SELECTION FOR AN INVARIANT CHARACTER, VIBRISSA NUMBER IN THE HOUSE MOUSE. V. SELECTION ON NON-TABBY SEGREGANTS FROM TABBY SELECTION LINES

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THE number of secondary vibrissae in unselected mice varies little. The normal number is 19: 2 supra-orbitals, 1 post-orbital, 2 post-orals and 3 ulna-carpals on each side with three inter-ramals which are median. About 15% of animals were found to have two rather than three inter-ramals but other variations are rare, particularly in noninbred mice (DUN and FRASER 1959).

As the Tabby gene reduces the number of vibrissae and increases their variance, selection for vibrissa number was practiced on Tabby mice and was successful in producing a response not only in Tabbies but also in non-Tabby sibs (DUN and FRASER 1959). This response was markedly asymmetrical (FRASER and KINDRED 1962). After 22 generations of selection on Tabby the mean vibrissa score of + segregants from the low line was 17.1, while the high line had a mean of 19.1. (Tabby is sex-linked and, as no differences were found in this experiment between +/Y males and +/+ females, they are grouped together and described as +.) Further, the mean of the high-line + mice had been 0.1 above the normal score for six generations and showed no indication that it would increase. At the time of writing, the line has been carried on for 34 generations and the mean of the high line is still 19.1. The first mice with extra whiskers had appeared in generation 6 and each subsequent generation included a few individuals with an extra supra-orbital or inter-ramal, but the bulk of the mice in this line had the normal 19. Compared to this very slight effect, the low line with a mean of 17.1 had very few mice with normal scores.

When the Tabby selection experiment was begun no attempt was made to select for vibrissa score of normal mice, as it was assumed that the variability was not great enough to permit successful selection. After selection on Tabby had produced + mice with varying scores, it was thought that selection on these should reveal something more of the process by which the normal phenotype is attained, despite the genetic variation which Tabby selection had demonstrated. It was also necessary to try selection on + mice from an unselected stock for comparison.

MATERIALS AND METHODS

Selection lines: 1. HST and LST (high selection Tabby and low selection Tabby) (DUN and FRASER 1959), selected for high and low numbers of secondary vibrissae in Tabby mice. Matings

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were *Ta*/+ × +/Y or +/+ × *Ta*/Y in alternate generations so that non-Tabby mice were produced each generation, but selection was on *Ta*/+ and *Ta*/Y only. Non-Tabby (+) mice that were the sibs of selected Tabby animals were used for mating.

2. HSN and LSN (high selection normal and low selection normal) selected for high and low numbers of vibrissae in normal mice. The Tabby gene was kept segregating, partly to keep the stocks as closely comparable as possible to HST and LST, and partly so that if selection were successful it would be possible to observe the effects on Tabby. The base population for HST and LST was not available, but an approximation to it was produced by using an unselected Tabby stock crossed with the 101 and CBA inbreds which predominated in the base stock.

3. HS+ and LS+ (high selection non-Tabby and low selection non-Tabby) derived from HST and LST, respectively, after generation 22 of selection. Matings and selection procedures were the same except that selection was on + mice.

4. XHST and XLST (relaxed selection) derived from HST and LST, respectively, after generation 12 of selection, but with random mating substituted for selection.

As a rule only first litters were used in all these selection lines, but in starting lines 3 and 4, second litters were taken from the ST parents so that there was no interference with the regular Tabby selection. In each line 30 females and ten males were mated each generation.

When Ragged (*Ra*) and crinkled (*cr*) were backcrossed to selection lines, five or six generations of *Ra*/+ or +/*cr* × + (selection line) matings were made so that the genetic background should have been predominantly that of the selection line. One generation of inbreeding was then carried out to produce *Ra*/*Ra* or *cr*/*cr* animals for scoring.

Scoring: Secondary vibrissae were scored under a desk lamp when the mice were 5 days old and checked at 10 days. Mystacials were scored at birth using a dissecting microscope as previously described (FRASER and KINDRED 1962). All vibrissae were equally weighted in determining the progenitors of the next generation.

