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Sex-linked variegation modified by selection in brindled mice

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SUMMARY

The sex-linked gene, *brindled*, in the mouse produces a coat-colour variegation in heterozygous females. There is much individual variation in the relative areas of mutant and wild-type colour, but it was not known if any of this variation was genetic. The main object, when the experiments were started, was to test the simple expectation of the Lyon hypothesis, that if *X*-inactivation is random the variegation should not be modifiable by selection. On the assumption that the variegation is due to *X*-chromosome inactivation, modification by selection would show that the inactivation process, or some property of the derived cell populations, is under genetic control. Heterozygous females were accordingly selected for the area of coat showing the mutant colour. Selection based on individual phenotypes was ineffective, but four cycles of reciprocal recurrent selection based on progeny-means produced a 'High' line with 64% mutant area and a 'Low' line with 30% mutant area, from a base population with 53% mutant area. Autosomal modifiers were not responsible for the response; the difference between the selected lines was entirely due to properties of the *X* chromosomes carrying the *brindled* gene. The changed properties of the *X* chromosomes were not restricted to the locus of *brindled*, but extended at least as far as the locus of *tabby*. The chromosomes carrying the wild-type allele of *brindled* were not altered by the selection, but normal *X* chromosomes from other strains affected the degree of variegation. It was concluded that the difference between the selected lines was due either to non-random inactivation or to somatic cell selection. It was not possible to distinguish between these two mechanisms. The results obtained in these experiments with a structurally normal *X* chromosome were in all essentials similar to those obtained by Cattanach with his *X*-autosome translocation.

1. INTRODUCTION

The hypothesis of *X*-chromosome inactivation in female mammals (Lyon, 1961; Russell, 1961) has received much support from subsequent work. (For reviews, see Lyon, 1968, 1970, 1972.) An expected consequence of the random and permanent inactivation of one or other *X* chromosome is that expression of a sex linked gene giving a variegated phenotype in heterozygotes should not be modifiable by selection, as pointed out by Grüneberg (1966). It was primarily to test this expectation that the experiment described here was started. After selection had

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been proved to be effective in modifying the phenotype, various tests were made with the object of finding out what had been the genetic basis of the response to selection.

Selection has been applied to sex-linked variegation in two previous experiments with mice, and a third published after the present work was completed. Selection on the effects of *tabby* (*Ta*) was successful in modifying the expression in heterozygous females (Dun & Fraser, 1959). There is evidence, however, that the expression of *Ta* can be influenced by autosomal modifiers (Sofaer, 1969), and that the response to selection was attributable to autosomal genes rather than to any effect on *X*-chromosome inactivation (Kindred, 1967*a*). Furthermore, the variegation caused by the *tabby* gene is complicated and does not conform to the expectation of the Lyon hypothesis in several respects (Grüneberg, 1966; Kindred, 1967*b*). For these reasons the experiments with *tabby* are not relevant to the problem of random inactivation.

The second experiment concerns the *flecked* translocation, *T*(1;*X*)*Ct* (Cattanach, 1961). The structurally abnormal *X* chromosome (X^T) has a large part of the autosome of linkage group I inserted into it, carrying the wild-type allele of *albino* (*c*) as well as those of several other known loci. A heterozygote, X/X^T, that has the mutant allele, *c*, on its normal autosomes shows variegation of *albino* and wild-type; areas in which the normal *X* is active are *albino* and areas in which X^T is active are wild-type. Selection for the expression of the variegation was successful in producting two lines with different proportions of *albino* in the variegation (Cattanach & Isaacson, 1965). Subsequent work showed that the different proportions of *albino* in the two lines was attributable to a 'controlling element', *Xce*, on the X^T chromosome close to the break-point and to the location of *Ta* (Cattanach, Perez & Pollard, 1970). Autosomal genes translocated to an *X* chromosome, however, are not always inactivated when the *X* chromosome carrying them is inactivated (Cattanach, 1961; Russell, 1963; Russell & Montgomery, 1970). Consequently the changes in the *flecked* chromosomes brought about by selection could have been connected in some way with the structural abnormality. The main reasons for doing the present experiment were to see if a structurally normal chromosome would respond to selection in the same way, and if a similar genetic mechanism was involved.

The third experiment (Krzanowska & Wabik, 1971) was done with a sex-linked gene *mosaic*, *Ms* (Radochonska, 1970), which produces a variegated phenotype very like that of *brindled*, with which the present experiments were done. Selection of heterozygous females was successful in separating lines with high and low proportions of mutant coat. This experiment establishes the fact that the variegation due to a structurally normal chromosome can be altered by selection.

Random and permanent inactivation leads to the expectation of a 'random phenotype' of heterozygous females, in which on average 50 percent of cells are of mutant phenotype and 50 percent wild-type, with individual variation following the binomial distribution corresponding to the number of primordial cells at the time of inactivation. There are several ways by which this random phenotype might

be altered. Here we need only be concerned with the two simplest, namely non-random inactivation, whereby one chromosome is inactivated more often than the other, and somatic cell selection, whereby the clones of the two cell-types after inactivation proliferate at different rates. The selection was planned with the idea of producing non-random inactivation and it will be described in these terms. It will be obvious, however, that most of what is said about non-random inactivation could equally well apply to differential cell selection.

Our work started with a simple procedure of individual selection which, unlike that of Krzanowska & Wabik (1971), failed to produce any significant change in the expression of a variegated phenotype. It was then realized that a special, and rather complicated, procedure might be needed to bring pressure to bear on differential inactivation or somatic cell selection. The main experiment was therefore the application of this special method of selection. We have given a short preliminary account of the main experiment in an abstract (Falconer & Isaacson, 1969).

2. MATERIAL AND METHODS

The gene used in these experiments was *brindled*, Mo^{br} (Fraser, Sobey & Spicer, 1953; Falconer, 1953). For simplicity, the symbol *Br* will be used throughout this paper. Heterozygous females have a variegated phenotype, seen in the pigmentation of the coat. There are patches of nearly white hair, like the phenotype of hemizygous *brindled* males, interspersed with patches of wild-type hair. Individuals vary widely in the relative amount of mutant hair in the coat; examples of the range of variation encountered are shown in Plate 1. *Brindled* males nearly all die at about 2 weeks of age and consequently the *brindled* gene can usually be transmitted only through heterozygous females. Though the most obvious effect of the gene is in the pigmentation of the hairs, the site of gene action is probably not in the melanocytes, because the skin is pigmented, and because the structure of the hairs is abnormal (Grüneberg, 1969). The pigmentary defect is probably the result of an abnormality of the hair follicles and it is the action of the gene in these cells that is relevant to the variegated phenotype. The fact that the gene is lethal in males shows, however, that there must be a more general effect and that hair-follicle cells are not the only cells affected. Some biochemical abnormalities have been found that suggest a neuroendocrine defect (Hunt & Johnson, 1972).

(i) *Classification of phenotype*

The relative amount of mutant skin was assessed visually. At first, the classification was made in five grades, illustrated in Plate 1. Later, a finer division of the classes was found to be desirable and classification was made by the estimated percentage of mutant skin in steps of 5%; thus each grade was subdivided into four classes on the percentage scale. The correspondence between grades and percentages is shown in Plate 1. The mice were classified between the ages of 6 and 8 weeks. All the classifications were made by one of us (J.H.I.), except for one generation of the main experiment, when they were made by Miss H. Macrae. The

mean of this generation was adjusted on the basis of the classification of the next generation, which was made by both observers.

