SELECTION FOR CANALISATION IN MICE

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THE term canalisation will be used here to describe a developmental buffering system which acts to reduce potential variation and produce a less variable phenotype. Unless such a system acts at the gene level it would act on variation due to either genotype or environment and canalising selection can aim at reducing the response to either genetic or environmental variation. Selection for canalisation has been successful in *Drosophila*. It was possible, by selecting for variance of scutellar bristle number in scute flies, to reduce variance and response to temperature (RENDEL and SHELDON 1960). Other attempts to select for canalisation in *Drosophila* have been made by WADDINGTON (1960) and KINDRED (1965). In each of these experiments a change in the response to temperature was produced.

Although differences in the degree of canalisation of mouse vibrissae have been shown (KINDRED 1963), canalising selection has not been attempted in mice. There is a need to demonstrate that canalising selection is possible in animals other than *Drosophila*. Furthermore, well canalised and poorly canalised lines would provide useful information about the canalisation system.

Mice do not produce large enough progenies to allow selection on within-litter variance, and it is extremely difficult to bring about a precise change in the environment of a developing mammal. Another indication of canalisation is symmetry, since presumably asymmetry indicates a response to some localised variable (VAN VALEN 1962). It should thus be possible to select for well canalised and poorly canalised lines by selecting for symmetry and asymmetry. A symmetrical animal may be one which is well canalised and doesn't respond to environmental changes, or it may not have been exposed to such changes. This dependence on chance differences may slow down response to selection, but it should not prevent a response. Unsuccessful attempts to select for symmetry in *Drosophila* have been made, but as WADDINGTON (1962) has pointed out, symmetry in *Drosophila* is complicated by the fact that each side of the adult develops from a different set of rudiments.

Much work has been done in this laboratory on the canalisation of secondary vibrissae in mice, and the character *secondary vibrissa number* was thus a logical choice for canalising selection. Because normal mice very rarely have asymmetrical vibrissae, the Tabby (*Ta*) mutant was used. Tabby has been employed to demonstrate that the number of secondary vibrissae is a canalised character (DUN and FRASER 1959).

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Another "invariant" character in the mouse is the number of toes. Luxate (*lx*), which affects the development of the whole hind limb, may reduce or increase the number of toes (CARTER 1950) and so provide variation which may be used for selection. Even in luxate mice, asymmetry is not sufficiently frequent to use as a character for selection, so it was decided to try selecting on the range of toe numbers within a litter as a means of canalising selection.

The present paper reports a response to selection altering the canalisation of both vibrissa number and number of toes. In both cases, the response has been produced in comparatively few generations.

MATERIALS AND METHODS

Tabby is a sex linked semi-dominant gene which reduces the number of secondary vibrissae on the face and fore-paws. Distributions of total vibrissa number for unselected $+/Y$, $Ta/+$ and Ta/Y are given in Table 1. The total vibrissa number given here includes the medially placed inter-ramals. The distribution of the total number of hind toes in unselected luxates is also given.

Selection for symmetry (ASL, asymmetry low) and asymmetry (ASH) was mainly on $Ta/+$, but Ta/Y were also used when they were produced. Matings of $Ta/+ \times +/Y$ produce $+/+$, $Ta/+$, $+/Y$ and Ta/Y . Only half of these ($Ta/+$ and $+/Y$) are available to set up similar matings. To increase the selection differential, $+/+ \times Ta/Y$ matings were used in alternate generations. Non-Tabby parents were chosen on the basis of performance of their Tabby sibs, but sib matings were avoided. The vibrissae scored were: supra-orbital (2), post-orbital (1), post-oral (2) and ulna-carpal (3). (See KINDRED 1948 for a diagram.) The number in brackets is the normal number at each site. The three inter-ramals were not included because they occur in one medially placed group. Vibrissae were counted under a desk lamp at five days of age and checked at ten days as described by DUN and FRASER (1959). If the two sides of the mouse differed at any site it was counted as asymmetrical; an asymmetry score of 2 meant that two sites differed, even if the difference was such that the total for each side was the same. All sites were given equal weight because it could not be determined *a priori* which sites should be given the greatest importance.