RESULTS

Selection for high and low numbers of secondary vibrissae produced very little result in normal mice (Figure 1). The slightly lower vibrissa numbers obtained in the low line were entirely due to increased numbers of animals with two rather than three inter-ramals. No animal with extra vibrissae was found. A very different picture emerged when selection was practiced on + mice from the Tabby selection lines (Figure 2). HST and LST were continued as before and are shown for comparison. The difference between HST and HS+ is striking, but that between LST and LS+ is slight. The increase in HS+ was at first mainly an increase in supra-orbitals, but within a few generations extra post-orals, inter-

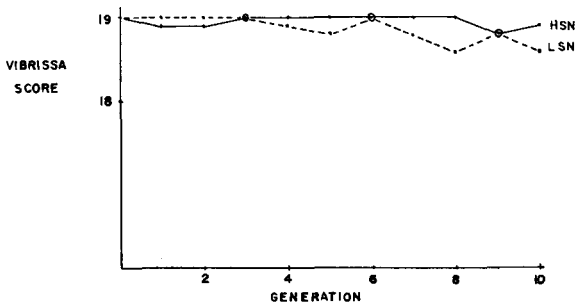

FIGURE 1.—Mean vibrissa scores for ten generations of selection on + mice. Scores are for SN (normal) +/Y males. Solid lines—high selection normal. Dashed line—low selection normal.

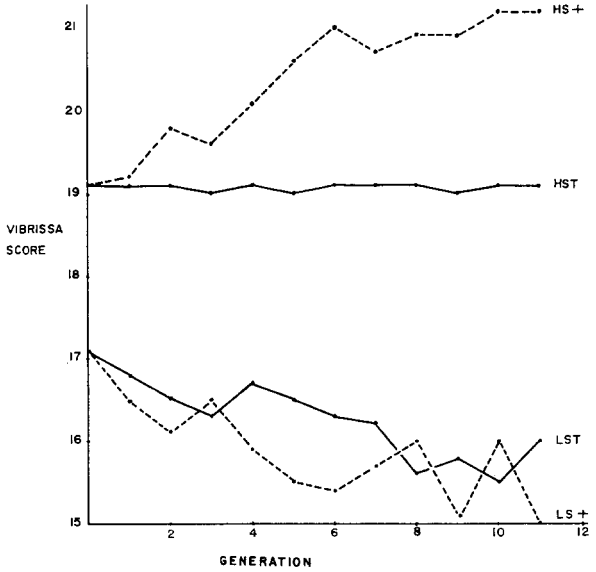

FIGURE 2.—Mean vibrissa scores for + mice from the S+ (non-Tabby) selection lines compared with + mice from the ST (Tabby) selection lines.

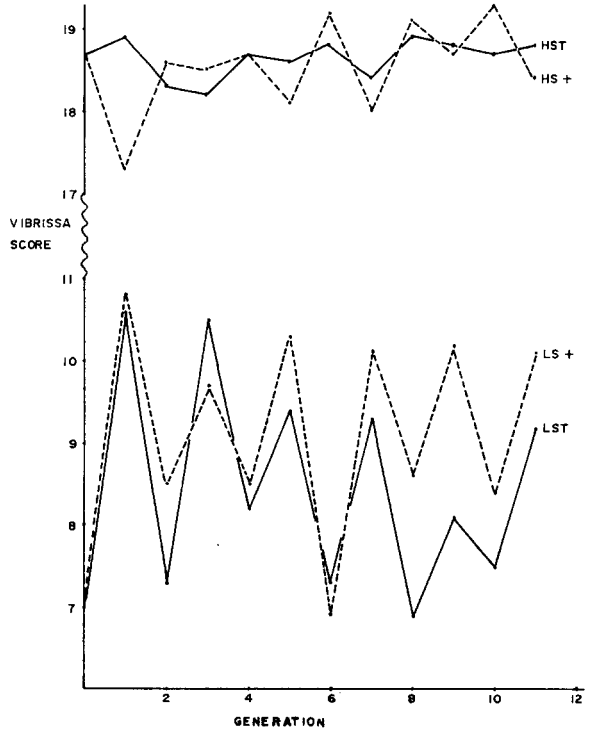

FIGURE 3.—Mean vibrissa scores of *Ta*/+ females from S+ (non-Tabby) and ST (Tabby) selection lines.