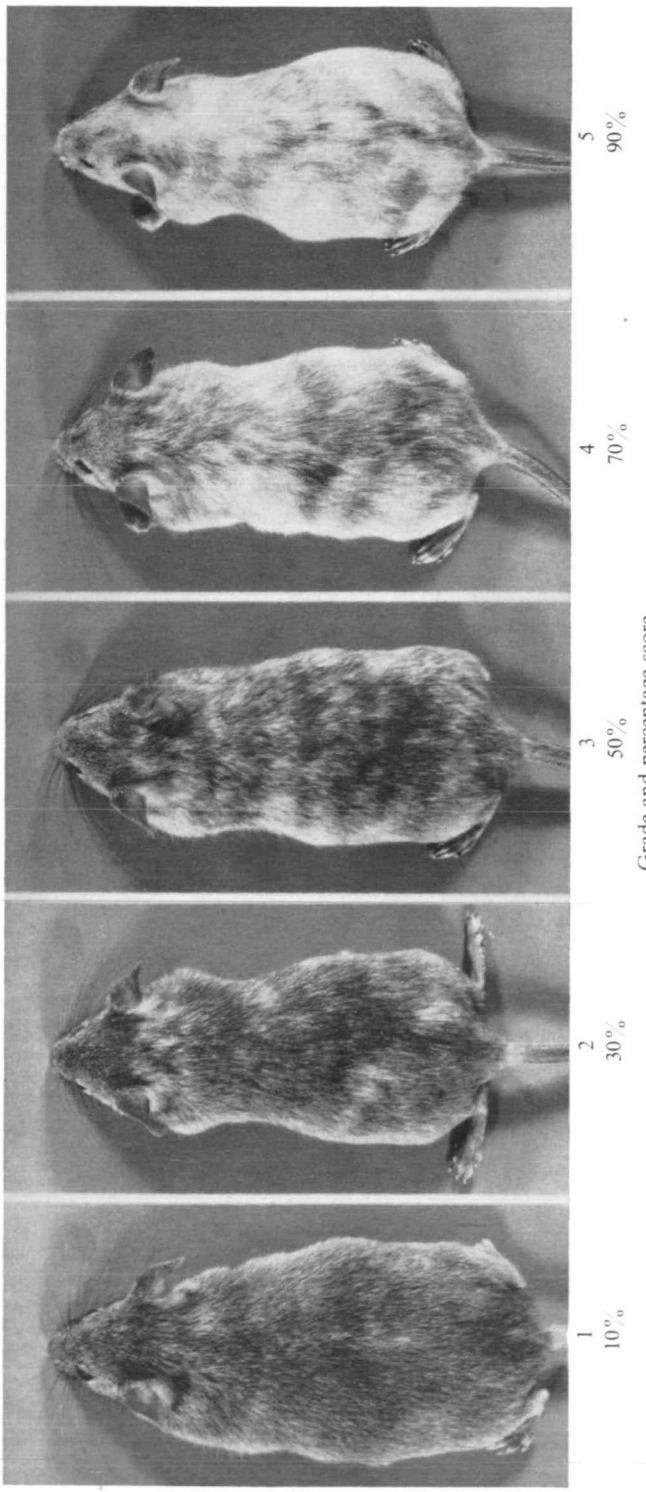
Visual classification in this way cannot be completely accurate, and in particular the percentage classification probably does not correspond very closely with the real percentage of mutant skin. An investigation of the correspondence between estimated and actual areas in the similar classification of the variegation of Cattanach's translocation (Cattanach & Isaacson, 1967) showed that the estimated areas were about 20 percentage units below the real areas over most of the range, though the correspondence was better at low values and the estimates were correct at values below about 20%. In order to estimate the reliability of the classification, two groups of mice were classified twice at an interval of 1 week. The correlation between the scores of the same animal, i.e. the repeatability, was 0·92 in one group of 33 mice and 0·95 in the other group of 44 mice. Thus, no more than about 5–8 percent of the variance was due to errors of estimation. In the two groups combined, 42 percent of mice had the same score on both occasions, 48 percent differed by one class-interval (i.e. 5% in the score) and 10 percent differed by two class-intervals (10%).

(ii) *Origin of stock*

The *brindled* gene came from a stock of mixed ancestry. *Brindled* females of this stock were crossed to *CBA* and *RIII* inbred males, and the *Br* female progeny were mated to *CBA* males. The progeny of these matings formed the foundation population of the first selection experiment. The background genotype for coat colour was wild-type (*agouti*), with *brown* (*b*) and *albino* (*c*) present at low frequencies. The construction of the foundation population can be seen in retrospect to have been far from ideal for the purpose. More details of the ancestry will be given later, when they become relevant to the conclusions to be drawn from the selection.

3. INDIVIDUAL SELECTION

Brindled females were classified in grades, and selected on the basis of their individual value without regard to their family. Wild-type males were taken, as far as possible, one from each family. Matings were made with least relationship. All matings were of necessity $Br/+ \, ♀ \times + \, ♂$. There were six matings in the foundation generation, from which high and low lines were established, each with six matings. The number of matings was increased to ten in the third and fourth selected generations, and the experiment was terminated after the fourth generation of selection. The results are given in Table 1. The high and low lines differed from each other in the direction expected from the selection, but the difference was small and non-significant. There was, however, a significant correlation between offspring and parents, when the data from all generations in both lines were combined. The mean grades of daughters grouped by the grade of their mothers are shown in Table 2. The regression of daughters on mothers was $0{\cdot}14 \pm 0{\cdot}045$, which is significant at $P = 0{\cdot}001$. The failure to modify the sex-linked variegation by selection contrasts with common experience of autosomal genes with variable



Variation in the degree of expression of *brindled* in heterozygotes. Representatives of the grades and percentages used for classification.

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Table 1. *Selection for individual values: mean grades of selected parents and their offspring*

(Numbers of offspring in parentheses. High line selected for increased, low line for decreased, amount of mutant coat.)

	Low line		High line		
Generation	Parents	Offspring	Parents	Offspring	Difference ($H-L$)
0	—	3·08 (12)	–	3·08 (12)	—
1	2·7	3·07 (45)	3·5	2·88 (49)	−0·19
2	2·2	2·78 (32)	3·3	3·03 (31)	+0·25
3	1·9	2·90 (63)	3·5	3·23 (52)	+0·33
4	1·7	3·07 (40)	4·0	3·32 (28)	+0·25

Table 2. *Mean grade of daughters classified by grade of mother over all generations of selection for individual values*

	Mean grade of daughters	
Grade of mothers	Low line	High line
5	—	$3·83 \pm 0·307$
4	—	$3·16 \pm 0·093$
3	$3·06 \pm 0·106$	$2·99 \pm 0·085$
2	$3·00 \pm 0·095$	—
1	$2·74 \pm 0·128$	—

Regression of daughters on mothers: $b = 0·140 \pm 0·045$.

expression, which are usually easily modified by selection. The difference between the results of this experiment and that of Krzanowska & Wabik (1971) will be discussed later. The failure of our individual selection to change the variegation led to the realization that individual selection does not fully test the possibility of modifying random inactivation or somatic cell selection. The reasons for this will be explained in the following section.

4. RECIPROCAL RECURRENT SELECTION

(i) *Theory*

Randomness of inactivation means equal probability of one or the other X chromosome being inactivated. If there is preferential, or non-random, inactivation, this must be a joint attribute of the two X chromosomes, since if one is more likely to be inactivated, the other is necessarily less likely. One can perhaps think of the interaction between the two X chromosomes as being some form of 'competition'. We shall discuss this in terms of competition for being active, rather than for being inactivated, or alternatively for faster proliferation.

Suppose there are two different X chromosomes, one (X^H) having a high competitive ability relative to the other (X^L) with a low competitive ability. Then the heterozygote, X^H/X^L, will have a non-random phenotype with a preponder-

Table 3. *Expected phenotypes in combinations of chromosomes with high or low 'competitive abilities'*

Genotype:	$\frac{BrL}{+H}$	$\frac{BrL}{+L}$	$\frac{BrH}{+H}$	$\frac{BrH}{+L}$
Proportion of *Br* in phenotype:	Low	Intermediate (= 'random')		High

ance of tissue in which the X^H chromosome is active and the X^L inactive. The two homozygotes, X^H/X^H and X^L/X^L, however, will have random phenotypes, since the two chromosomes in each are of equal competitive ability. Table 3 shows the expected phenotypes when *brindled* is associated with high and with low competitive abilities of the X chromosomes carrying it. The symbols *Br* and + indicate the chromosomes carrying the *brindled* gene and the wild-type allele respectively; the associated symbols, H or L, designate these chromosomes as having high or low competitive abilities.

Selection for a non-random phenotype, i.e. for high or low expression of *Br* in the variegation, will result in the selection of heterozygotes for chromosomes with different competitive abilities. Thus, for example, selection of individuals with a high proportion of *Br* coat will select simultaneously *BrH* and $+L$ chromosomes. If the competitive ability is determined by some part of the chromosome itself, crossing-over will take place in the oogenesis of the selected individuals, and the effect of the selection will be lost in the progeny unless the controlling loci are close to *Br*. Therefore if selection is to act effectively on the inactivation process, or on cell selection, it will be necessary to preserve the X^H and X^L chromosomes separately, and prevent their properties from being continually disrupted by crossing over in heterozygotes. This can be done by reciprocal recurrent selection, as described below.

