

Selection for Constancy of Score and Pattern of Secondary Vibrissae in *Ta/Ta-Ta/Y* and *Ta/+* Mice

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Abstract

In wild-type mice, total secondary vibrissa score is almost invariably 19. This score is made up of the scores at three paired sites and one median site on the head and one paired site on the fore paws, namely supraorbital (2/2), postorbital (1/1), postoral (2/2), inter-ramal (3) and ulnacarpal (3/3). When the sex-linked gene Tabby (*Ta*) is introduced, total score is reduced in all genotypes and total and individual site scores become more variable. In *Ta/Ta* females and *Ta/Y* males total score becomes about seven as a result of score reductions at all sites. The most common scores at these sites are: supraorbital (1/1), postorbital (0/0), postoral (1/1), inter-ramal (1), and ulnacarpal (1/1). In *Ta/+* females total score becomes about 15, largely as a result of score reductions at the sites around the mouth. The most common scores at these sites are postoral (1/1) and inter-ramal (1).

Lines selected for constancy of total secondary vibrissa score in *Ta/Ta-Ta/Y* mice (ASH line) and for constancy of total score in *Ta/+* mice (ASL line) were examined for evidence as to whether constancy of total score was the result of regulation of total scores or of regulation of scores at the individual whisker sites. In the ASH line it was found that the increase in the proportion of *Ta/Ta-Ta/Y* mice with seven vibrissae was due, almost exclusively, to an increase in the frequency of animals with scores characteristic of standard *Ta/Ta-Ta/Y* animals at the individual whisker sites. In the ASL line, also, the increase in the proportion of *Ta/+* mice with 15 vibrissae was due largely to a rise in the frequency of mice with individual site scores characteristic of the standard *Ta/+* mouse. In neither line was there any evidence of negative correlations between site scores in either the selected or the unselected genotypes.

It was concluded that constancy of total score in mice carrying the *Ta* gene is due to regulation of scores at the individual whisker sites rather than to regulation of total vibrissa score.

Introduction

The total secondary vibrissa score in the mouse is made up of the sum of the scores at three paired sites and one median site on the head (supraorbital, postorbital, postoral and inter-ramal) and at one paired site on the fore paws (ulnacarpal) (Dun and Fraser 1959). In wild-type mice total score is almost invariably 19. When the sex-linked gene Tabby (*Ta*) is introduced, scores are reduced and become more variable. In *Ta/Ta* females and *Ta/Y* males, scores at all whisker sites are markedly reduced. In *Ta/+* females the sites around the mouth are more seriously affected than those near the eyes or those on the fore paws. *Ta/+* females, therefore, tend to have scores typical of *+/+* and *+/Y* mice at the orbital and ulnacarpal sites ('+' sites) and tend to have scores typical of *Ta/Ta* and *Ta/Y* mice at the oral sites ('*Ta*' sites).

The constancy of the total secondary vibrissa score in *+/+* mice could be due to a system regulating total score irrespective of scores at the individual sites, or it could

be due to regulation of the scores at the individual sites. Regulation of total score has been shown to be responsible for control of scutellar bristle numbers in *Drosophila* (Rendel 1965) and of total vertebral scores in the mouse (Pennycuik, unpublished data). However, the spatial separation of the secondary vibrissa sites, the relatively late appearance of the vibrissae during embryogenesis (12 days +) and the dependence of vibrissa development upon dermal-epidermal interactions (e.g. Kollar 1972) make it quite possible that constancy of total score is the outcome of constancy of scores at the individual whisker sites.

In 1970 we reported the results of an experiment in which *Ta/Ta* and *Ta/Y* mice were selected for total secondary vibrissa scores of seven (ASH line) and in which *Ta/+* mice were selected for total secondary vibrissa scores of 15 (ASL line) (Johnston *et al.* 1970). In both lines there was an increase in the frequency of animals of the selected genotype which had scores in the modal class for the selected genotype. The scores of mice from these two lines have now been examined for evidence as to whether constancy of score was due to regulation of total score or to regulation of scores at the individual whisker sites.

Materials and Methods

Scoring

In all genotypes, secondary vibrissae were counted first when the pups were 5 days old. These scores were checked when the pups reached 10 days of age; if the score had increased, the second score was taken as being the score for the site. A vibrissa was considered to be absent if no fibre occupying the position was more than half the normal length. Any fibre less than half the length was taken to be a hair. Scores at each site were recorded separately as follows: a/a_1 , b/b_1 , c/c_1 , d , e/e_1 where a is the left supraorbital score, a_1 the right supraorbital score, b and b_1 postorbital scores, c and c_1 postoral scores, d the score at the inter-ramal site, and e and e_1 ulnarpal scores. Animals with the most common scores for the genotype at all sites were said to have total scores of the standard pattern for that genotype. In estimating the scores at the five different kinds of site, scores at the paired sites were added together but inter-ramal numbers were taken as scored. Total secondary vibrissa scores were calculated by adding scores for all sites. Kindred (1967) omitted inter-ramal sites in calculating total secondary vibrissa scores. Her 14-class and 6-class closely approximate to but do not exactly coincide with the 15- and 7-class respectively of Johnston *et al.* (1970).

