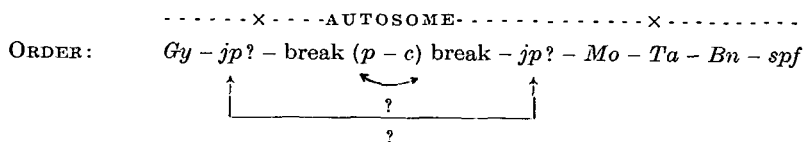
In the present experiments clustering was also found but since no unchanged Dp males were found in the affected families (Fig. 2, Case A and B) the data can most simply be explained by the occurrence of single changes in earlier generations. More at variance with the conclusions deduced from the earlier experiments was the finding that the changes did not appear to occur independently of the normal X, i.e. exceptional males were only found in the line carrying the high 'state' in the X^T. Admittedly, the observed frequency of independent changes in the H line (3/108) was so low that the absence of any changes in the L line (0/115) does not establish conclusively that such changes could not have occurred. However, the fact that the normal X of each line carries the low 'state' of the element establishes with near-certainty that the nature of the normal X is indeed important. Changes only occurred in females which were heterozygous for the 'state' of the controlling element and hence it is most likely that crossing over in the region of the X between the translocation breakpoint and the locus of the controlling element is the responsible mechanism of change. The fact that all three changed 'states' could be shown to be altered with respect to their influence upon the Ta locus confirms the validity of the argument.

In spite of the likelihood that crossing over is the mechanism responsible for the changes detected in the present experiments, it is still difficult to interpret the earlier data on this basis. For example, both changed and unchanged Dp males were detected in each of two generations in one pedigree and, at minimum, three changes must have occurred. This constitutes clear evidence of a clustering of new changes, a finding which cannot easily be reconciled with the low crossover frequency calculated from the results of the present experiments. It is therefore still possible that some of the changes in the 'state' of the mouse X-chromosome controlling element do occur by a process analogous to that of the 'changes in

state' of the maize controlling elements (McClintock, 1950, 1965). Perhaps the presence of a second element is required for maize-type changes. Such an element could have been present in the original selection lines in which the changes were first detected (Cattanach & Isaacson, 1967), but either was not introduced into the sublines along with X^T or was not maintained during the repeated backcrossing to the inbred.

Table 5. *Order of genes and breakpoint in X-chromosome linkage map*

Recombination frequencies from Cattanach (1966).

$$- - - - - - \times - - - - \text{AUTOSOME} - - - - - - - - - - - - - \times - - - - - - - - - - -$$

ORDER: $Gy - jp? - \text{break}\ (p - c)\ \text{break} - jp? - Mo - Ta - Bn - spf$

Recombination frequencies:
Break – *Gy*: approximately 20 %
        jp: < 0·5 %*
        Mo: 2·16% (lower and upper 95% confidence limits, 0·59 and 5·45, respectively)
        Ta: 4·11% (lower and upper 95% confidence limits, 1·90 and 7·66, respectively)
        Bn: approximately 20%
        spf: 50% ± 14%
Break – controlling element: 2·78% (lower and upper 95% confidence limits, 0·57 and 8·54, respectively)

    * Only one recombinant detected in over 200 progeny of $Dpjp^+/\cdot jp$ females.

By whatever mechanism or mechanisms the changes occur it is clear from the data presented that the locus of the controlling element must lie close to the translocation breakpoint and this is known to be located near the centre of the linkage group (Cattanach, 1966). The order of the genes and the breakpoint in the linkage map and the recombination frequency of each tested gene with the breakpoint is shown in Table 5 and it can be seen that the element must lie in or very close to the *Mo-Ta* region on one side of the autosomal insertion or in the *Gy-jp* region on the other. The decision as to which side of the insertion the element lies cannot be made on the basis of these data alone, but there is some justification for considering that it must be located on the same side as *Ta*. Kindred (personal communication) has found that there are X-linked modifiers of *Ta* which are closely linked to *Ta* and since this gene is one of those influenced by the controlling element system it is probable that Kindred's *Ta* modifiers represent the X-chromosome controlling element (Cattanach *et al.* 1969). In addition, M. F. Lyon (personal communication) has observed that the 'penetrance' of *Gy* in the female may change as a result of crossing over with other X chromosomes in the *Gy-Ta* region. This suggests another example of X-linked modification of an X-linked gene and again points to a region near *Ta* as being the location of the modifying locus, i.e. the controlling element. Linkage tests are now under-way to determine the precise location of the element. Should the proposed location be correct it will

be interesting to see if it maps close to the breakpoint of Searle's X-autosome trans-
location (Lyon *et al.* 1964). Females heterozygous for this translocation have the
rearranged X active in almost all their cells and since the controlling element is
considered to be responsible for the inactivation of the X chromosome (Cattanch
et al. 1969) a break at or near the locus of the element may impair its function.

Russell (1963, 1964) has proposed that there is a spread of inactivation along
the X, as along associated autosomal regions in the X-autosome translocations,
the origin being the postulated inactivation centre (Grumbach, 1964; Lyon, 1964;
Russell, 1964), and she has concluded that in X chromosomes split by rearrange-
ment only the part bearing the centre undergoes the inactivation process. If
this were true, only one part of the X in the *flecked* translocation would be inacti-
vated for the 'spreading effect' (Cattanach, 1961, 1963) indicates that there is
only a limited spread of inactivation across the length of autosomal material
inserted into the X. Both *Mo* and *Ta* lie on one side of the insertion and when
either mutant allele is present in the heterozygous condition with the translocation,
the characteristic 'variegated' phenotype of the heterozygote may be observed
(Cattanach, 1966). This indicates that the *Mo-Ta* region is subject to inactivation
and hence by Russell's hypothesis the inactivation centre, i.e. the controlling
element, would again be placed on this side of the insertion. However, although the
hypothesis provides a pleasingly simple model for X-chromosome inactivation,
there is yet no evidence of a spread of inactivation along the X or that any X-
linked gene in either rearranged or normal chromosomes is free of the inactivation
process (Lyon, 1966) and the concept that X-chromosome regions isolated from
the inactivation centre are not inactivated is based on the behaviour of a single
autosomal gene in one X-autosome translocation. Perhaps the latter point is
correct for reciprocal translocations but with the insertional rearrangement,
$T(1;X)Ct$, observations on *pink-eye* (p)-variegation (Eicher, personal communi-
cation) and *ruby-eye-2* ($ru-2$)-variegation (B. M. Cattanach, unpublished; Eicher,
personal communication) suggest that there is spread of inactivation into the $ru-2$
side of the insertion as well as into the c side and this would indicate that both
parts of the split X are subject to inactivation.

These observations are clearly at variance with Russell's (1964) hypothesis
that there is spread of inactivation along the X from a single inactivation centre.
Either there must be a second inactivation centre (at minimum) on the Gy side
of the insertion (Cattanach & Isaacson, 1965) or inactivation of the X is not
accomplished by any kind of spread from the centre. The fact that the low 'state'
of the controlling element permits 'incomplete' inactivation of Ta and Vbr, two
genes which are located centrally in the linkage group and very close to the con-
trolling element, is difficult to reconcile with a spread of inactivation (Cattanach
et al. 1969) and this is especially true since more remote loci can still be shown to
undergo the inactivation process (Lyon, 1966). It would therefore seem that
each locus, or perhaps inactivators scattered over the length of the X (Lyon, 1968)
respond independently to the controlling element (inactivation centre) whether or
not separated from the centre by autosomal material. If this is so, the spread of

inactivation into the *ru-2* side of the insertion should be influenced by the controlling element system just as the spread into the *c* side and this is being investigated.

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