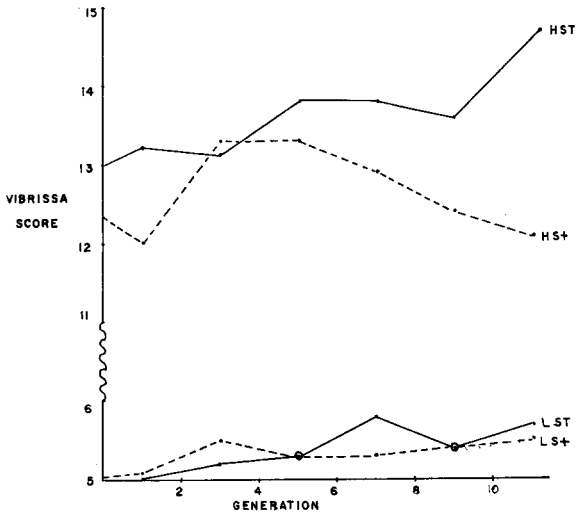

FIGURE 4.—Mean vibrissa scores of *Ta*/Y males from the S+ (non-Tabby) and ST (Tabby) selection lines.

ramals and ulna-carpals were also occurring. The only site not involved was the post-orbital.

Since the Tabby gene had been kept in these lines it was possible to compare the effects on *Ta*/+ and *Ta*/Y mice as well as + (Figures 3, 4). From the means there appears to be little difference between the lines selected on + and those

TABLE 1

Variance of Ta/+ *and* Ta/*Y mice from non-Tabby, Tabby and relaxed selection lines*

Genotype	Generation of of selection	Selection line					
		HST	HS+	XHST	LST	LS+	XLST
Ta/+	1*(23)	0.60	4.88	2.63	4.91	4.32	8.50
	2	1.24	2.58	1.50	4.42	5.59	6.05
	3	1.26	2.17	3.18	5.27	5.77	6.05
	4	0.46	2.35	2.56	5.26	5.66	7.06
	5	0.63	2.57	2.55	7.20	6.26	7.53
	6	0.44	1.30	2.54	4.99	4.65	5.21
	7	0.98	3.02	3.64	4.96	5.14	6.27
	8	0.22	1.79	1.21	2.82	4.53	7.60
	9	0.28	2.34	2.03	4.70	4.52	4.86
	10	0.59	3.61	2.80	3.22	1.96	5.42
Ta/Y	1(23)	2.91	4.48	3.83	0.52	0.79	0.88
	3	1.67	1.56	2.75	0.69	0.74	0.68
	5	1.77	1.61	2.92	0.50	0.63	1.58
	7	1.54	1.67	2.22	0.55	0.52	0.93
	9	1.32	1.65	2.45	0.74	0.54	1.12

* Generation 1 of HS+ is equivalent to Generation 23 of HST.
HST=high selection Tabby. HS+=high selection non-Tabby. XHST=relaxed selection derived from HST.LST=low selection Tabby, etc.

TABLE 2

Distributions of total vibrissa scores for Ta/+ *and* Ta/Y *selected for Tabby and normal compared with unselected animals*

Genotype	Selection line	Vibrissa score																	
		21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3
Ta/+	Unselected	.	.	13	15	36	58	28	15	8	4	4	1	.	1	.	.	.	.	.
	HST	1	2	34	3	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.
	HS+	.	2	17	6	3	1	1	2	1	2	.	.	.	.	.	.	.	.	.
	LST	.	.	.	.	.	.	.	2	4	6	9	3	6	.	1	2	1	.	.
	LS+	.	.	.	.	.	.	.	1	5	5	10	3	1	2	2	.	1	.	.
Ta/Y	Unselected	.	.	.	.	.	.	.	.	3	7	9	22	30	21	20	15	3	.	.
	HST	.	.	.	.	.	4	7	10	10	7	4	2	1	.	.	.	.	.	.
	HS+	.	.	.	.	.	2	.	9	5	10	7	4	3	.	1	1	.	.	.
	LST	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5	15	3	1
	LS+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	9	18	4	1