Tabby had previously been backcrossed into five inbred strains and two other lines (FRASER and KINDRED 1962). Variance of total vibrissa number and symmetry were scored for $Ta/+$ and Ta/Y mice from these lines, in order to compare the two methods of measuring canalisation (Figure 1). The correspondence between the two is very good, correlation coefficients being 0.88

TABLE 1

Distribution of vibrissa number in unselected Tabby mice (including inter-ramals), and distribution of toe numbers in unselected luxate mice

Genotype	Vibrissa number														
	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5
$+/Y$	270	43	1	..	..	..	..	..	..	..	..	..	..	..	..
$Ta/+$	13	15	36	58	28	15	8	4	4	1	..	1	..	..	..
Ta/Y	..	..	..	..	..	..	3	7	9	22	30	21	20	15	3
	Toe number														
	13		12		11		10		9		8				
$+/+$	..		..		..		86		..		..				
$+/lx$	1		4		12		67		..		..				
lx/lx	..		..		11		57		12		2

FIGURE 1.—Variance of total vibrissa score plotted against mean asymmetry score for $Ta/+$ and Ta/Y mice from inbred backcross lines.

for $Ta/+$ females and 0.91 for Ta/Y males.

The toe number of luxate mice was scored at three weeks of age. A separate claw was counted as a toe, but for convenience in scoring a broad toe which may have contained extra bones was counted as only one.

X-ray treatment consisted of 200r whole body irradiation given at a rate of 30r/minute with the generator operating at 180kv and 6ma. A filter of 0.85 mm copper and 0.5 mm aluminum with HVL 1.535 mm³ was used. For heat treatment the females were placed in an incubator at 28°C for the whole of the second week of pregnancy. Mice used for heat and X-ray treatments were from selection generation 11.

Probit transformation is a very convenient method for comparing nonlinear genotype/phenotype relationships of different lines. The method used has been described by RENDEL (1959). The width of a class depends on the frequency of individuals falling into that class and its relation to the mean of the distribution. The width of a class is measured as the difference in standard deviations between successive cut-off points, the position of each cut-off point being measured from the mean of the distribution. The width of a class can only be measured if the class is complete, i.e. if some individuals fall into the classes on either side.

Unfortunately, the laboratory moved while these experiments were in progress. Moving, air conditioning problems, and blasting as a road was built upset the mice badly. From generation 5 to 8 virtually no selection was possible; all mice had to be mated to keep the lines going. The chief cause of the low numbers produced during this period was females who deserted their young, although the total number of females producing litters was also reduced. After generation 8, the number of litters and the number of young weaned returned to the pre-moving level. All mice were moved at the same time and were subjected to the same disadvantages.

Since generation 12, the lines have not been kept in the same manner and it has therefore not been possible to obtain additional data.

RESULTS

Vibrissa number: In the asymmetry selection lines the number of asymmetrical sites was slow to change, but a convincing difference between the lines is appearing at Generation 12 (Figure 2; Table 2). Figure 3 shows that the overall variance corresponds very well with symmetry, as it does in the Tabby-inbred backcross lines (Figure 1); as symmetry increases the variance decreases. The probit distance of a vibrissa class shows the proportion of the population that falls into

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FIGURE 2.—Mean asymmetry score for 12 generations of the asymmetry selection lines. Selection was effectively suspended from generation 5 to generation 8. ASL = Low asymmetry. ASH = High asymmetry.

that class in terms of standard deviations. The modal vibrissa class for $Ta/+$ females in the base population was 14 and this has remained the modal class in ASL (asymmetry low line) and for most generations of ASH (asymmetry high line). The probit distance of this class is shown plotted against generation in Figure 4. From generation 5 to generation 8 no selection was possible. The effects of this are obvious in Figures 2, 3, and 4.

Several different arrangements of vibrissae could make up a total of 14, but in ASL the increase in the 14 class is entirely an increase in one particular pattern, i.e. mice with 2 supra-orbitals, 1 post-orbital, 1 post-oral and 3 ulna carpals on each side 2113/2113. The incidence of mice with this pattern is shown in Figure 5. The only difference between this and the normal pattern is in the number of post-orals; usually there are two.

A maternal effect has been described (KINDRED 1961) such that $Ta/+$ daughters

FIGURE 3.—Variance of total vibrissa score for 12 generations of the asymmetry selection lines.