(ii) *Procedure*

The object was to make two lines, one of which would contain both *BrH* and $+H$ chromosomes (to be called the High line) and the other both *BrL* and $+L$ chromosomes (the Low line). This was done as follows. The base population consisted of all the parents and their progeny in the last generation of the individual selection described above. There were altogether 17 families, 8 from the high line and 9 from the low. The mean phenotype of the *brindled* daughters in each family was calculated, and used as a test of the genotypes of the parents. A family with a high mean (i.e. a high proportion of *Br* in the variegation) indicated a genotype $BrH/+L$ for the individuals in the family. This meant that the mother transmitted a *BrH* chromosome and the father a $+L$ chromosome. (The + chromosome of the mother was untested except in so far as it had contributed, by recombination, to the H property of the *BrH* chromosome transmitted.) Thus the mother was selected to be a parent of the High line, to which it contributed a *BrH* chromosome, and the father was selected to be a parent of the Low line to which it contributed a $+L$ chromosome. In the same way a family with a low mean identified a $BrL/+$ mother

for use as a Low line parent and a $+H$ father for use as a High line parent. The parents of the families with the highest and the lowest means were therefore remated to make 'line' matings which had been subjected to one generation of selection. Sixteen of the seventeen available families were selected as high or low, so that eight matings were made in each line. The progeny of the line-matings were expected to have more or less intermediate phenotypes, as shown in Table 3. Therefore the effect of the selection was not expected to show in a difference between the High line and the Low line progeny.

To see whether the selection had been effective it was necessary to cross the lines reciprocally. High ♀ by Low ♂ would give $BrH/+L$ daughters with a high phenotype, and the reciprocal would give $BrL/+H$ daughters with a low phenotype. The reciprocal crosses served also as test-matings for the second cycle of selection, which was made in the same way as the first selection described above. Thus the cycle of selection consisted of (i) crosses, made reciprocally, between the lines to assess progress and to provide test-progeny for selection of the parents, (ii) selection of the best parents on the basis of their cross-bred progeny, (iii) remating the selected parents within their own lines, the progeny of these line-matings providing the animals for crossing in stage (i) of the next cycle.

Selection by this procedure was carried through four cycles. The numbers of line-matings made in each line were 8 in the first generation, as already mentioned, 11 in the second and 13 in the third and fourth generations. All the available *brindled* females in the progeny of the line matings were used in cross-matings; the numbers of females of each line that were cross-mated varied between 20 and 26. Thus about 50 percent of tested parents were selected for remating in lines. After the fourth cycle of selection, line-matings were made simultaneously with cross-matings in order to obtain a more reliable comparison between the lines and the crosses. Thereafter, from generation 6 to 12, no crosses were made, but the lines were continued by ten matings each, with selection of the best females from each family.

(iii) *Results*

Fig. 1 shows the results of the selection. The selection was clearly effective; but, contrary to expectation, it was almost as effective in differentiating the lines as the crosses. The low crosses were, in fact, lower than the Low line in all four generations, and the high cross was higher than the High line in the first generation. These differences are in the expected direction, but are not significant. Generation 5 provides the most reliable comparison of the lines with the crosses because they were reared and scored contemporaneously. The means are given in Table 4, and the distributions of the scores are shown in Fig. 2. The distributions of the Low line and cross show a marked bimodality, which was found also in the later generations.

The fact that the crosses were not intermediate between the lines proves that autosomal genes played no part in the response to selection, from which it can be concluded that the variegation of *brindled* is not subject to autosomal modifiers.

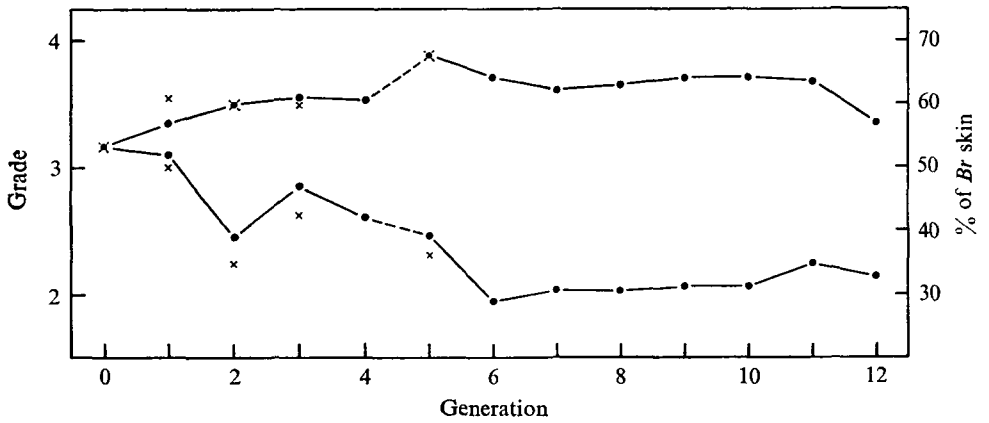

Fig. 1. Mean grades or percentage scores in successive generations. Dots are the progeny of line-matings and crosses are cross-bred progeny. Up to generation 4, all the points are plotted against the number of generations of selection to which the parents had been subjected. At generation 5 the lines and crosses were unselected and were reared and scored contemporaneously. Up to generation 4 classification was by grades, shown on the left-hand scale; from generation 5 on, classification was by percentage of *Br* skin, as shown on the right-hand scale.

Table 4. *Mean percentage scores in generation 5, i.e. after four generations of reciprocal recurrent selection*

	Low line	Cross $L♀ \times H♂$	High line	Cross $H♀ \times L♂$
No. scored	56	71	59	68
Mean	39·3	36·2	67·7	67·6
S.E.	2·08	2·11	1·59	1·44

The fact that the crosses closely resembled the lines from which the *brindled* female parent came proves that the difference between the lines was either a maternal effect or was due to changes in the *X* chromosome carrying *Br*; the male parent, or the normal *X* chromosome that he transmitted, had no effect on the variegation. The two alternatives – a maternal effect or changes in the *Br* chromosome – cannot be distinguished without further tests because *Br* was always introduced by the female parent.

After the reciprocal recurrent selection was discontinued the lines maintained their levels with very little change, at least up to generation 10. Individual selection within families was applied from generation 5 onwards, and it produced no response at all, which confirms the conclusion from the first experiment, described in the previous section. The mean percentage scores of generations 6–10 were $63 \pm 0{\cdot}8$ % in the High line and $30 \pm 1{\cdot}1$ % in the Low line.

The distributions of the scores are shown in Fig. 3. The Low line again shows the bimodality seen in generation 5. No explanation of the bimodality was found. The difference between the high and low modal groups was not transmitted to their daughters. It was therefore not attributable to the segregation of two different

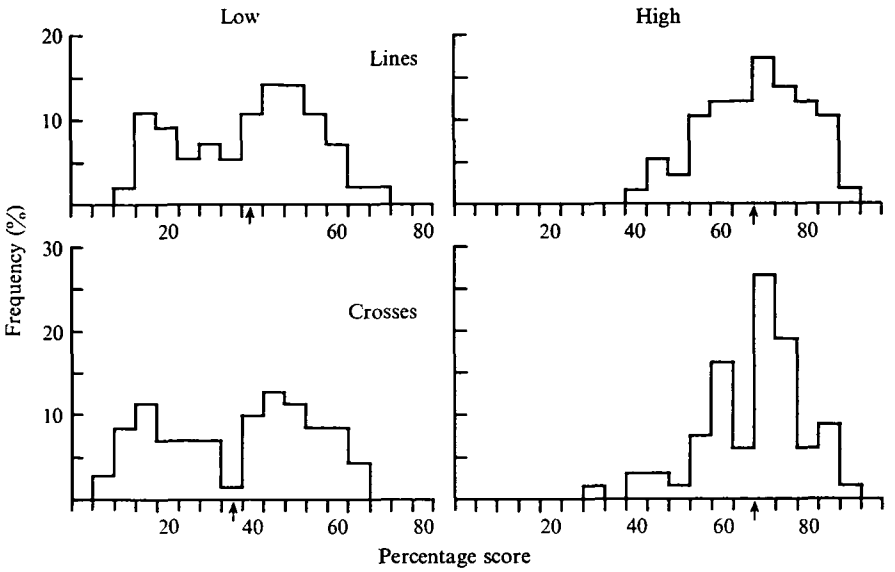

Fig. 2. Distributions of individual scores of lines and crosses in generation 5. The arrows mark the means.

BrL chromosomes. It could not have been due, either, to different $+L$ chromosomes because it did not show in the cross of Low-line males to High-line females.

