# SELECTION FOR AN INVARIANT CHARACTER, VIBRISSA NUMBER, IN THE HOUSE MOUSE. IV. PROBIT ANALYSIS

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FOR any canalised character the genotype may be considered as having two aspects: firstly, the genes controlling the potential variability of the character and secondly, the genes which act during development to canalise or limit that variability. That a normally invariant character may have underlying genetic variability has been demonstrated for a number of characters (WADDINGTON 1953; RENDEL 1959; DUN and FRASER 1959) but the actual genetic mechanism by which underlying variability is channelled into phenotypic invariance has received less attention. FRASER (1960) has shown by means of a computer model how an epistatic genotype could work to produce a curvilinear genotype-phenotype relationship, i.e., a zone of canalisation in which change in the genotype dose does not result in phenotypic change. RENDEL and SHELDON (1960) have actually produced a new canalisation zone by selecting, in a scute stock of Drosophila which normally shows canalisation at four scutellar bristles, for low variance in number of scutellar bristles around a mean of two bristles.

The shifting of a character away from a canalisation zone can be achieved either by selection for a basic genotype which is so extreme that the canalisation system cannot cope with it, or by selecting for altered canalisation. Such an alteration may be a change in the intensity of canalisation or a change in the level of expression at which canalisation occurs. If selection is carried out simply for high or low expression of the character and if both genetic systems are available for selection, then it is to be expected that both systems will respond to a greater or lesser degree (WADDINGTON 1955).

The secondary vibrissae of mice are canalised at a total of 19 (DUN and FRASER 1959). However, the Tabby gene reduces the number of these vibrissae and at the same time increases their variance. Selection on this exposed variation has produced a marked response not only in Tabby mice but also in non-Tabby sibs (DUN and FRASER 1959; FRASER and KINDRED 1960). A probit analysis of the data from this and other selection experiments was undertaken to compare the intensity of canalisation of different lines and to detect any change in the pattern of canalisation which may have accompanied the change in mean vibrissae score.

## MATERIALS AND METHODS

*The mouse stocks:* The stocks used in this work have been described in detail elsewhere (DUN and FRASER 1959; FRASER and KINDRED 1960; KINDRED 1961).

The main selection stock is segregating for Tabby, a sex-linked semidominant gene (+/+, *Ta*/+, +/· and *Ta*/· individuals are produced). Selection is for high and low vibrissa score and is practised on Tabby mice only. Matings of the types *Ta*/+ × +/· and +/+ × *Ta*/· are used in alternate generations to obtain the greatest selection differential. The score used is the total number of vibrissae in five groups on the face and fore limbs (Figure 1). The high and low selection lines are termed HST and LST respectively and have been selected for 21 generations. After 12 generations a pair of lines (XHST, XLST) were derived from these and maintained for nine generations with the same mating plan, but without selection. A third pair of lines (HME, LME) were also derived from the main selection lines and maintained with selection on Tabby mice but with *Ta*/+ × +/· matings each generation. The high and low lines are not strictly comparable as the base stock was not very well mixed (DUN and FRASER 1959), but the three high and three low lines provide a useful comparison. The response of mean vibrissa score in the lines above is shown in Figure 2.

The normal vibrissa score for a mouse is 19 but occasionally in unselected stocks of non-Tabby mice, individuals occur with 18 or 20 vibrissae. These rare deviants have been used as a basis for lines which were selected for the number of vibrissae at a particular site. The sites were supraorbital (HB, LB) and interramal (HD, LD). After several generations, the Tabby gene was crossed into these lines so that the same three levels of expression (+/−, *Ta*/+, and *Ta*/·) which had been used in the main selection lines, could be studied. Selection was still practised only on +/− mice.