selected on Tabby. There is only a tendency for LS+ *Ta*/+ females and HS+ *Ta*/Y males to move back towards the mean values for unselected mice. However, in HS+ there is a very noticeable effect on variance. In both *Ta*/+ females and *Ta*/Y males variance has increased even in the first generation of selection on +. No such effect on variance was found in LS+. This is what happened when selection was relaxed in the ST lines and random mating was substituted (Table 1). More detail is given for the first generation in Table 2. There was, at first, little change in the mean of *Ta*/+ or *Ta*/Y although in time the means moved slowly toward the unselected value but there was an immediate increase in variance. In the relaxed lines, however, the variance changed in the low line as well as in the high. This indicates that in HS+ but not in LS+, the response to selection in + was accompanied by suspension of selection on Tabby. It will be noticed that the variance of LST *Ta*/+ females is much greater than that of HST *Ta*/+ and that this is reversed for *Ta*/Y males. This is a consequence of the relationship of the mean to the canalisation zone (KINDRED 1963). Since the means of the ST, S+, and XST lines differ little, in the first few generations at least, this matter does not complicate the comparison of these lines.

There are two other mutants which affect the number of vibrissae: Ragged (CARTER and PHILLIPS 1954) and crinkled (FALCONER, FRASER and KING 1951). Crinkled (*cr*), an autosomal recessive, is a mimic of Tabby and homozygotes cannot be distinguished phenotypically. A *Ta/Ta cr/cr* mouse also looks exactly like *Ta/Ta* or *cr/cr*. Further, when crinkled is backcrossed to + mice from the HST and LST selection lines the scores of *cr/cr* are comparable to those of *Ta*/Y (KINDRED unpublished).

When Ragged (*Ra*) and Tabby are compared, a very obvious interaction is found (Table 3). No *Ta/Ta Ra/Ra* animals have been produced, as both *Ta/Ta Ra*/+ and *Ta*/Y *Ra*/+ animals are very poorly fertile. *Ta/Ta* and *Ta*/Y have not been found to differ in hair or vibrissa growth, so it is unlikely that *Ta/Ta Ra/Ra* would differ from *Ta*/Y *Ra/Ra*. Backcrossing Ragged to HST and LST +

TABLE 3

Comparison of the effects of Tabby and Ragged combinations on vibrissae and hair

Genotype	Mystacials (scored at birth)	Secondaries (scored at 10 days)	Hair
+/Y *Ra*/+	48 (normal—no variance)	19 (normal—no variance)	Reduced number of zigzags causes rough looking coat.
+/Y *Ra*/*Ra*	mean 6.4	Usually born dead.	Animals which survive are almost naked. Most follicles are present (SLEE 1962).
Ta/+ +/+	48 (normal—no variance)	mean 14.2 variance 9.4	Coat appears striped. The frequency of hair types is altered and abnormal hairs are present.
Ta/+ *Ra*/+	48 (normal—no variance)	mean 13.1 variance 8.4	Coat both rough and striped.
Ta/+ *Ra*/*Ra*	mean 4.3	Usually born dead.	Resemble +/+ *Ra*/*Ra*
Ta/Y +/+ *Ta*/*Ta* +/+	48 (normal—no variance)	mean 8.0 variance 3.2	Smooth looking coat composed of abnormal hairs.
Ta/Y *Ra*/+ *Ta*/*Ta* *Ra*/+	mean 46.8	mean 6.1 variance 1.4	A few abnormal hairs only.
Ta/Y *Ra*/*Ra*	No external sign of follicles—only one retarded follicle found in one mouse which was sectioned.	Born dead. No external sign of follicles.	No external sign of follicles. Sections show a few condensations in the Malpighian layer of the skin at birth.

Variances are given only when more than 20 animals were scored.

mice resulted in practically no effect on the number of secondary vibrissae, and little effect on the mystacials, of *Ra/Ra* (FRASER and KINDRED 1962). This suggests that the modifiers which affect Tabby do not have much effect on Ragged. The results of backcrossing Tabby and Ragged to inbred lines confirm this. With Tabby the highest scores were found in TaDBA, followed by TaC57, Ta101, TaCBA and TaA in that order. With Ragged the order was Ra101, RaC57, RaCBA, RaDBA and RaA.

After six generations of S+ selection, Ragged was also backcrossed to these lines. With the HS+ background the mean secondary vibrissa score for +/+ (21.00) is appreciably different from *Ra*/+ (19.9). The difference is not significant, as the variance of *Ra*/+ is high, but compared to LS+, where the means for +/+ and *Ra*/+ are 15.6 and 15.4 respectively, it is quite large. Secondary vibrissae are not scored on *Ra/Ra*, and the difference in the mystacial scores was no greater than it was when Ragged was backcrossed to HST and LST.