5. TESTS OF THE SELECTED CHROMOSOMES

The selection described in the previous section produced lines with a substantial difference in the degree of variegation, and the crosses between the lines proved that the degree of variegation characteristic of each line was transmitted, along with the *Br* gene, by the female parent. The experiments now to be described were aimed at finding out more precisely what had been changed by the selection. The first question to be answered was whether the difference between the lines was a maternal effect or was due to changed properties of the X chromosome carrying *Br*. In other words, does the mother impart some quality to the chromosome she transmits that makes it more often, or less often, active than the chromosome transmitted by the father, or is the difference inherent in the chromosome itself, irrespective of which parent transmits it? This question can be partially answered by seeing if high and low females transmit normal (i.e. non-*brindled*) chromosomes with different activities. The second question, which is related to the first, was whether the normal chromosomes had really been unaffected by the selection, as appeared from the comparisons of the lines and the crosses. The third question, also concerning non-*brindled* chromosomes, was whether normal X chromosomes from other strains had any effect on the variegation when combined with the *Br* chromosome from the two selected lines. The final question was whether the difference between the two lines was confined to the *brindled* locus or whether it

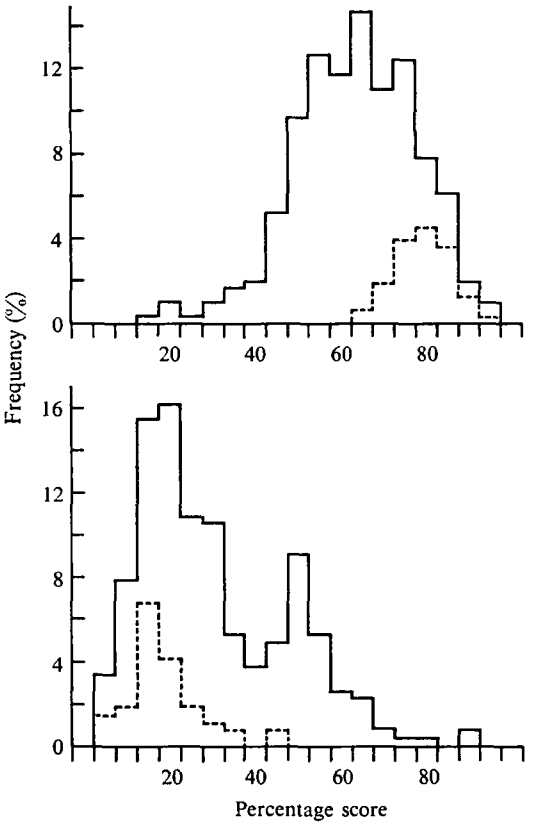

Fig. 3. Distributions of individual scores of High line (above) and Low line (below) in generations 6–10 combined. Numbers of individuals: 309 High, 265 Low. The dotted distributions are the individuals used as parents.

Table 5. *Tests of normal chromosomes by crosses with* blotchy (Blo)

(Mean percentage scores of *Blo*-variegation with standard errors, and numbers of animals in parentheses.)

	Origin of + chromosome	
Parents	Low line	High line
$+/+$ ♀ × *Blo*♂	57 ± 2·3 (44)	53 ± 2·5 (44)
Blo/*Blo* ♀ × +♂	59 ± 2·5 (23)	57 ± 3·6 (11)
Combined	58 ± 1·7 (67)	54 ± 2·1 (55)
Sex-difference (+♂ − *Blo*♂):		3·1 ± 2·6
Line-difference (L − H):		3·4 ± 2·7

affected neighbouring loci on the *X* chromosome: in other words was it a property of the locus or of the chromosome.

(i) *Crosses with* blotchy

Blotchy (*Blo*) is a sex-linked gene with a variegated phenotype (Grahn, Fry & Hamilton, 1969). It is located very close to *Br* and is almost certainly allelic (Lyon, personal communication). It differs from *Br* in that males are viable and fertile. It was therefore possible to use *Blo* to compare the normal *X* chromosomes from the two lines, and to see whether there was any difference in their effects when transmitted by males or by females. *Blo* males were crossed with normal females from the two lines, and *Blo*/*Blo* females with normal males from the two lines. The *Blo*/+ daughters were scored for the amount of variegation in the same way as the *brindled* variegation was scored. A high *Blo* score means low activity of the + chromosome tested, and a low score means high activity of the + chromosome. The pattern of variegation is somewhat different from that of *Br*, being more diffuse. The percentage scores are probably less reliable, and are not directly comparable with those of *Br*. We are, however, only concerned with comparisons between *Blo* scores in different groups, and there is no reason to think that these are unreliable. The results are given in Table 5. The differences between the groups are small and non-significant. There is no evidence that the + chromosome differs according to whether it is transmitted by the mother or the father. Thus there is no support for a maternal effect being the cause of the difference between the selected lines. This conclusion, however, will have to be qualified later, when the results of another experiment are presented. The comparison of the + chromosomes from the two lines confirms the conclusion that the + chromosomes were very little, if at all, affected by the selection: the difference between them, though again in the expected direction, was far from significant.

(ii) *Crosses with* tabby

Tabby (*Ta*) is linked with *Br* at a distance of 4 % (Falconer, 1953). It causes abnormalities of coat structure including the lack of some of the facial vibrissae (Dun & Fraser, 1959). Heterozygous females also lack some vibrissae, but to a lesser extent than hemizygotes or homozygotes, and the number of missing vibrissae provides a convenient means of quantifying the degree of expression in heterozygotes. The expression, measured in this way, has been shown to be influenced by the 'controlling element', *Xce* (Cattanach, Pollard & Perez, 1969) and so is at least in part related to sex-linked variegation. In order to find out if *Ta* would be differentially affected by the *X* chromosomes from the selected lines, *Ta* males were mated to *Br*/+ females from both lines. Each male was mated to one High line and one Low line female. This was done contemporaneously with generation 10 of the lines. The expression of *Ta* in the female progeny was scored by the number of vibrissae that were missing out of a total of 13 sites examined (supra-orbitals, postorbitals, postorals and interramals). All the female progeny were, of course, heterozygous for *Ta*, half of them in combination with the *Br* chromosome and

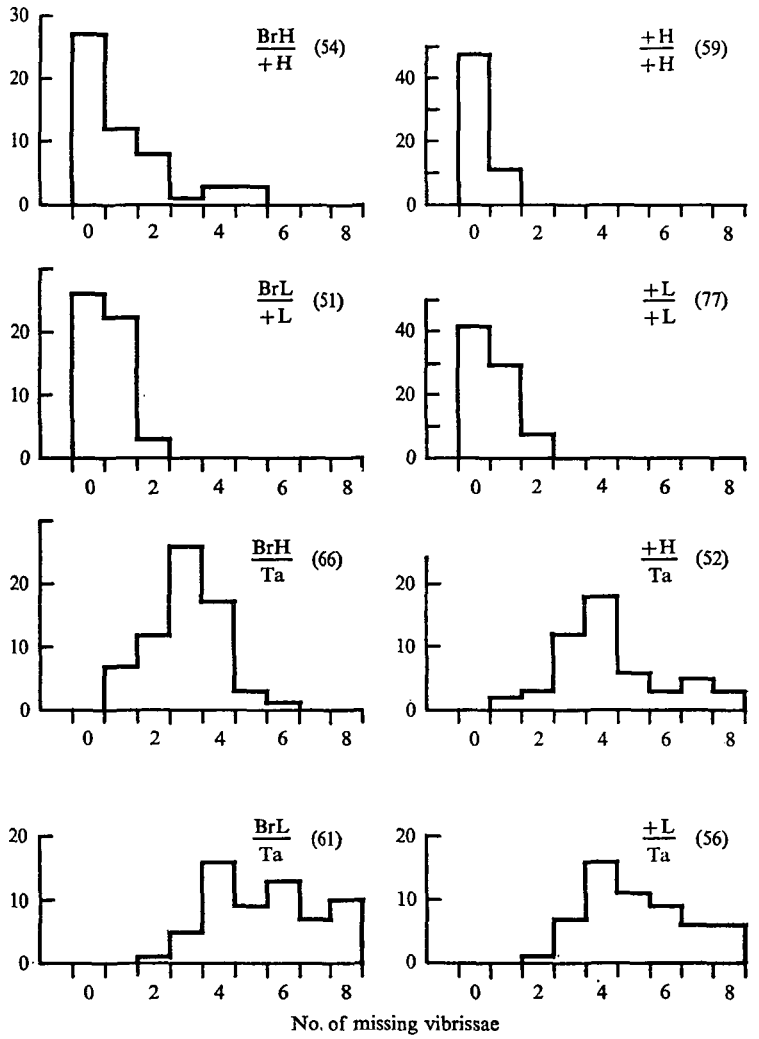

Fig. 4. Distributions of numbers of missing vibrissae. The vertical scales are the numbers of mice. The total number of mice in each group is shown in parentheses.

half with the + chromosome from the lines. Both *brindled* and non-*brindled* progeny were scored in order to assess the effects of the *Br* and of the normal chromosomes from the lines. A large number of missing vibrissae means high expression of *Ta*, which in turn means a low activity of the line-chromosome 'competing' with the *Ta* chromosome. The interpretation of the results was slightly complicated by the facts that *Br* itself results in some vibrissae being absent, and that occasionally even normal mice have a few missing. It was therefore necessary to score individuals of the lines for missing vibrissae. The results of all the vibrissa counts are given in the form of frequency distributions in Fig. 4.