*Probit analysis:* A probit transformation can be used to give a linear measure of a frequency distribution. The method has been described in detail by RENDEL (1959) who used the technique in his studies of the canalisation of Drosophila scutellar bristles. The probit value for a particular class depends on the frequency of individuals falling into that, or a lower class, therefore the difference between successive probit values (called by RENDEL the probit distance of the class)

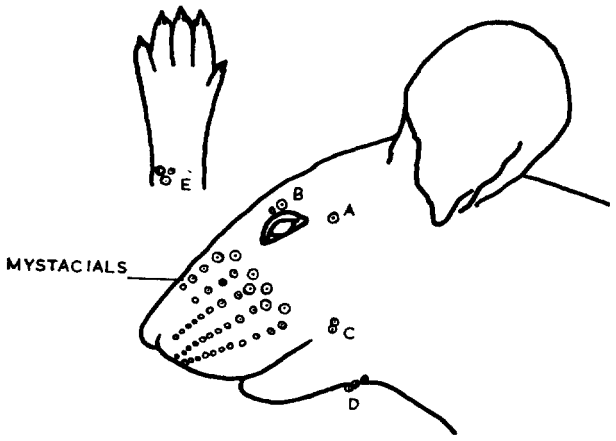



FIGURE 1.—Distribution of primary (mystacial)and secondary vibrissae.

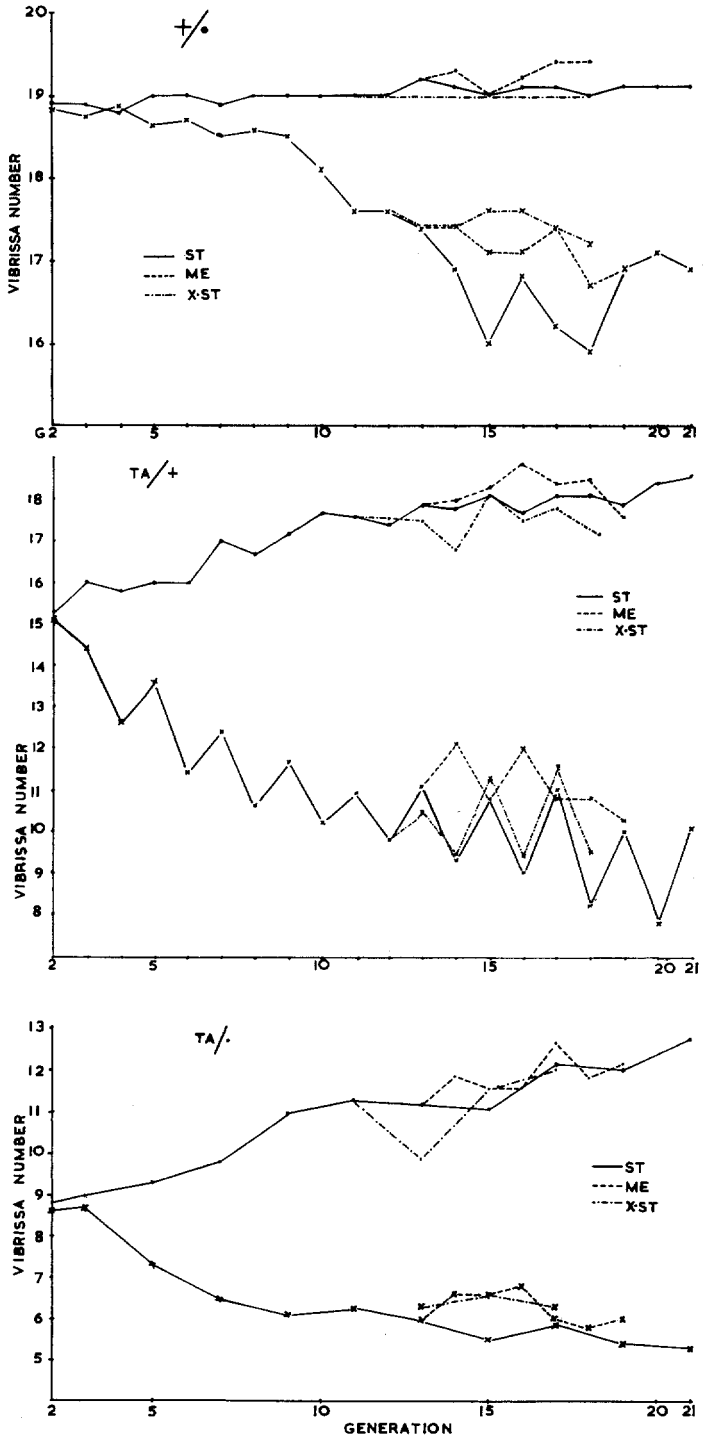



FIGURE 2.—Mean vibrissa scores of $+/\cdot$, $Ta/+$ and $Ta/\cdot$ mice for 21 generations of selection.