History of the Lines

Kindred (1967) established the base stock for the selection lines described in this paper by crossing mice from a random breeding stock to *Ta* mice from a strain in which the gene had been backcrossed to the inbred strain CBA. She maintained this base stock for three generations prior to and while the selection lines were being established. In generations 1 and 3 she mated $+/Y$ males to *Ta/+* females; in generation 2 she mated *Ta/Y* males to $+/+$ females. Generations 1 and 3 produced $+/+$, *Ta/+*, $+/Y$ and *Ta/Y* offspring born to *Ta/+* mothers (Kindred 1967). In the present experiment the scores of the offspring of these two latter generations were used as base stock scores partly because the range of genotypes correspond more closely with those produced in later generations, and partly because Kindred (1961) had demonstrated that *Ta/+* offspring of *Ta/+* mothers had higher vibrissa scores than *Ta/+* offspring of $+/+$ mothers.

Kindred established two selection lines; one line, ASH, was selected for a high degree of asymmetry of scores, and the other, ASL, was selected for a low degree of asymmetry of scores. In both instances selection was based primarily on symmetry or asymmetry of *Ta/+* scores but *Ta/Y* scores were taken into account where these were available. In both lines the genotypes of the parents in the odd generations were *Ta/+* \times $+/Y$ and the genotypes of the parents in the even generations were $+/+$ \times *Ta/Y*. Both selection lines were maintained for 13 generations under this regime (Kindred 1967).

Johnston *et al.* (1970) continued Kindred's two lines but changed the selection procedure. In the ASH line, selection was for seven vibrissae in *Ta/Ta* and *Ta/Y* mice. Thirty families were ranked

on the basis of the proportion of $Ta/Ta-Ta/Y$ mice with scores of seven and mice with scores of seven were selected from the high-ranking families as parents for the next generation. In the ASH line selection was for 15 vibrissae in $Ta/+$ females. Families were ranked on the proportion of $Ta/+$ mice with scores of 15 and females with scores of 15 were selected from the high-ranking families as parents for the next generation. In addition to these changes in the selection procedure, the genotypes used as parents were changed; instead of using Kindred's alternating system, $Ta/+$ females were mated to Ta/Y males in all generations, i.e. the offspring produced were $+/Y$, $Ta/+$, Ta/Y and Ta/Ta . The selection lines were maintained for a further 10 generations under this regime.

Selection Procedure from Generation 24 to Generation 33

At generation 24 the selection procedure in both lines was again changed: instead of basing selection solely on total score, the pattern of the score was also taken into account. In the ASH line 30 matings were ranked according to the fraction of Ta/Ta and Ta/Y offspring that had seven vibrissae arranged in the standard pattern for these genotypes. From the 10 litters with the highest fraction, 15 Ta/Y males were selected with seven vibrissae arranged in this pattern. From the same litters 30 $Ta/+$ females were taken at random. These animals were the parents of the next generation.

In the ASL line 30 matings were ranked according to the fraction of $Ta/+$ females that had 15 vibrissae arranged in the standard pattern for $Ta/+$. Thirty $Ta/+$ females with 15 vibrissae arranged in this pattern were taken from the 10 matings with the highest fraction of this sort of $Ta/+$ female, and 15 Ta/Y males were taken at random from the same litters. These were the parents of the next generation. Since almost all mice with seven or 15 vibrissae had these arranged in the standard pattern (Table 2), there was little difference in practice between this and the previous selection procedure.

Data Presented

The effects of the three different selection procedures on the *total* score of the three genotypes was assessed by examining mean scores, variances and probit widths of the modal score characteristic of the genotype in question (i.e. $Ta/Ta-Ta/Y$, 7; $Ta/+$, 15; $+/+ - +/Y$, 19). The probit widths of the modal classes are presented for all generations. Means and standard deviations are given for the base generation (generations 1 and 3, see above) and for the final generation of each selection regime, namely generations 14, 23, and 33. Generation 14 was chosen in preference to generation 13 to illustrate the effects of selection for asymmetry and symmetry because Kindred used different genotypes as parents from those used by Johnston *et al.* (1970). By using generation 14 instead of generation 13 it was possible to standardize the genotypes of the parents and to standardize the genotypes of the young scored.

The effects of the three different selection procedures on scores at the five different vibrissa sites was assessed by measuring the proportion of mice with site scores characteristic of the genotype under consideration. Probit widths of the modal class could not be used for individual site scores because classes on one side or the other of the modal class were very frequently empty. The percentages of mice with the standard score at each site are presented for all generations. Detailed results of scores at each site are presented for the base generation and for generations 14, 23, and 33, i.e. at the end of each selection regime.