Consider first the lines themselves, without *tabby*. The non-*brindled* mice had some missing vibrissae and the lines differed significantly. In the High line 19 per-

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cent of mice had one missing, and in the Low line 47 percent had one or two missing. ($\chi^2_{(1)} = 11{\cdot}7$; $P < 0{\cdot}001$). This difference is presumably an effect of the autosomal background on the development of vibrissae. The *brindled* mice of the Low line did not differ from the non-*brindled*; 49 percent had one or two missing. The *brindled* mice of the High line, however, were more strongly affected; 50 percent had between 1 and 5 missing. This reflects the high expression of the *brindled* phenotype in the High line. Consider now the crosses with *tabby*. The main question at issue is answered by the comparison of the *brindled* progeny of the two crosses, i.e. of the cross with the High line and the cross with the Low line. These are the two lower distributions on the left. They clearly differ in the number of missing vibrissae, and in the expected direction. *Ta* in 'competition' with *BrH* has a mean $3{\cdot}0 \pm 0{\cdot}13$ missing, and in competition with *BrL* has $5{\cdot}5 \pm 0{\cdot}21$ missing. The difference is highly significant, being nearly 10 times its standard error ($t_{(125)} = 9{\cdot}8$; $P < 0{\cdot}001$). The effects of the *Br* gene itself noted above will have tended to give a difference in the opposite direction – more missing with *BrH* than with *BrL*. The conclusion is therefore not affected by this complication. The meaning of the difference observed is that the normal allele of *Ta* is more active in the *BrH* chromosome than in the *BrL*. Thus the difference between the selected lines is not confined to the *Br*-locus, but extends to neighbouring loci at least as far as *Ta*. Selection has therefore affected the *X* chromosome as a whole, or at least a region of it, and not just the *Br* gene. In this respect the genetic basis of the change made by selection has been the same as with the *flecked* translocation (Cattanach *et al.* 1969).

The two lower distributions on the right of Fig. 4 compare the $+H$ and $+L$ chromosomes. The $+H$ chromosome results in fewer missing vibrissae than the $+L$ chromosome, indicating a higher activity of $+H$ than of $+L$ when in competition with the *Ta* chromosome. The mean numbers of missing vibrissae were $4{\cdot}3 \pm 0{\cdot}24$ and $5{\cdot}1 \pm 0{\cdot}21$. The difference is significant at $P = 0{\cdot}02$ ($t_{(106)} = 2{\cdot}57$). In this case, however, the difference of background between the lines is working in the same direction and the difference between the crosses, though perhaps suggestive again of the $+H$ chromosome having a higher activity than the $+L$, is more probably due to the background genotype of the lines.

Another point of interest concerns the difference between the normal chromosome and the *Br* chromosome of each line. In the High line the *BrH* chromosome is more active than the $+H$, as expected, the mean number of missing vibrissae being $3{\cdot}0 \pm 0{\cdot}13$ for *BrH/Ta* and $4{\cdot}3 \pm 0{\cdot}24$ in $+H/Ta$; the difference is highly significant ($t_{(118)} = 4{\cdot}8$, $P < 0{\cdot}001$). In the Low line, however, the two are not very different, the number of missing vibrissae being $5{\cdot}5 \pm 0{\cdot}21$ in *BrL/Ta* and $5{\cdot}1 \pm 0{\cdot}21$ in $+L/Ta$; the difference is not significant. From this it seems to follow that the *Br*-variegation in the Low line, with a mean score of 30%, represents a nearly 'random' phenotype with each chromosome being about equal in 'competitive ability'. A random phenotype with less than 50% mutant area can be readily explained by a threshold effect in hair follicles of mixed cell-type. *Brindled* almost certainly affects the cells of the hair follicles, and follicles of mixed cell-type may

Table 6. *Variegation of* brindled *in the progeny of crosses with the* tabby*-strain, compared with the selected lines in the contemporaneous generation*

(Mean percentage scores with standard errors, and numbers of animals in parentheses.)

	Low line	High line
Lines	$\frac{BrL}{+L} = 31 \pm 2{\cdot}3$ (65)	$\frac{BrH}{+H} = 64 \pm 1{\cdot}9$ (63)
Crosses	$\frac{BrL}{Ta} = 43 \pm 1{\cdot}5$ (61)	$\frac{BrH}{Ta} = 71 \pm 1{\cdot}4$ (62)
Difference	$12 \pm 2{\cdot}7$	$7 \pm 2{\cdot}4$
t	4·3	2·8
P	$< 0{\cdot}001$	$< 0{\cdot}05$

produce hairs that appear normal, though the pigmentation may be reduced. If only pure *Br* follicles produce mutant hairs, then 30% mutant area would imply that 40 percent of follicles are of mixed cell type (30 percent pure *Br*, 30 percent pure +, 40 percent mixed). Gartler *et al.* (1971) found an average of 25 percent of scalp follicles of mixed cell-type with respect to G-6-PD in eight human subjects.

The crosses with *Ta* provide evidence on whether the variegation of *Br* can be affected by normal chromosomes from other strains. The variegation of the *Br* progeny was classified in the normal way and the results are given in Table 6, with the means of the lines in the contemporaneous generation for comparison. In both cases the crosses had higher scores than the lines. The differences were quite large and significant. The differences mean that the *Ta* chromosome was less active than the $+L$ chromosome when competing with *BrL*, and less active than the $+H$ chromosome when competing with *BrH*. It is possible that the differences were not in the inactivation properties of the *Ta* chromosome but were due to the presence of *Ta* itself in repulsion with *Br*. The *Ta* patches have less hair and this might give an impression of larger *Br* areas. But it seems unlikely that such large differences would result from this cause and the conclusion to be drawn is that the expression of *Br*-variegation can be affected by the normal chromosome with which *Br* is associated in the heterozygote. The conclusion was confirmed by the crosses to be described next.

(iii) *Crosses with* C57BL

Brindled females from generation 5 of the High and the Low lines were crossed with males of the inbred strain *C57BL/Fa*, and the *Br* variegation of the F_1 daughters was scored. The means, with the contemporaneous generation 6 of the lines for comparison, are given in Table 7. Both crosses showed lower scores than the corresponding line, thus confirming the conclusion drawn above that the expression of *Br* can be influenced by the normal *X* chromosome in the heterozygote. In contrast to the *Ta* chromosome, the *C57 X* chromosome proved to be more active in competition than the $+H$ or the $+L$ chromosomes.

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Table 7. *Variegation of* brindled *in the progeny of crosses with the* C57BL *strains compared with the selected lines in the contemporaneous generation*

(Mean percentage scores with standard errors, and numbers of animals in parentheses.)

	Low line	High line
Lines	$\frac{BrL}{+L} = 29 \pm 2{\cdot}2$ (71)	$\frac{BrH}{+H} = 64 \pm 2{\cdot}0$ (59)
Crosses	$\frac{BrL}{C57} = 24 \pm 1{\cdot}8$ (61)	$\frac{BrH}{C57} = 51 \pm 2{\cdot}2$ (54)
Difference	$4{\cdot}5 \pm 2{\cdot}8$ m	$13{\cdot}5 \pm 3{\cdot}0$
t	1·6	4·5
P	0·1	$< 0{\cdot}001$

Table 8. *Variegation of* brindled *in the progeny of* Br *males of the Low line mated to* $+/+$ *females of both lines, compared with the selected lines* (Br ♀♀ × + ♂♂) *over generations* 6–10

(Mean percentage scores with standard errors, and numbers of animals in parentheses. The sex-symbol in the chromosomal constitution indicates the parental origin of the chromosome.)