measures the percentage of animals falling into the class in question; the greater the proportion of animals in a class the wider will be the probit distance for that class. Consequently, the probit distance is a measure of the relative difficulty of passing from one class to the next under selection. Thus, if it is fairly easy for, say *Ta*/+ mice to be moved by selection through the 15-vibrissa class to the 16-vibrissa class then the probit distance for the 15 class will be small and similarly, if the stock is canalised at 19 vibrissae, a high proportion of mice will fall into the 19-vibrissa class and the probit distance for this class will be large.

The probit distance of a class cannot be estimated unless a probit value can be obtained for each of the adjoining classes. If the vibrissa score is completely invariant at 19 no probit distance can be estimated as there is no indication of the limits of the 19 class. Only when animals with 18 and 20 vibrissae also occur can the probit distance for 19 be measured.

If an additive genetic system canalised by a secondary epistatic system is postulated, probit analysis allows measurement of the effects of the epistatic system and there is no reason why the probit distance of a class should change under selection unless selection affects the epistatic system.

## RESULTS

A probit analysis was carried out for each of the stocks described. The three genotypes, +/− (+/+ and +/· show no differences), *Ta*/+ and *Ta*/· were considered separately for each generation. As the numbers were small compared with RENDEL's Drosophila (RENDEL 1959) it is hardly surprising that there is a fair amount of fluctuation in the probit distance for each vibrissa number from generation to generation. However, the mean probit distances which are shown plotted against vibrissa class in Figures 3 and 4, for the ST stock and for the ME and XST stocks derived from it, compare very well, indicating that the mean probit distance is a fairly good measurement of the canalisation pattern.

The normal, almost invariant, number of secondary vibrissae is 19, and the very marked peak at the 19 vibrissa class (Figure 3) shows the extent of the canalisation at this number. The existence of secondary canalisation zones at 16 vibrissae in the low-line +/− mice and at seven and five vibrissae in low-line *Ta*/· males are also obvious.

Downward selection could, then, be partly selection for canalisation at a lower zone. Table 1 gives the probit distances of LST for 21 generations of selection. There is a small increase in the probit distance of the 16 class as selection proceeds and at the same time a small decrease in the probit distance of the 18 class, i.e., selection for low vibrissa score has increased the degree of canalisation at 16 while it has become even easier than before to pass through the 18 class. The regression for the 18 class is significant, but the one for the 16 class which could not be measured for the first eight generations does not quite reach significance. Unfortunately, the 19 class cannot be measured to see if it has been reduced as no animals in this line have been found with 20 vibrissae. The LME and XLST lines were not continued long enough to be used to check such small effects.