Both ST and S+ lines were selected on secondary vibrissae but some changes were found in the mystacials (Table 4). High mystacial scores are much more common in HS+ than in HST. Mystacials were scored at birth, and low scores found in both LST and LS+ were due to slow growth of fibres; a few days later these vibrissae were present.

DISCUSSION

Considering HS+ first: when selection pressure is applied to + mice instead of Tabby, the scores of *Ta*/+ and *Ta*/Y sibs of the + mice behave as if selection has been suspended, yet there is a very rapid response in +. This immediately suggests that selection has been transferred to a different system. It is also striking that selection on + produces such very different results after preliminary selection on Tabby. All these data can be explained if there are two systems contributing to the development of follicles and vibrissae, the products of which must be present in balanced amounts. If the Tabby mutant affects one of these (the Tabby system) by reducing its efficiency, this system will then become limiting and vibrissa growth will be reduced. Selection for high scores in Tabby mice will then be selection for modifiers which tend to restore the efficiency of this system. Presumably they could also increase the efficiency of the Tabby

TABLE 4

Mystacial scores of + mice from + and Tabby selection lines

Mystacial number	Selection line			
	HST	HS+	LST	LS+
52	.	3	..	...
51	.	8	..	...
50	2	14	..	...
49	6	41	..	...
48	48	104	60	112
47	..	...	..	...

system in normal sibs of selected mice, but this would not result in extra vibrissae because the other system would become limiting; extra whiskers would only appear in those animals which by chance had a high rating in this system as well. As soon as selection is practised on these, a rapid response ensues, since the level of the Tabby system is already high. In HSN where there was no prior selection on Tabby, selection was unsuccessful.

The type of response found in the low vibrissa selection lines fits into this theory too. By selecting for low vibrissa scores of Tabby mice the efficiency of the Tabby system in + sibs is also lowered and vibrissa scores are reduced. Subsequent selection on + does not then produce the striking effects found in the high line, because with the Tabby system reduced and the second system normal, selection on + will still affect the Tabby system. Hence in LS+ the system on which selection acts is not changed and Tabby mice do not show the increase in variance found in HS+.

Crinkled, if it is not the same mutant as Tabby, affects the same system and is acted upon by the same modifiers. Ragged is not greatly affected by the Tabby modifiers, but there is a considerable difference in Ragged mice after backcrossing to HS+ and LS+. It seems likely, in fact, that Ragged is a mutant on the second pathway. This would explain the drastic effects of the *Ta/Y Ra/Ra* genotype. As vibrissa follicles are present in both *Ta*/Y and *Ra/Ra*, their almost complete absence in *Ta*/Y *Ra/Ra* seems disproportionate but would be reasonable if two major systems were reduced. Another piece of evidence, unconvincing by itself but adding to the idea outlined here, comes from backcrossing Tabby and Ragged to inbred strains. In DUN's (1958) original survey of vibrissa numbers, he found in the A strain far more low vibrissa scores than in any other stock, and it is on an A background that the lowest vibrissa scores for both Tabby and Ragged mice are found. The mystacials are practically unaffected by selection on Tabby, but in HS+ the numbers are higher. It could be coincidence that the Tabby gene has no effect on the mystacials of unselected mice, while homozygous Ragged mice have greatly reduced mystacial numbers.

This immediately demonstrates that the idea of two systems is an oversimplification, as Tabby has a greater effect on some vibrissae than on others. Perhaps this is a matter of timing, but a simple balance between two systems could not explain the normal invariant phenotype either. It may be that a balance must be struck at different stages of development and that appropriate gradients must be established. Alternately, each system might be of the type proposed by RENDEL, SHELDON and FINLAY (1966) to explain the invariant scutellar bristle number in *Drosophila melanogaster*, a system involving more direct control on the gene product.

SUMMARY

Selection for the number of secondary vibrissae of Tabby mice has produced a good response in the low line and a much smaller response in the high. In these lines a marked difference in the mean scores of non-Tabby mice also appeared,

although very little variation in vibrissa numbers occurs in unselected mice and an attempt to select on this was unsuccessful. After Tabby selection had been practised for 22 generations, selection on + mice produced rapid and striking results.

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