Male parent	Source of female parent: Low line	Source of female parent: High line
+	$\frac{BrL\ (♀)}{+L\ (♂)} = 30 \pm 1{\cdot}1$ (265)	$\frac{BrH\ (♀)}{+H\ (♂)} = 64 \pm 0{\cdot}8$ (309)
BrL	$\frac{+L\ (♀)}{BrL\ (♂)} = 56 \pm 2{\cdot}3$ (45)	$\frac{+H\ (♀)}{BrL\ (♂)} = 57 \pm 1{\cdot}7$ (76)
Difference	$26{\cdot}0 \pm 2{\cdot}5$	$6{\cdot}5 \pm 1{\cdot}9$
t	10·4	3·5
P	$< 0{\cdot}001$	0·001

Cattanach & Williams (1972) have shown that normal X chromosomes can influence the variegation of the *flecked* translocation and of *Ta* and Mo^{vbr} (an allele of *Br*). Our *Br* chromosomes thus behave in the same way in relation to the normal X with which they are associated.

(iv) *Transmission through males*

About 1–2 percent of *brindled* males survive to adulthood and a few of these breed. In the whole of these experiments five survivors have bred. The survival of males will be dealt with more fully later; here we are concerned only with those that bred. All five of the fertile males came from the Low line, one in generation 6, three in generation 8, and the fifth in generation 9. These fertile *brindled* males gave the opportunity to look further into the question of whether the properties of the selected chromosome are influenced by the sex of the parent that transmits them. The crosses with *blotchy* proved that + chromosomes of the two selected

Table 9. *Crosses of* Br *males and* Br *females of the Low line with* C57BL

(Mean percentage scores with standard errors, and numbers of animals in parentheses. The reciprocal crosses were not contemporaneous.)

Parents of F_1	F_1
Br ♀ × *C57BL* ♂	24 ± 1·8 (61)
Br ♂ × *C57BL* ♀	37 ± 2·1 (56)
Difference	13·0 ± 2·75
t	4·7
P	< 0·001

lines were not so affected. The *brindled* males provided a means of testing whether the *BrL* chromosome was influenced by the sex of the parent transmitting it. Unfortunately the value of these males was not fully appreciated at the time and the matings made were not as informative as they might have been. Three *Br* males were mated to normal (non-*brindled*) females of both High and Low lines. The mean percentage scores of their daughters are given in Table 8, with the means of the lines over generations 6–10 for comparison. We may note first that the matings with High line and with Low line females gave almost identical mean scores in the daughters, thus confirming again the conclusion that the $+H$ and $+L$ chromosomes differed very little from each other when transmitted by females. Comparison of the reciprocal matings within the Low line shows a striking difference. The line itself, in which all matings were *Br* ♀ × + ♂, gave an average score of 30 %, but the reciprocal matings of + ♀ × *Br* ♂ gave an average of 56 %, and the difference is highly significant ($P < 0{\cdot}001$). The three males were entirely consistent, the means of their daughters being, respectively, 56 %, 56 % and 55 %. The families from which the males came were in no way remarkable, the mean of the males' sisters, all combined, being 31 %.

Confirmation of the difference between reciprocal matings was obtained from crosses with *C57BL*. Four of the *Br* males were crossed with *C57BL* females, and the comparison with the reciprocal cross, described in the previous section, is made in Table 9. This comparison is not altogether reliable since the two crosses were made at different times separated by roughly three generations. If the possibility of non-genetic change with time is discounted, the results clearly confirm the conclusions from the crosses in the Low line. Transmission through the female gave a mean score of 24 % whereas transmission through the male gave a mean score of 37 %, and the difference is significant at $P = 0{\cdot}001$.

A difference between reciprocal matings in the degree of expression of sex-linked genes in heterozygotes is not a new observation. The degree of variegation of *albino* produced by the *flecked* translocation differed according to the sex of the parent transmitting the translocation *X*-chromosome (Cattanach & Perez, 1970) and the number of missing vibrissae in *tabby* heterozygotes differed according to whether *Ta* was introduced by the father or the mother (Kindred, 1961). All three

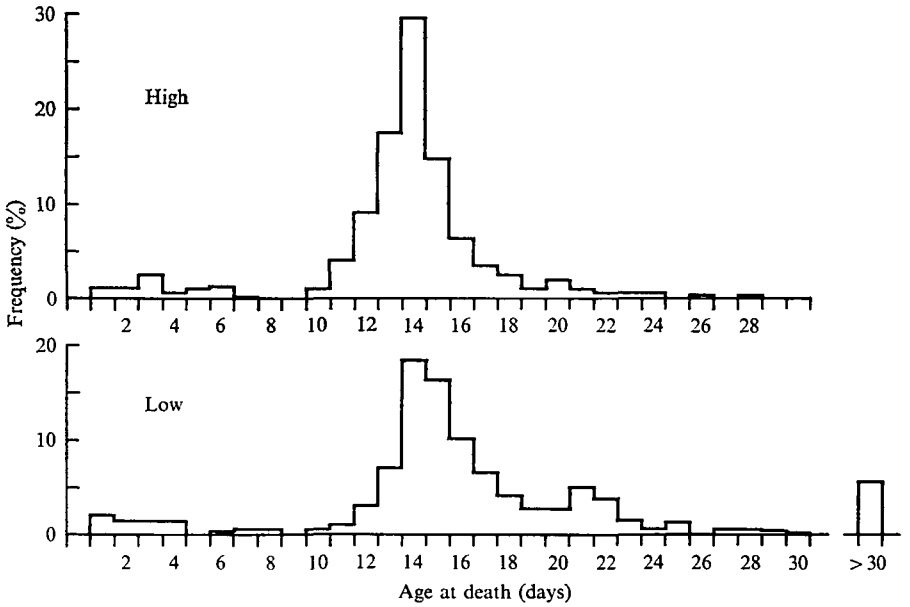

Fig. 5. Distributions of ages at death of *brindled* males in generations 6–10 of the selected lines. The High line distribution is based on 528 individuals and the Low line on 464.

cases – *Br*, *flecked* and *Ta* – are consistent in showing a higher activity of the chromosome when derived from the father than when derived from the mother. The reciprocal difference found with *Br*, however, seems to be a good deal larger than that found with *flecked*. The difference in the variegation score for *flecked* amounted to about 5 percentage points, whereas the difference with *brindled* was 26 percentage points.

6. OTHER FEATURES OF THE SELECTED LINES

(i) *Survival of* Br *males*

The difference between the selected lines in the variegation of *brindled* heterozygotes has the superficial appearance of a difference in the severity of the defect caused by the *Br* gene. Though the difference in variegation has been shown beyond doubt to be an effect of the *X* chromosome not confined to the *Br* locus, it is still possible that what selection has changed is not the relative competitive ability for activation or for cell proliferation but the functional activity of a region of the chromosome in the synthesis of gene products. It therefore seemed worth while to investigate the possibility by comparing the survival times of *brindled* males in the two lines. If the severity of the gene action had been altered, the *Br* males would be expected to be more severely affected and so to die earlier in the High line than in the Low.

The viability of males may also be influenced by maternal effects and by autosomal modifiers in the genetic background. Therefore a longer survival time in the

Table 10. *Ten-day weights in generation 8*

(Mean weights in grams with standard errors, and numbers of animals in parenthesis.)

Genotype and sex	Line		Difference (L − H)
	Low	High	
+/+ ♀	5·16 ± 0·117 (50)	5·19 ± 0·127 (87)	−0·033 ± 0·173
+ ♂	5·07 ± 0·109 (57)	5·00 ± 0·180 (50)	0·068 ± 0·211
Br/+ ♀	4·97 ± 0·108 (50)	4·67 ± 0·121 (64)	0·307 ± 0·162*
Br ♂	4·40 ± 0·087 (60)	4·25 ± 0·111 (70)	0·149 ± 0·141
Differences			
+/+ − *Br*/+ ♀	0·184 ± 0·160	0·524 ± 0·176**	
+ − *Br* ♂	0·674 ± 0·140***	0·755 ± 0·212***	

Significance levels: *5 %, **1 %, ***0·1 %.

Low line will not be conclusive of altered gene action unless it can be shown not to be due to a maternal effect, or to a difference of genetic background.

The ages at death of all *brindled* males were recorded in generations 6 to 12 of the selection lines. The *brindled* males still alive at 3 weeks were not weaned in the normal way, but were left with their mothers until they died, or in the case of the few 'long-term' survivors until they were 5 or 6 weeks old. The distributions of age at death are shown in Fig. 5. These are based on 528 High line males and 464 Low line males. The main distribution of deaths specifically attributable to the *Br* gene begins at about 10 days, increases to a mode at 14 days, and then tails off to about 30 days. Beyond about 30 days there are a few 'long-term' survivors, which have clearly survived well beyond the normal weaning age. Of these there were 25 animals (5·4 percent) in the Low line, with survivals ranging from 32 to 78 days, but there were none in the High line surviving beyond 28 days. The two lines were thus clearly different in the occurrence of long-term survivors.