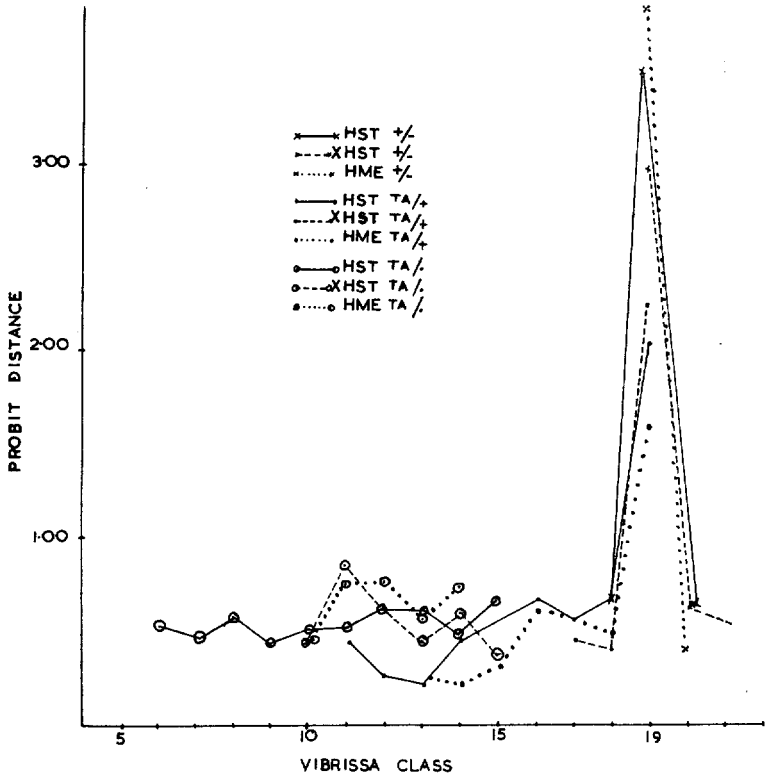



FIGURE 3.—Probit distances plotted against vibrissa class for HST, XHST and HME.

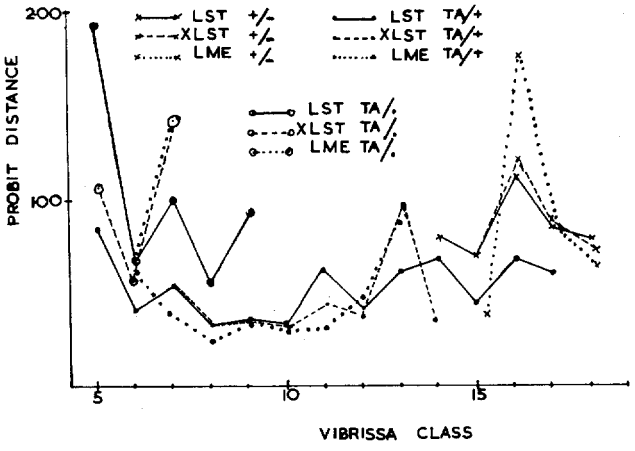



FIGURE 4.—Probit distances plotted against vibrissa class for LST, XLST and LME.

TABLE 1

*Probit distances for +/— mice of the LST line*

| Generation | Vibrissa class | | | | | | |
|---|---|---|---|---|---|---|---|
| | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
| 3 | ... | ... | ... | ... | 0.44 | 0.93 | 3.63* |
| 4 | ... | ... | ... | ... | ... | 1.31* | 3.69* |
| 5 | ... | ... | ... | ... | 0.87* | 0.88 | 3.25* |
| 6 | ... | ... | ... | ... | 1.40 | 0.70 | 2.90* |
| 7 | ... | ... | ... | 0.27* | 0.78 | 1.01 | 2.94* |
| 8 | ... | ... | ... | ... | 0.96* | 0.88 | 3.16* |
| 9 | ... | ... | ... | 1.00 | 0.42 | 0.61 | 2.97* |
| 10 | ... | ... | ... | 1.11 | 0.49 | 0.70 | 2.70* |
| 11 | ... | ... | 0.28* | 1.19 | 0.88 | 0.80 | 1.85* |
| 12 | ... | ... | 0.29* | 1.04 | 0.60 | 1.00 | 2.07* |
| 13 | ... | ... | 0.28* | 1.22 | 0.89 | 0.71 | 1.90* |
| 14 | ... | ... | 1.17 | 0.65 | 0.76 | 0.76 | 1.66* |
| 15 | ... | 0.59 | 0.84 | 1.16 | 0.84 | 0.82 | 0.72* |
| 16 | ... | ... | 0.52* | 1.53 | 1.05 | 0.62 | 1.28* |
| 17 | ... | 0.95 | 0.44 | 0.97 | 0.91 | 0.76 | 0.97* |
| 18 | ... | 0.84 | 0.68 | 0.99 | 1.13 | 0.77 | 0.59 |
| 19 | ... | ... | 0.31* | 1.70 | 1.12 | 0.46 | 1.41* |
| 20 | ... | ... | ... | 1.72 | 0.90 | 0.67 | 1.71* |
| 21 | ... | ... | 0.99 | 1.03 | 0.90 | 0.54 | 1.54* |
| Mean | ... | 0.79 | 0.68 | 1.11 | 0.84 | 0.79 | ... |

* Incomplete classes—not included in means.