Results

Total Scores in the ASH Line

Total scores of mice selected for asymmetry of $Ta/+$ and Ta/Y (generations 1–13), for Ta/Ta and Ta/Y with seven vibrissae (generations 14–23), and for Ta/Ta and Ta/Y with seven vibrissae in the standard pattern (generations 24–33) in the ASH line are given in Table 1. This table illustrates that all selection procedures used led to constancy of mean score in the selected genotype ($Ta/Ta-Ta/Y$), that variance changed little when selection was for asymmetry but that variance declined rapidly when selection was for score constancy, and that the probit width of the 7-class increased from 1.2σ in the base generation to 4.12σ at generation 33 (Fig. 1). Table 2 shows that the increase in the probit width of the 7-class was due to an

Table 1. Means, variances and probit distances of each genotype in the ASH and ASL lines
 Probit values marked with a dagger were for vibrissa classes at the end of the range for that generation

Selection line	Generation	Ta/Ta and Ta/Y				Ta/+				+/Y		
		No. of mice	Mean	σ^2	Probit 7-class	No. of mice	Mean	σ^2	Probit 15-class	No. of mice	Mean	σ^2
Base		38	7.34	1.37	1.20	54	15.22	5.04	0.76	92	18.97	0.03
ASH	14	52	7.12	1.47	2.00	29	14.59	6.23	0.53	36	18.91	0.08
	23	83	7.18	0.22	2.85	36	13.64	5.44	0.38	36	18.94	0.05
	33	102	7.05	0.11	4.12†	56	12.29	8.61	0.42	63	18.95	0.05
ASL	14	31	8.53	1.67	1.30†	33	15.29	2.09	1.22	76	18.82	0.17
	23	104	8.82	2.13	1.63	63	14.86	1.51	1.73	63	18.65	0.39
	33	116	7.85	1.00	2.37†	61	14.87	0.68	2.18	60	18.76	0.21

increase in the proportion of mice with seven vibrissae and that, in almost all instances this increase in the frequency of mice with seven vibrissae was due to an increase in the number of mice with seven vibrissae arranged in the standard pattern.

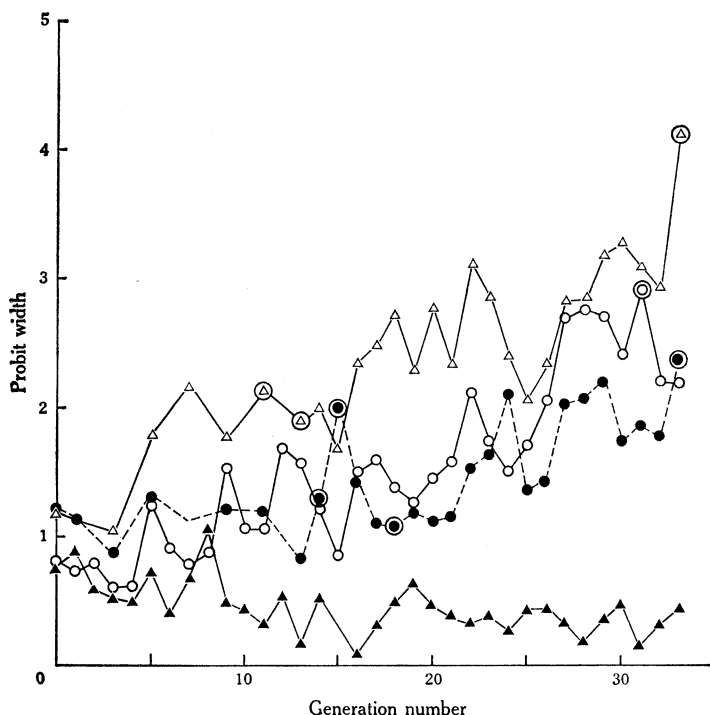


Fig. 1. Probit distances of the modal classes of $Ta/Ta-Ta/Y$ mice (7 vibrissae) and $Ta/+$ mice (15 vibrissae) in the ASH and ASL selection lines. \triangle $Ta/Ta-Ta/Y$ (selected genotype), ASH line. \blacktriangle $Ta/+$, ASH line. \circ $Ta/+$ (selected genotype), ASL line. \bullet $Ta/Ta-Ta/Y$, ASL line. Circled values were for vibrissa classes which were at the end of the range for that generation.

In the unselected Tabby genotype ($Ta/+$) mean score declined, variance increased and the probit width of the 15-class declined (Table 1). In the unselected genotype, $+/Y$, mean score, variance, and the probit width of the 19-class remained fairly constant (Table 1).

Scores at each Site in the ASH Line

Fig. 2 illustrates the changes in the frequency of mice with modal scores at each whisker site characteristic of the selected genotypes, $Ta/Ta-Ta/Y$, and Table 3 shows the frequencies of the different score classes at each site at the end of each selection regime. In the base stock, scores at all sites other than the postoral tended to be above the mode. The most significant changes with selection in this genotype were a rise in postoral scores and a fall in ulnacarpal scores. At the other sites there was an overall reduction in the number of mice with scores differing from modal scores; this was apparent at the inter-ramal site during the last period of selection only.

Fig. 3 illustrates the changes in the frequency of mice of the unselected genotype, $Ta/+$, with modal scores characteristic of the genotype. Clearly, selection for constancy in $Ta/Ta-Ta/Y$ caused a fall in the frequency of animals with modal scores at the supraorbital, postorbital and ulnarpal sites, i.e. at sites at which $Ta/+$ mice have modal scores like those of $+/Y$ mice. Postoral and inter-ramal scores, on the other hand, showed some fluctuations, but no definite trend in frequency. Table 3, which gives the scores at each whisker site in the base stock and at the end of each selection regime, shows that the decline in the frequency of the modal class at the orbital and the ulnarpal sites was due to an increase in the frequency of classes below the mode; in the case of ulnarpal scores, the modal score dropped from 6 to 4, half-way between the modal scores of $Ta/Ta-Ta/Y$ and $+/Y$. The changes in the frequencies of scores in the various classes at the oral sites were not significant (Table 3).