The main distributions of deaths, between 10 and 30 days, are skewed, particularly in the Low line, and the comparison between the lines was made with log-transformed data. The means of the logarithms, converted back to days, were 14·2 in the High line and 16·1 in the Low line, and the difference is highly significant ($t_{(891)} = 10{\cdot}4$; $P < 0{\cdot}001$). This difference, with the difference in long-term survivors, leaves no doubt that the survival time was shorter in the High line than in the Low, as expected from a difference in gene-activity.

In order to find out if the difference in survival time could be attributed to a maternal effect, all the young mice of generation 8 were weighed at 10 days of age. The means are given in Table 10. The non-*brindled* mice did not differ between the lines, so there was no evidence of any difference in maternal effect on the growth of litters up to 10 days. The *brindled* mice differed from non-*brindled*, and differed between the lines, in the manner to be expected from the expression in the phenotype. The differences are given in the table and need no further comment. Though the 10-day weights did not reveal any difference in maternal effect between the lines, the survival time of *Br* males is undoubtedly influenced by the maternal, or

Table 11. *Survival of* Br *males in backcrosses to* C57BL

Backcross...	1st		2nd and 3rd	
Line of origin...	Low	High	Low	High
N	99	63	125	103
Mean of logs	1·155	1·126	1·154	1·154
Days	14·3	13·4	14·2	14·2
Line-difference (logs)	0·0287		0·0004	
S.E. of diff.	0·0094		0·0057	
t	3·06		0·06	

litter, environment. No detailed study was made, but it was obvious from the records that the age at death was correlated within the litters; it was found also to be correlated negatively with litter size at birth in the High line, but not in the Low line. It is possible, therefore, that the difference in survival time did result from some unidentified maternal effect.

The possibility that the difference of survival of *brindled* males was due to autosomal modifiers was tested by comparison of back-crosses to *C57BL*. If it was due to autosomal modifiers, the difference should disappear progressively with repeated backcrosses. The F_1 *brindled* females from the cross to *C57BL* described earlier were used to make the first backcross. The survival of *Br* males was not recorded in the F_1 but was recorded in three successive backcrosses, and the mean survival times are given in Table 11. The means were calculated from the log-transformed data of deaths between 10 days and the latest death at 26 days. In the 1st backcross there was a significant difference in the same direction, and about half as great, as the difference between the lines. There was no difference in the 2nd or in the 3rd backcross generations, and these are pooled in the table. The fact that the difference of survival times disappeared when *Br* from the two sources was backcrossed on to the same inbred strain, and the fact that there were no long-term survivors in the backcross generations, point very strongly to autosomal modifiers being the cause of the difference between the selection lines. These autosomal differences might be in the viability of the *Br* males themselves, or in a maternal effect. This study of the survival of *Br* males therefore provides no evidence that the selection affected the functional activity of the *Br* gene.

(ii) *Segregation*

The segregation of *brindled versus* non-*brindled* was examined in generations 6–12 of the selected lines, in order to see whether the segregation had been affected by the changes made in the *Br* chromosomes. The data are given in Table 12. There was a small but non-significant deficiency of *Br* progeny classified in both sexes of both lines, probably due to reduced viability. There was no difference in the segregation ratios between the sexes or between the lines. The segregation of *Br* was thus not affected by the selection.

Table 12. *Segregation of* Br *in generations 6–12*

	Low line				High line			
	Females		Males		Females		Males	
	Br	+	*Br*	+	*Br*	+	*Br*	+
Numbers...	412	429	467	492	524	552	551	559
χ^2_1 (deviation from 1:1)	0·34		0·65		0·73		0·06	
χ^2_6 (het. between generations)	4·73		10·20		6·25		3·79	
χ^2_1 (het. between sexes)	0·02				0·19			
χ^2_1 (het. between lines)								
Females	0·02							
Males	0·18							

Table 13. *Fate of the* Br *chromosomes in the selected lines*

(The chromosomes surviving the second generation of selection are identified by letters, A–E in the High line and F–J in the Low line. The figures in the table are the number of representatives of each chromosome among the parents of the numbered generation, out of the total number of female parents in each line given at the top of each column.)

	Generation										
	2	3	4	5	6	7	8	9	10	11	12
Total...	11	13	13	18	10	10	10	10	10	10	10
Chromosome											
High A	3	5	7	11	5	7	5	5	5	6	6
B	2	3	4	5	4	2	3	4	4	4	4
C	3	3	1	1	1	1	2	1	1	—	—
D	1	1	1	1	—	—	—	—	—	—	—
E	2	1	—	—	—	—	—	—	—	—	—
Low F	5	3	2	7	6	10	10	10	10	10	10
G	1	4	6	7	3	—	—	—	—	—	—
H	1	4	4	4	1	—	—	—	—	—	—
I	2	2	1	—	—	—	—	—	—	—	—
J	2	—	—	—	—	—	—	—	—	—	—

(iii) *Origin of chromosomal differences*

The changes in the properties of the *Br*-chromosomes brought about by the selection raise the question of the origin of the differences between the chromosomes on which selection acted. If the differences arose by fairly rare events, such as the changes of 'state' described by Cattanach & Isaacson (1967), then most of the differences will have been present at the beginning, and selection will have resulted in the spread of one particular chromosome throughout the selected line. This was found by Cattanach & Isaacson (1967) to be what happened in the line selected for the variegation caused by the *flecked* translocation. If, on the other hand, the differences arose by constantly recurring common events, they need not

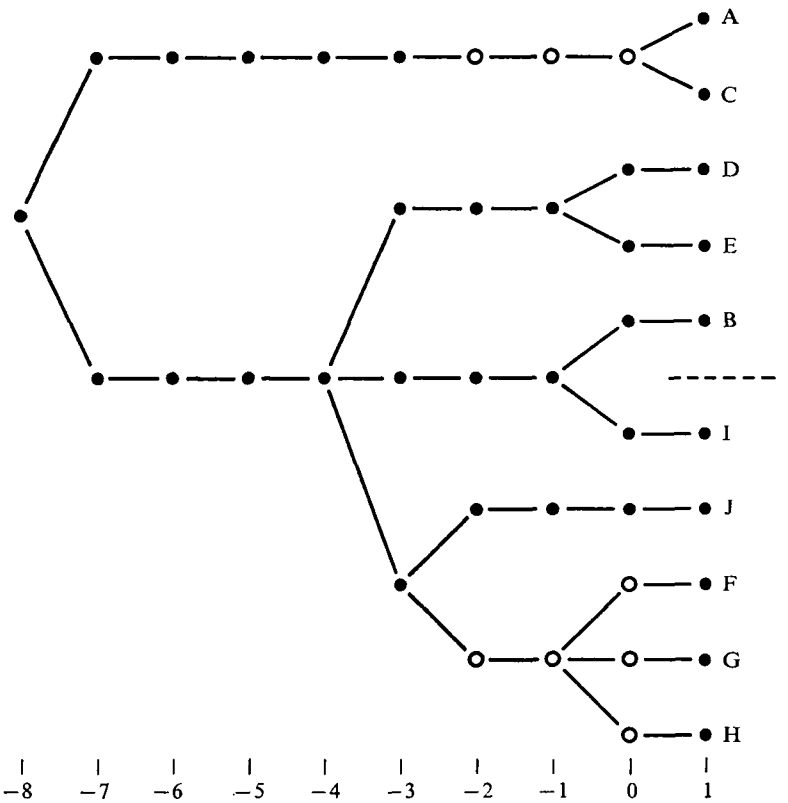

Fig. 6. Pedigree of *X* chromosomes marked by *Br* in the generations before the start of the reciprocal recurrent selection. Each circle represents the *Br* female parent of the generation numbered below. Only those chromosomes that survived the second selection are shown. The letters identifying them are as in Table 13, A–E being the High line and F–J the Low line. Generations −3, −2 and −1 were the lines subjected to individual selection, and the low-line females of these generations are indicated by open circles.

have been present at the beginning and they could have arisen during the course of the selection. In this case many, or most, of the original chromosomes could have been retained in the lines, with their properties being progressively altered.

The history of the representatives of the *Br* gene marking the *Br X* chromosomes in the present selection experiment was as follows. (Reference to a '*Br* chromosome' means here only a segment in the neighbourhood of *Br* which has not recombined with a non-*brindled X* chromosome.) In the base population of the reciprocal recurrent selection there were 17 *Br* females, and so 17 initially distinct *Br* chromosomes. The first selection retained all but one of these 17 females, assigning eight to the High line and eight to the Low line. The second selection, to provide parents of generation 2, eliminated three from each line, leaving five represented. The subsequent fate of these five chromosomes in each line is given in Table 13. The last reciprocal recurrent selection was generation 4. By then each line was composed mainly of two of its original chromosomes. The subsequent individual

selection had little effect on the distribution of the chromosomes in the High line. In the Low line, however, the two common ones at generation 4 were subsequently lost and one of the rare ones spread throughout the line. These results are not conclusive, but they do suggest that the differences between the *Br* chromosomes were mainly present at the beginning of the selection.