These results are reflected in the mean vibrissa scores for LST +/— (Figure 2). For several generations the mean score was about 19, then it moved quite rapidly to about 16, where it has remained since.

There are slight indications that similar changes of probit distance with selection have occurred in HST and LST *Ta*/+, but many more generations will need to be produced before the importance of these can be estimated.

The secondary zones at five and seven probably constitute a single broad zone. Mice with five vibrissae almost always have one vibrissa in each supraorbital and ulna carpal group and the central inter-ramal, those with seven vibrissae have one post-oral on each side as well. Symmetry of distribution is probably an aspect of canalisation, and at low vibrissa-numbers seems to be important. Animals with six vibrissae are always asymmetrical, as no mouse has yet been found lacking the central inter-ramal, and this probably accounts for the paucity of mice in this class.

It has been postulated (DUN and FRASER 1959) that the Tabby gene does not simply shift the vibrissa number outside the canalisation zone so that variability which is normally masked can be expressed, but that it also changes the actual canalisation. Probit analysis reveals that this is probably correct; the Tabby gene changes both the level of expression and the degree of canalisation of the character. In Figure 3 the peaks for *Ta*/+ mice at 19 vibrissae are much lower than those for +/— mice, and all other stocks in which the main canalisation zone

can be measured for both +/− and *Ta*/+ show the same phenomenon: *Ta*/+ mice do not appear to be nearly so well canalised as +/−. The same thing holds for the secondary zone at 16 vibrissae which is clearly marked in +/− mice, but not in *Ta*/+. Surprisingly, *Ta*/+ are again less well canalised than the more extreme phenotype, *Ta*/·, at the 5 and 7 classes. The curves for *Ta*/+, are in fact, consistently lower than those for +/− or *Ta*/·. This may be partly an artifact due to the greater variance of *Ta*/+, but the difference for the 19 class at least is large enough to show a real difference in canalisation. The poor canalisation of *Ta*/+ accounts for their susceptibility to maternal effects. Two maternal effects have been reported (KINDRED 1961, 1962) both acting on *Ta*/+ mice but not on +/− sibs. The canalisation system will act on variation of any kind whether of genetic origin, such as has been exposed in the Tabby selection lines, or of environmental origin. It is not easy to affect the intra-uterine environment of a mammal by experimental means, but if the mother is subjected to stress conditions, effects on the young can be produced.

One treatment which the mother's physiological homeostatic mechanisms does not alleviate is irradiation. HST and LST *Ta*/+ females which had been mated to +/· males were given 200r on the 13th day of gestation. This time was chosen because it is at the end of the period when the secondary vibrissae are sensitive to irradiation (FRASER and HALL 1958) and therefore it is possible to affect the vibrissae by X rays without causing so much damage that small differences in the response of genotypes are lost. Table 2 shows the mean vibrissa scores for irradiated and control mice. Normal mice from the high line and *Ta*/· males from the low line which are in marked canalisation zones show less response to radiation. This indicates that there is some ability to buffer development even against the damaging effects of X rays. All vibrissae were not equally affected: The D vibrissa consistently show a marked response while other groups are affected less frequently (Table 3). The unexpectedly low response in LST *Ta*/+ ♀♀ is almost certainly due to the increase in the D vibrissae of the X-rayed mice. So far no explanation of this can be offered.