In the unselected $+/Y$ genotype, which shares no modal scores with $Ta/Ta-Ta/Y$, scores at all sites were unaffected by selection (Table 3).

Table 2. Frequencies of $Ta/Ta-Ta/Y$ mice with total scores of 7 and with scores of 7 arranged in the standard pattern in the ASH line, and frequencies of $Ta/+$ mice with total scores of 15 and with scores of 15 arranged in the standard pattern in the ASL line

Generation	$Ta/Ta-Ta/Y$ mice (ASH line)			$Ta/+$ mice (ASL line)		
	Total No. of mice	Percentage with total score of 7	Percentage with vibrissae in standard pattern	Total No. of mice	Percentage with total score of 15	Percentage with vibrissae in standard pattern
Base	38	42.1	42.1	54	29.6	27.8
14	52	67.3	67.3	33	45.5	42.4
23	83	78.3	78.3	63	60.3	58.7
33	102	96.1	95.1	61	72.1	72.1

Relationship in the ASH Line between the Scores at each Vibrissa Site and Total Score in the Selected Genotype ($Ta/Ta-Ta/Y$) and the Unselected Genotype ($Ta/+$)

If regulation of total score as such were keeping total score constant, site scores would tend to compensate for each other; when one site score was high another would be low. On the other hand, if constancy of total score were a byproduct of constancy at each site and site scores were independent of one another, the distribution of total scores would be predictable from the distributions of site scores; the frequency of total scores with values of 0, 1, 2, 3, 4 etc. could be estimated from the frequency of site scores with values of 0, 1, 2 etc. The distribution of total scores estimated from the distribution of site scores on the assumption that these are independent of each other can then be compared with the actual distribution of total scores and the significance of departures from the estimated distribution can be tested by χ^2 .

Generations 14–33 were included in these estimations. Generations 14–20, 21–26 and 27–33 were pooled. The significance of χ^2 values are given in Table 4. In the selected genotypes Ta/Ta and Ta/Y observations fit estimations almost exactly. In $Ta/+$ genotypes, on the other hand, there was a tendency for scores at all sites to move in the same direction regardless of genotype. If scores were low at one site there was a tendency for scores to be low at all sites and vice versa. These positive relationships were highly significant. This analysis reveals no sign of any overall

control of variation in total score but does suggest that mean score has some overall influence acting on it in *Ta/+*.