TABLE 2

Frequency of vibrissae and width of vibrissa classes in standard deviations in Ta/+ and Ta/Y mice

Population	Vibrissa number								
	8	9	10	11	12	13	14	15	16
<i>Ta</i> /+ Base population	2	4	8	4	25	20	52	13	8
	..	(0.48)	(0.44)	(0.15)	(0.63)	(0.39)	(1.11)	(0.55)	
ASL G.11	..	..	..	1	1	1	20	5	..
					(0.33)	(0.23)	(2.16)		
ASL G.12	..	..	2	..	5	5	37	6	..
						(0.36)	(2.01)		
G(11 + 12)				(0.18)	(0.57)	(0.32)	(2.03)		
ASH G.11	..	1	1	..	7	5	4	9	9
						(0.39)	(0.28)	(0.68)	
ASH G.12	2	1	3	5	13	23	26	8	7
		(0.18)	(0.33)	(0.34)	(0.55)	(0.69)	(0.86)	(0.46)	
G(11 + 12)		(0.29)	(0.33)	(0.27)	(0.63)	(0.60)	(0.65)	(0.50)	
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		3	4	5	6	7	8	9	10
<i>Ta</i> /Y Base population		1	1	4	16	12	2	1	1
			(0.32)	(0.62)	(1.20)	(1.05)	(0.37)	(0.32)	
ASL G.11	..	..	1	1	9	3	5	5	4
				(0.33)	(1.20)	(0.27)	(0.46)	(0.61)	
ASH G.11	..	..	1	..	16	6	5	1	..
						(0.60)	(1.00)		

ASL = Asymmetry Low. ASH = Asymmetry High. G = Generation of selection.
Numbers in brackets are the intervals in standard deviations between vibrissa classes.

FIGURE 4.—Probit distance of the 14 vibrissa class for 12 generation of the asymmetry selection lines.

FIGURE 5.—Percentage of mice with the 2113/2113 vibrissa pattern for 12 generations of the asymmetry selection lines.

of *Ta*/+ females have higher vibrissa scores than those of +/+ females. As *Ta*/+ and +/+ mothers have been used in alternate generations, the graph of mean vibrissa score plotted against generation has a zigzag appearance. This is much more pronounced in ASH than in ASL (Figure 6), showing that the asymmetrical line is more affected by this aspect of maternal environment.

Vibrissa scores of developing mice can be affected by heating or by irradiating pregnant females at about 12½ days gestation (KINDRED 1963). The effects of

FIGURE 6.—Mean vibrissa scores for the asymmetry selection lines showing that the fluctuation due to the *Ta*/+ maternal effect is greater in ASH.

TABLE 3

The effects of heat and X-ray treatment on variance, frequency of mice with the 2113/2113 pattern, and probit width of the modal class in $Ta/+$ females

Selection line	Control	Heated	X-rayed
Variance of vibrissa score			
ASL	1.1	0.32	2.49
ASH	2.8	3.07	6.71
2113/2113 frequency			
ASL	0.67	0.85	0.25
ASH	0.24	0.25	0.19
Probit distance of the 14 class			
ASL	2.0	2.93	...
ASH	0.85	0.88	...

ASL=Asymmetry Low. ASH=Asymmetry High.

such treatments on these selection lines is shown in Table 3. Heat has had little effect on ASH but seems to have improved the canalisation of ASL. This is not accompanied by any reduction in mean litter size or in the ratio of Tabby to normal mice, so it cannot be due to differential death of foetuses. Irradiation has a marked effect on the variance of both lines, but this is greater in the asymmetrical line. Irradiation at this time reduces the number of vibrissae and in neither line were mice with more than 14 vibrissae produced. Therefore no estimate can be made for the cut-off between 14 and 15, and the probit distance for the 14 class cannot be calculated.

So far only $Ta/+$ females have been considered, although $+/Y$ males are produced each generation and $+/+$ females and Ta/Y males every second generation. Selection or heating had no effect whatever on $+/+$ or $+/Y$. Only an occasional animal had a score which differed from the normal 16, and it was always a post-oral or ulna-carpal that was missing.

After irradiation, vibrissa numbers at all sites were reduced; the mean scores being 14.9 and 14.2 for ASH and ASL respectively. This is the opposite of the finding in $Ta/+$ females, where the effect on ASH was greater. The difference between ASH and ASL $+/Y$ males was entirely due to a reduction in the post-orals of the latter, and mice with the 2113/2113 score which was common among ASL $Ta/+$ females, were again common among ASL $+/Y$ males. The frequencies of normal and 2113/2113 were 0.19 and 0.41 in ASL; 0.41 and 0.05 in ASH.