A comparable set of mice were placed in a temperature of 96°F for the second week of pregnancy. Obviously the effect of this treatment on the environment of the foetus will depend on the mother's ability to control her temperature and on the metabolic by-products of this process, but as it was sufficiently severe to kill two of the females, some effect on the foetal environment should occur. With such

TABLE 2

*Mean vibrissa scores of X-rayed mice*

| | | Genotype | | |
|---|---|---|---|---|
| | | +/− | *Ta*/+ | *Ta*/· |
| HST | Treated | 18.5 | 15.2 | 7.7 |
| | Control | 18.9 | 17.7 | 13.3 |
| LST | Treated | 15.9 | 10.3 | 5.1 |
| | Control | 16.7 | 11.1 | 5.6 |

TABLE 3

*Means of individual vibrissa groups for X-rayed mice*

| | Genotype | | Vibrissa group A | B | C | D | E |
|---|---|---|---|---|---|---|---|
| HST | *Ta*/+ | X-rayed | 1.6 | 3.9 | 2.3 | 1.7 | 5.7 |
| | | Control | 1.8 | 4.0 | 3.3 | 2.7 | 5.8 |
| | +/— | X-rayed | 2.0 | 4.0 | 4.0 | 2.7 | 5.8 |
| | | Control | 2.0 | 4.0 | 4.0 | 3.0 | 5.9 |
| | *Ta*/· | X-rayed | 0.1 | 2.8 | 1.2 | 1.1 | 2.6 |
| | | Control | 0.7 | 4.0 | 1.8 | 2.0 | 4.7 |
| LST | *Ta*/+ | X-rayed | 1.6 | 3.1 | 1.7 | 1.5 | 2.4 |
| | | Control | 1.8 | 3.6 | 2.1 | 1.2 | 2.5 |
| | +/— | X-rayed | 3.9 | 2.0 | 3.8 | 2.2 | 4.1 |
| | | Control | 4.0 | 2.0 | 4.0 | 2.7 | 4.1 |
| | *Ta*/· | X-rayed | 2.0 | 0.0 | 0.1 | 1.0 | 1.9 |
| | | Control | 2.0 | 0.1 | 0.8 | 1.0 | 1.8 |

imprecise treatment it is impossible to predict in which direction a change might be expected or, indeed, if changes might occur in different directions in different individuals. Consequently, it is not surprising that while no changes in mean were found, the mice which are poorly canalised show an increase in variance. Variances for all groups are given in Table 4.

A few mice with three rather than two supraorbital vibrissae on one or both sides were found in normal stocks. These animals were tested to determine whether the extra vibrissae were due to the action of a single gene. When it was clear that several genes were involved, the descendants which now had a mean supraorbital number of 5.7, were used as the basis of the HB, LB selection lines.

When probits are calculated for the HB line it is revealed that the main canalisation zone is now at 21 instead of 19 vibrissae. This is not an extra zone, as the normal canalisation at 19 is absent. In LB, the mean vibrissa score rapidly returned to the normal number, four, and has remained at this value since. Similarly, the probit distance of the 19 class increased rapidly (the distance for the 21 class could not be measured) i.e., the position of the canalisation zone returned to that usually found. If the main canalisation zones of the HB and LB are matched (Figure 5) the probit distances for the other classes coincide quite well,

TABLE 4

*Variance of vibrissa scores of heat-treated mice*

| | | Genotype +/— | *Ta*/+ | *Ta*/· |
|---|---|---|---|---|
| HST | Control | 0.09 | 0.55 | 2.48 |
| | Heated | 0.09 | 1.56 | 3.60 |
| LST | Control | 1.30 | 2.48 | 1.02 |
| | Heated | 1.25 | 3.66 | 0.88 |