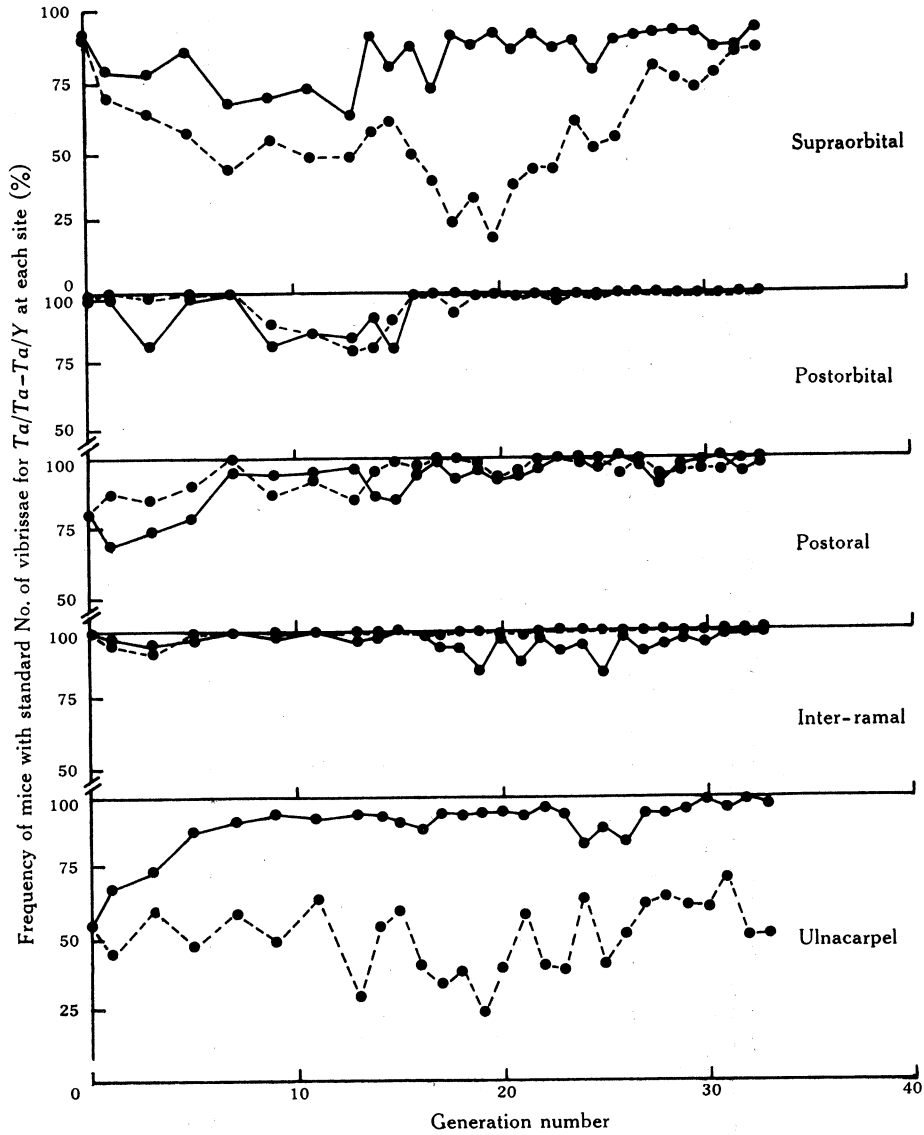


Fig. 2. Frequencies of *Ta/Ta-Ta/Y* mice with modal scores at the five individual vibrissa sites in the ASH line (●—●, *Ta/Ta-Ta/Y* selected) and the ASL line (●--●). Modal scores at each site were: supraorbital (2), postorbital (0), postoral (2), inter-ramal (1), ulnacarpal (2).

Total Scores in the ASL Line

Total scores of mice selected for symmetry in *Ta/+* and *Ta/Y* (generations 1–13), selected for 15 vibrissae in *Ta/+* (generations 14–23) and for 15 vibrissae in the standard pattern (generations 24–33) in the ASL line are also given in Table 1. This table illustrates that all selection procedures used led to constancy of mean score in

Table 3. Number of mice in each class of the individual site scores in the ASH line

Genotype	Gener- ation	Supraorbital				Postorbital				Postoral				Intra-ramal				Ulnacarpal					
		1	2	3	4	0	1	2	3	4	0	1	2	3	1	2	3	4	5	6			
Ta/Ta-Ta/Y	Base																						
	14	36	1	1		37	1			3	3	31	1										
	23	49	2	1		47	4	1		3	3	45	1			38							
	33	1	74	8		83						83				51	1						
χ^2		99		3		102						2	100			77	6						
		3.77										20.03***				1	101						
Ta/+	Base																						
	14	1	4	49		3	3	48				41	6	7									
	23	4	1	24		1	4	24				20	6	3									
	33	2	4	30		2	5	29				28	2	6									
χ^2		14	9	33		11	14	31				50	5	1									
		21.40**										9.09											
+/Y	Base																						
	14											1	90										
	23																						
	33																						

** 0.001 < P < 0.01. *** P < 0.001.

the selected genotype ($Ta/+$), that variance declined from 5.04 to 0.68 over the 33 generations of selection, and that the probit width of the 15-class increased steadily.