Ta/Y males do not show a response comparable to that of $Ta/+$ females, in symmetry, in variance or in probit distances. In fact the only consistent change has been a slow increase in the mean vibrissa score of ASL.

Toes of luxate mice: The results of selection for within-litter range of toe numbers of lx/lx mice are shown as overall variance in Figure 7. There has been no change in the line selected for low range, but the high-range line does show an increase in variance. As with Tabby selection, probit analysis shows a consistent change in only one class. The probit distance for ten toes is decreasing in

FIGURE 7.—Variance of toe number in lines of luxate mice selected for high and low range of within-litter toe number.

the high-range line as the variance increases, but it is not changing in low-range line (Table 4).

DISCUSSION

Selection for symmetry has begun to produce a new canalisation zone in *Ta/+* mice. The criteria of variance, probit distance and response to environmental variables all confirm that canalisation at 14 vibrissae is taking place in ASL. Canalisation of a mutant phenotype at a level of expression differing from the normal canalisation zone has already been demonstrated in *Drosophila* (RENDEL 1959) by selecting for within-bottle variance, but selection for high variance was unsuccessful. In the line selected for asymmetrical vibrissae it does appear that there is some reduction in canalisation, but the evidence for decanalising selection is more convincing in the luxate lines. It seems that characters like vibrissae of Tabby mice or scutellar bristles of scute *Drosophila*, in which the mutant moves the expression of the character away from the normal canalisation zone, can be canalised at a new level, particularly if the level is one at which the expression

TABLE 4

Probit values for the 10 class in lx/lx mice for ten generations of selection

Generation	Low-range line	High-range line
1	1.32	2.64
2	1.15	1.57
3	1.43	1.37
4	2.00	0.99
5	1.38	0.79
6	1.17	0.88
7	1.19	1.08
8	1.01	0.52
9	1.75	1.10
10	1.51	1.04

is symmetrical. However, when in such cases canalisation has already been upset by a mutant gene, further decanalisation is difficult to produce. The toe number of luxates is another matter. The normal canalisation class is apparently still at the middle of the distribution. Ten hind toes is a highly invariant character in the mouse (a survey of 44,184 mice revealed only five deviations) and it seems reasonable that increasing canalisation at 10 will be difficult even in the presence of a mutant. In this case it is easier to reduce canalisation.

It is impossible to predict how long selection would have to be applied to alter the canalisation of $+/+$ or $+/lx$ animals. In fact, it may not be possible to do this with one treatment. In such a complex organ as the foot it may be necessary to use different treatments simultaneously.

The difference in response of ASL and ASH $+/Y$ males to irradiation suggests that the increase in $Ta/+$ canalisation has been achieved at the cost of $+/Y$ canalisation, but this would not explain why nearly half of the $+/Y$ males have the 2113/2113 pattern. The effect cannot be simply due to a greater susceptibility to radiation damage, as this would also be apparent at sites other than the post-oral. It seems likely that this pattern is being taken as "normal" in ASL so that radiation damage to the post-orals is not being repaired. It is even possible that the scores of Ta/Y males are tending towards the same pattern.

The milder heat treatment actually reduced the number of $Ta/+$ females with vibrissa patterns other than 2113/2113. This was surprising, because the heating of pregnant females is known to increase the variance of $Ta/+$ progeny (KINDRED 1963). Possibly the treatment caused enough damage to activate repair mechanisms without being too severe, and minor differences were compensated for at the same time.

Selection has been successful in producing differences in canalisation between lines. The asymmetry selection lines differ in symmetry, variance, probit value of the modal class, and response to radiation damage. Decanalising selection has also been successful in the lx/lx selection lines. These results were achieved in comparatively few generations of selection. If changes in canalisation can be produced so readily, it would appear that the genetic control of canalisation depends on relatively few genes.

SUMMARY

The methods of canalising selection which have been used with *Drosophila* cannot be used in mice, because of the smaller numbers of progeny. In two selection experiments which aim at altering the canalisation of (a) secondary vibrissae by selecting for asymmetry, and (b) the number of toes on the hind feet by selecting on the range of toe numbers found within a litter, selection has produced results in the mutants Tabby and luxate. There is insufficient variation in normal animals to permit selection.

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