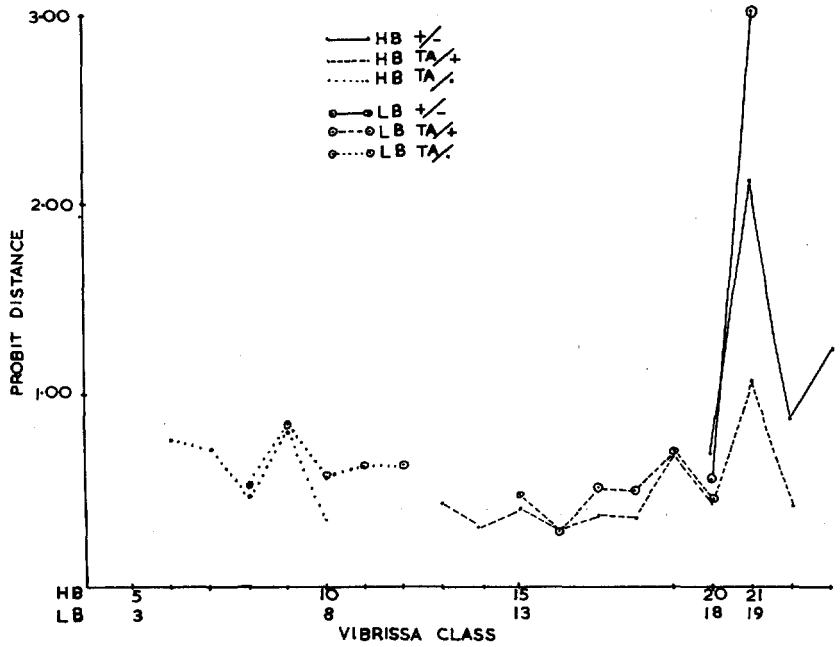



FIGURE 5.—Probit distances plotted against vibrissa class for HB and LB, showing how the probit distances coincide if the canalisation classes are matched.

suggesting that there has been simply an addition of two more vibrissae, virtually a change in the zero point of the scale of vibrissa classes, rather than a change in the canalisation pattern.

The inter-ramal (D) selection lines were also based on rare deviants but in this case selection lines were established from the beginning. As the high and low deviants were found in the same stock the lines are comparable. In these lines, there has not been any apparent change in the class at which canalisation occurs. The high line still shows canalisation at 19 vibrissae, although many animals with higher scores are found, but in the low line the canalisation zone is broadened to include the 18 class (Figure 6). This could be a change in the class at which canalisation occurs, a change which is proceeding slowly and is as yet incomplete, but it seems more likely, since the lines have been continued for nine generations, that here is a change in the intensity of canalisation. Additional evidence for this idea comes from the response of vibrissa groups other than the one under selection. HB, LB, and HD show no change in the mean vibrissa score which is not due to the selected vibrissae, whereas in LD other vibrissa groups are also reduced, a further indication that the canalisation is weakened not simply moved.

## DISCUSSION

The original model of a smooth canalisation curve or surface for vibrissae (Figure 7) (DUN and FRASER 1959; FRASER and KINDRED 1960) was later modi-