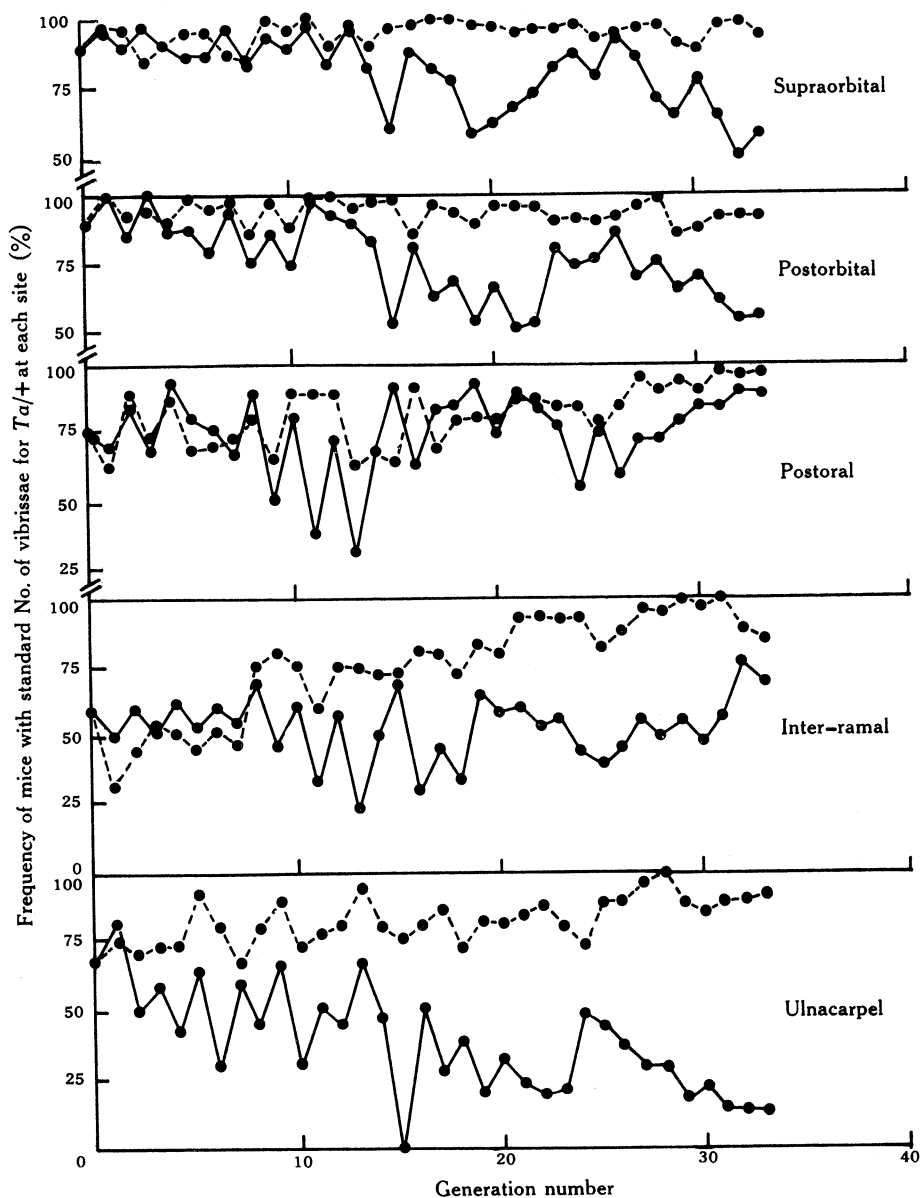


Fig. 3. Frequencies of $Ta/+$ mice with modal scores at the five individual vibrissa sites in the ASH line (●—●) and the ASL line (●---●, $Ta/+$ selected). Modal scores at each site were: supraorbital (4), postorbital (2), postoral (2), inter-ramal (1), ulnacarpal (6).

However, this increase (from 0.76 to 2.18 σ) was small by comparison with the increase in the probit width of the 7-class in $Ta/Ta-Ta/Y$ mice from the ASH line (Table 1, Fig. 1). Table 2 shows that the increase in the probit width of the 15-class in $Ta/+$ mice was due to an increase in the proportion of mice with 15 vibrissae, and

that almost all of these mice with 15 vibrissae had the vibrissae arranged in the standard pattern.

In the unselected Tabby genotypes ($Ta/Ta-Ta/Y$), mean scores rose and then fell again, variance was high by comparison with that of $Ta/Ta-Ta/Y$ mice from the ASH line, but the probit width of the 7-class increased (Fig. 1). This rise in probit width was due to a fall in the number of mice with scores below seven rather than to a rise in the frequency of mice in the 7-class. Only at generation 33 was the frequency of mice in the 7-class above that in the base generation (49 v. 42%).

In the second unselected genotype of this line, $+/Y$, mean score declined and variance increased over the first 23 generations. By generation 33, mean score had increased slightly and variance had declined (Table 1).

Table 4. Contingency table for the ASH line

Contingency tables were prepared to show the interrelationships between scores at one whisker site with scores at other sites in mice from the ASH line. This table shows the significance of the χ^2 values calculated from these tables

Vibrissa group	Generation	$Ta/Ta-Ta/Y$				$Ta/+$			
		Post-orbital	Post-oral	Inter-ramal	Ulna-carpel	Post-orbital	Post-oral	Inter-ramal	Ulna-carpel
Supraorbital	14-20	—	—	—	—	***	***	***	***
	21-26	—	—	—	—	***	*	***	***
	27-33	—	—	—	—	***	***	***	***
Postorbital	14-20		*	—	—		***	***	***
	21-26		—	—	—		***	***	***
	27-33		—	—	—		***	***	***
Postoral	14-20			—	—			***	***
	21-26			—	—			***	***
	27-33			—	—			***	***
Inter-ramal	14-20				—				***
	21-26				**				**
	27-33				—				***

* $0.01 < P < 0.05$. ** $0.001 < P < 0.01$. *** $P < 0.001$.

Scores at each Site in the ASL Line

Fig. 3 illustrates the increase, over 33 generations of selection, in the frequency of mice with modal scores at each site characteristic of the selected genotype ($Ta/+$). At the orbital sites the frequencies of mice with scores in the modal class were high in the base stock and remained at a high level over the 33 generations of selection. At the oral and ulnarpal sites frequencies of mice with scores in the modal class were relatively low in the base stock. Selection over 33 generations caused an increase in the frequency of animals in the modal classes. Table 5, which gives the number of animals in each score class for the five vibrissa sites in the base stock and at the end of each selection regime, shows that the changes in the class frequencies with selection were significant for the oral and ulnarpal sites. Changes at the orbital sites, both of which have $+/Y$ modal scores, were not significant.

Fig. 2 illustrates the changes in the frequencies of $Ta/Ta-Ta/Y$ mice (unselected genotypes) with modal scores for each of the whisker sites. The site which showed the most dramatic change was the supraorbital: numbers in the modal class at this

Table 5. Number of mice in each class of the individual site scores in the ASL line