The probable presence of differences between the *Br* chromosomes at the start of the selection raises the question of the number of generations that had elapsed since these chromosomes had a common origin, during which time the differences must have arisen. Fig. 6 shows the pedigrees of the five chromosomes in each line that survived the second selection. All, from both lines, trace back to a single *Br* female eight generations before the start of the reciprocal recurrent selection. The origin of the differences between the chromosomes must therefore depend on events that are very much more frequent than gene mutation.

(iv) *Origin of non*-brindled *chromosomes*

We turn now to the question of why the + chromosomes were not affected by the selection. An acceptable reason can be found in the origin of these chromosomes. They all came from two inbred lines, *CBA/Fa* and *RIII/Fa*, introduced five generations before the start of the selection. The procedure of reciprocal recurrent selection results in the + chromosomes being exposed to selection only when carried by males, and each individual chromosome is thus exposed to selection only in alternate generations. Four generations before the start all the males used were *CBA*. Consequently in each alternate generation thereafter, the selection on + chromosomes was only between *CBA* chromosomes which were of recent common origin and therefore probably did not differ one from another. Simultaneous exposure of *CBA* and *RIII* chromosomes to selection only occurred twice, in the parents of generations 2 and 4 of the reciprocal recurrent selection. No change in their relative frequencies resulted from these selections. Thus the failure of selection to alter the properties of the + chromosomes may be attributed either to the fact that there were only two generations of selection instead of four in the case of the *Br* chromosomes, or to there being no difference in competitive ability between the *X* chromosomes of the *CBA* and *RIII* strains.

7. DISCUSSION

The chief points to be discussed are the efficiency of different methods of selection and the nature of the differences between the selected chromosomes. First, however, it may be helpful to summarize the main experimental results.

(i) *Summary of main experimental results*

1. Individual selection was unsuccessful, despite a significant correlation between mothers and daughters. In contrast, reciprocal recurrent selection was successful, and it produced a change in both directions.

2. The changes produced by selection were not due to autosomal genes, nor to

changes in the *Br* gene itself; they were due to changes in the properties of the chromosomes marked by *Br*.

3. The selection had very little or no effect on the non-*brindled* chromosomes, but non-*brindled* chromosomes from other strains did influence the degree of variegation.

4. The 'competitive abilities' of the *Br* and + chromosomes in the Low line were equal. The mean phenotype of the Low line (30 % *Br*) therefore represented the outcome of random inactivation and no differential cell selection.

5. The degree of variegation in the Low line differed widely according to whether the *Br* chromosome was derived from the mother or the father; derivation from the father gave more of the *Br* phenotype in the daughters. No data on this point were obtained from the High line.

(ii) *Selection*

The notion of competitive interaction between the two *X* chromosomes in heterozygous females satisfactorily accounts for the failure of our individual selection and the success of the reciprocal recurrent selection. We have, however, no evidence that the success of the reciprocal recurrent selection was really due to selection on crossing performance rather than to selection based on family averages. Selection on crossing performance would be more effective than selection on family averages only if there were variation between the + chromosomes as well as between the *Br* chromosomes. Since there appeared to be no variation between the + chromosomes in the strain used, the failure of the individual selection was probably due to the individual phenotypes being a poor guide to the identification of desirable *Br* chromosomes.

The failure of our individual selection is in marked contrast to the success reported by Krzanowska & Wabik (1971), who obtained lines with 73 % and 35 % mutant areas, after four generations of within-family selection. The only variation on which within-family selection can act is variation in the properties of the *X* chromosomes arising by recombination, or variation due to autosomal genes. All heterozygous females within a full-sib family must have identical + chromosomes received from the father, and must have mutant chromosomes that are identical except in so far as recombination in the mother has taken place. Krzanowska and Wabik's results therefore show that a considerable amount of variation can be released by recombination. This variation was presumably not present in our strain, or at least no suitable cross-overs occurred.

(iii) *Differences between selected chromosomes*

There are four possible ways in which a non-random phenotype might be produced by differences between the two *X* chromosomes in a heterozygote. These are by alteration of a threshold effect, by incomplete inactivation or reactivation, by non-random inactivation, and by differential cell selection. The first two can be rejected with little comment.

The way in which a threshold effect could give rise to a random phenotype with less than 50 percent of mutant area was mentioned in an earlier section. Selection might modify the threshold in two ways. First, the degree of intermingling of the cells during development could be affected, leading to a change in the number of hair follicles of mixed cell-type. Any change in the migrational behaviour of cells would, however, almost certainly be mediated by autosomal genes and not by the properties of the *X* chromosome. A modification of the threshold effect by this means is therefore ruled out. Second, the functional activity of the mutant gene, or the chromosome carrying it, might be affected, leading to a change in the proportion of cells in a mixed-type follicle needed to produce a mutant hair. This possibility is made very unlikely by the comparison of the viabilities of *Br* males in the two selected lines.

The idea that some cells might have both *X* chromosomes active, whether by failure of inactivation or by reversal of inactivation, arose from studies of autosomal segments translocated to the *X* (Cattanach, 1961; Russell & Montgomery, 1970). There are strong reasons for thinking it does not happen with structurally normal chromosomes, because cloned cell cultures from heterozygotes have never shown a heterozygous phenotype (for references and comments, see Lyon, 1972). Reversal of inactivation was disproved as the cause of modification of the *flecked* variegation (Cattanach & Williams, 1972).

The remaining two possible causes of a non-random phenotype – non-random inactivation or differential cell selection – cannot be distinguished by the evidence available. Cattanach & Williams (1972) conclude that one or other of these mechanisms is responsible for modification of the variegation of the *flecked* translocation, of *Ta* and of *Vbr*, but they also are unable to discriminate between the two. Some cases are known where non-random inactivation has been presumed, particularly with structurally abnormal *X* chromosomes, but in none of these has cell selection been disproved (Lyon, 1972). On the other hand, there are several cases where cell selection has been proved to operate (see Lyon, 1972), and on *a priori* grounds therefore cell selection seems the more likely mechanism. The only observation that might argue in favour of non-random inactivation is the 'parental-source' effect, i.e. the fact that the variegation is influenced by the sex of the parent transmitting the chromosome. It is easier to imagine how the parental source might influence the probability of inactivation, by some mechanism such as that proposed by Cooper (1971), than to imagine how it might influence the cell's rate of proliferation.

If differential cell selection is the mechanism by which a non-random phenotype is produced, it is interesting to note that only a very small selective advantage of one cell-type over the other would suffice to produce the difference between the selected lines. For example, if the cells in which the mutant-bearing *X* chromosome is active went through one cell division more than the cells with the other chromosome active, there would be twice as many mutant as wild-type cells in the adult. So, if the random phenotype were 50 %, one additional cell division would give a phenotype with 67 % mutant area. The variegation of *Br* is complicated by

the threshold effect, giving a random phenotype of 30 % mutant, but at most two additional cell divisions would be ample to produce the non-random phenotype of the High selected line. The very small difference in the proliferation rates of the two cell-types would, unfortunately, make it very difficult to use *in vitro* culture methods to detect differential cell selection in cells cultured from the selected *brindled* lines.

The well-established cases of cell selection refer to differences between a normal gene and a major mutant causing an enzyme deficiency, or to chromosomes of different specific origin (horse and donkey). It is easy to understand how differential rates of cell proliferation could result from these major differences between the two *X* chromosomes in a heterozygote. The cases of the *Br* chromosomes, and *flecked* translocation chromosomes, however, are different, because the modified phenotypes have been developed by selection without any alteration of the marker genes themselves. If there are different rates of cell proliferation, these must therefore be attributed to allelic differences between genes elsewhere on the chromosome which have no other visible effect. Since only a very small selective advantage of one cell-type over the other is required, it seems reasonable to suppose that there might be many loci on the *X* chromosome capable of modifying the variegation in this way. Perhaps, when more *X*-linked biochemical polymorphisms are known, one or more of these may be found to affect the rate of cell proliferation. If the existence of genes affecting the rate of cell proliferation could be established through the study of sex-linked variegation, this would be of great interest in connection with the study of growth. It would show that the genetic variation among normal individuals can affect the rate of cell proliferation through the functional activity of the cell itself.

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