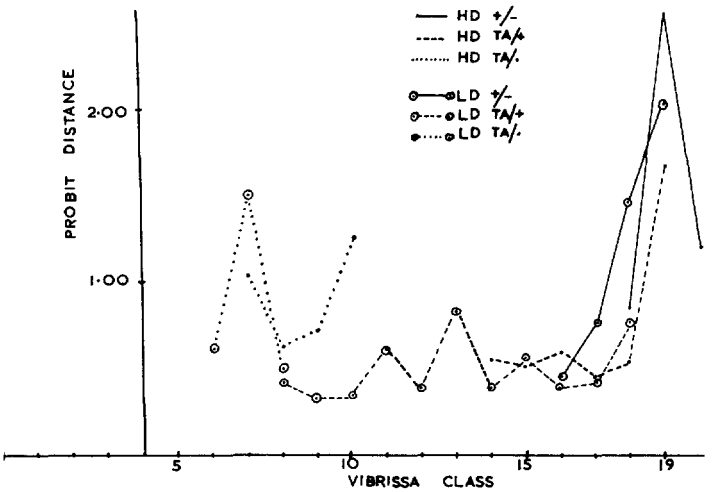
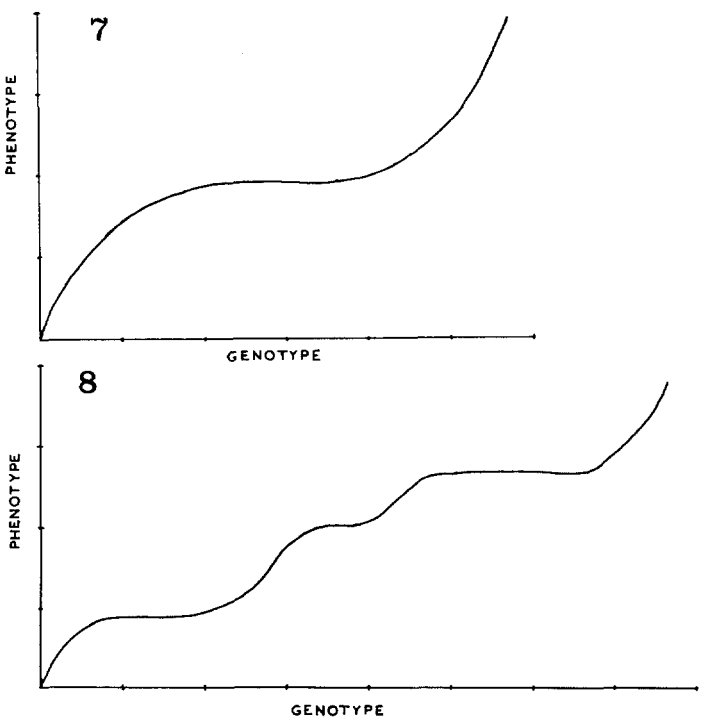



FIGURE 6.—Probit distances plotted against vibrissa class for HD and LD.




FIGURES 7 and 8.—Curve relating genotype to phenotype as suggested by DUN and FRASER (1959) (top). Modified genotype-phenotype curve to allow for secondary canalisation zones (bottom).

fied (FRASER and KINDRED 1962) as it became apparent that local alterations of the surface were possible. Now it seems that the curve resembles a series of steps representing the secondary canalisation zones (Figure 8). Whether these secondary zones would be shown by the very tightly canalised primary (mystacial) vibrissae and the apparently uncanalised main coat, which are the other aspects of hair growth used in the canalisation surface model, is unknown. It seems reasonable to expect that if the primaries can be changed sufficiently, they will show similar effects and as the main canalisation zone has not been detected with the coat, it is unlikely that secondary zones would be found.

Any suggestion as to why secondary zones occur at particular vibrissa classes is, at present, purely speculative, but it could well be that symmetry of development is involved. At the main canalisation zone, invariance is found for vibrissa symmetry as well as vibrissa number. Again at five, seven and 16 vibrissae the pattern usually found is symmetrical. It may be that the preservation of symmetry is an important function of canalisation.

In these experiments, selection for vibrissa number has been brought about by changes in all the systems available for change. The selection response in HST, LST and HD has been mainly a response of the basic genotype, although in LST there has also been a tendency towards canalisation at a lower zone. LD shows an alteration in the intensity of canalisation together with a change in the basic genotype. The most interesting finding is that in HB there is no change in either the canalisation pattern or the basic vibrissa genotype (there are no changes in other vibrissae) but instead, what is apparently the simple addition of extra vibrissae into the system. These then respond to selection as an ordinary additive variable without affecting the canalised variability at all. In fact supraorbital number in the high selection line behaves as a simple additive character. Nevertheless probit analysis and downward selection show that at lower numbers the character is still canalised. This is a clear demonstration that a character does not need to be canalised *or* additive and it could well be that many characters which are thought to vary in a simple additive manner, uncomplicated by epistasis, appear to do so only because efforts have not been made to determine if canalised variation also occurs.

## SUMMARY

Probit analysis of several lines of mice selected for total number of secondary vibrissae or for the number of vibrissae at a particular site reveals changes of different kinds in the canalisation of these lines. The Tabby gene reduces canalisation in all lines, as shown by probits and by increased reaction to environmental change.

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