Genotype	Gener- ation	Supraorbital				Postorbital				Postoral				Intra-ramal				Ulnacarpal						
		1	2	3	4	0	1	2	3	4	0	1	2	3	1	2	3	4	5	6				
Ta/Ta-Ta/Y	Base	36	1	1		37	1			3	3	31	1			38			1	21	14	2		
	14	18	8	5		25	4	2			1	30				31				17	7	7		
	23	47	26	31	3	101	3				1	103				104			1	42	32	19	8	2
	33	103	9	4		115	1					116				116				61	31	22	2	
χ^2		183.87***				23.61***				—				—				10.60						
Ta/+	Base	1	4	49		3	3	48				41	6	7		32	10	12			10	7	37	
	14		2	30			1	32				23	7	3		24	8	1			1	5	27	
	23	1	1	61		3	3	57				54	6	3		58	5				2	10	51	
	33		4	57		5	5	56				59	2			52	9				2	3	56	
χ^2		2.14				1.90				17.92**				39.62***				18.90**						
+/Y	Base				91			91				1	90				2	89					91	
	14				76			1	75			3	73				9	67				1	75	
	23				63				63			2	2	59			1	14	48				63	
	33				60				60			9	51				6	54					60	
χ^2		—				—				13.33**				17.83***				—						

** 0.001 < P < 0.01. *** P < 0.001.

site declined until generation 20, then rose again to base stock levels at generation 33. The decline in frequency of the modal class at the supraorbital site was accompanied by an increase in the frequency of mice with scores which were higher than the mode; mean scores, therefore, increased accordingly. At the ulnarpal site, too, the frequency of mice in the modal class (2) declined up to generation 23 and then reverted towards preselection frequencies. However, the changes at this site were not significant. At the postorbital site and at the two sites with scores common to $Ta/Ta-Ta/Y$ and $Ta/+$ (postoral and inter-ramal) modal scores characteristic of $Ta/Ta-Ta/Y$ were found in all mice by generation 16 or sooner and the frequency of mice in these classes remained high until generation 33 (Fig. 3). In $Ta/Ta-Ta/Y$ mice, therefore, the increase in the total score over the first 23 generations of selection (Table 1) was due largely to an increase in the frequency of mice with high ulnarpal scores. The increase in probit width of the 7-class with increasing generation number (Table 1) was due to an increase in the frequency of mice with modal scores characteristic of $Ta/Ta-Ta/Y$ in *all* sites: it was not due, solely, to increasing stability at the sites with modal scores common to $Ta/Ta-Ta/Y$ and $Ta/+$.

In the unselected genotype, $+/Y$, the frequency of the modal classes at sites with scores common to $+/Y$ and $Ta/+$ was unchanged over the 33 generations of selection. At the postoral and inter-ramal sites, however, the frequency of animals with 4 postorals and 3 inter-ramals declined over the first 23 generations of selection. This decline in oral scores was responsible for the fall in total score in this genotype.

Relationship in the ASL Line between the Scores at each Vibrissa Site in the Selected Genotype ($Ta/+$) and the Unselected Genotypes ($Ta/Ta-Ta/Y$)

The relationship between the scores at the various secondary vibrissa sites was estimated from contingency tables. As in the ASH line, only generations 14–33 were considered in these estimates and these generations were pooled into three groups: generations 14–20, 21–26, 27–33. The significance of the χ^2 values of the selected genotype, $T/+$, and the unselected Tabby genotypes ($Ta/Ta-Ta/Y$) are shown in Table 6.

In the selected genotype, $Ta/+$, the relationships between the scores at the '+' sites (supraorbital, postorbital and ulnarpal) were positive and highly significant, and the relationships between the scores at the 'Ta' sites were also highly significant, but '+' scores showed no tendency to move in concert with 'Ta' scores. In this, $Ta/+$ mice from the ASL line differed from those of the ASH line. The strong relationship between scores at '+' sites and between scores at 'Ta' sites in the ASH line was established by generations 14–20 and this relationship was retained for generations 21–26 and generations 27–33.

Although scores were more variable in $Ta/Ta-Ta/Y$ mice of the ASL line than in those of the same genotype from the ASH line, scores at one site showed little tendency to move in the same direction as those at another. The only exception to this was that in the period when total scores for $Ta/Ta-Ta/Y$ tended to rise (generation 23, see Table 1), an increase in postoral scores tended to accompany a rise in ulnarpal score.

Discussion

The constancy of mean total scores of mice of the selected genotypes in both the ASH and ASL lines, the low variances and the large probit widths of the modal score

classes, all confirm our earlier findings that total score stability can be improved by selecting for score constancy in both $Ta/Ta-Ta/Y$ and $Ta/+$ mice, and that, contrary to expectation, selection in $Ta/+$ mice for constancy at 15 vibrissae increased the probit width of the 7-class in $Ta/Ta-Ta/Y$ (Johnston *et al.* 1970). The rise in the frequency of mice with scores of the standard pattern which accompanied the decline in the variance and the increase in the probit width of the 7- and 15-classes, in selected genotypes in the two lines, suggested that total score stability was achieved by increasing the stability of the scores at the individual whisker sites. This suggestion was supported by the observation that the relationships between the individual site scores in mice of the selected genotype were never negative. It is, therefore, unlikely that constancy of total score was due to a regulatory system in which an increase in score at one site was balanced by a decline in score at a second.

Table 6. Contingency table for the ASL line

Contingency tables were prepared to show the interrelationships between scores at one whisker site with scores at other sites in mice from the ASL line. This table shows the significance of the χ^2 values calculated from these tables

Vibrissa group	Generation	$Ta/Ta-Ta/Y$				$Ta/+$			
		Post-orbital	Post-oral	Inter-ramal	Ulna-carpel	Post-orbital	Post-oral	Inter-ramal	Ulna-carpel
Supraorbital	14-20	—	—	—	—	***	—	—	*
	21-26	—	—	—	—	***	—	—	***
	27-33	—	—	—	*	***	—	—	***
Postorbital	14-20		—	—	—		—	—	***
	21-26		—	—	—		—	—	***
	27-33		—	—	—		—	—	***
Postoral	14-20			—	—			***	*
	21-26			—	**			***	—
	27-33			—	—			***	—
Inter-ramal	14-20				—				—
	21-26				—				—
	27-33				—				—

* $0.01 < P < 0.05$. ** $0.001 < P < 0.01$. *** $P < 0.001$.

Embryologically, total secondary vibrissa score is dependent upon three processes: the determination of the areas where groups of vibrissae will develop (8 days), the initiation of one or more follicles in each of these areas (12 days +), and the successful development or regression of the follicles which have been initiated (12-19 days) (Jacobson 1966). Since total score stability seems to be due to increased stability at the individual whisker sites rather than to balancing of one group against another, it is unlikely that the genes regulating the areas where whisker groups will develop are involved in determining score stability. Rather, score stability at the individual whisker sites must depend upon genes which regulate follicle initiation, and development or regression of these follicles, at the individual whisker sites. These genes regulating final follicle number could possibly be common to all whisker groups but it is unlikely that the set regulating scores in $+/+ - +/Y$ mice at the '+' score level are the same as those involved in regulating scores in $Ta/Ta-Ta/Y$ mice at the 'Ta' score level, for constancy of scores in wild-type mice does not promote constancy in $Ta/Ta-Ta/Y$ mice from the same stocks (Dun and Fraser 1959).

Regulation of individual sites scores in $Ta/+$ mice from the ASL line must be more complex than that in $+/-+/Y$ and in $Ta/Ta-Ta/Y$ mice from the ASH line, for females of this genotype are composed of mosaics of X^{Ta} and X^+ cells (e.g. McLaren *et al.* 1973). The fact that the orbital and ulnacarpal sites usually have scores which approach the '+' level suggests that the effects of the X^+ cells predominate at these sites. Regulation of scores at these '+' sites could be effected by a regulator system similar to that in $+/-+/Y$ mice, but the genes accumulated during selection for 15 vibrissae in the ASL line, which promoted constancy at '+' sites in $Ta/+$ animals, had no observable effects on scores in their $+/-+/Y$ sibs. Similarly, the fact that scores at the oral sites in $Ta/+$ mice are usually at the 'Ta' level, suggests that X^{Ta} cells exert a greater effect on oral scores than X^+ cells. Regulation of scores at these 'Ta' sites could be due to a regulator system similar to that accumulated in $Ta/Ta-Ta/Y$ mice in the ASH line, but the fall in the oral site scores in $+/-+/Y$ mice from the ASL line suggests that regulation of scores at 'Ta' sites in $Ta/+$ mice might have been due, in part at least, to the accumulation of modifiers peculiar to vibrissae at the oral sites (cf. i.r. line described by Dun and Fraser 1959). One further mechanism remains which may possibly promote constancy of score in $Ta/+$ mice: because they are mosaics of X^{Ta} and X^+ cells some genetic mechanism may operate which influences the frequency of active X^{Ta} and X^+ cells at the different whisker sites. It is now well established that the proportion of maternal X to paternal X cells in certain tissues is not always 1 : 1 (for example Lyon 1972; Takagi and Sasaki 1975). If the ratios of $X^{Ta} : X^+$ cells at the orbital and ulnacarpal sites in $Ta/+$ mice were distorted by selection in favour of X^+ and the ratio of $X^{Ta} : X^+$ cells at the oral sites were distorted by selection in favour of X^{Ta} , score constancy at these sites would not be unexpected.

When the effects of selection on both the selected genotypes and the unselected genotypes in the two lines are taken together, they suggest that although the number of positions at which secondary vibrissae groups will appear is regulated by the embryos as a whole, regulation of numbers at each site is under the control of factors operating within that site. It is the individual whisker group score, therefore, rather than the sum of the secondary vibrissa sites, which is equivalent to scutellar score in *Drosophila* or to total vertebral score in the mouse.

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References

- Dun, R. B., and Fraser, A. S. (1959). Selection for an invariant character, vibrissae number, in the house mouse. *Aust. J. Biol. Sci.* **12**, 506-23.
- Jacobson, C. M. (1966). A comparative study of the mechanisms by which X-irradiation and genetic mutation cause loss of vibrissae in embryo mice. *J. Embryol. Exp. Morphol.* **16**, 369-79.
- Johnston, P. G., Pennycuik, P. R., and Rendel, J. M. (1970). Selection for constancy of expression of the *Tabby* gene in the mouse. *Aust. J. Biol. Sci.* **23**, 1061-6.
- Kindred, B. M. (1961). A maternal effect on vibrissae score due to the *Tabby* gene. *Aust. J. Biol. Sci.* **14**, 627-36.

- Kindred, B. M. (1967). Selection for canalization in mice. *Genetics* **55**, 636–44.
- Kollar, E. J. (1972). The development of the integument: spatial, temporal and phylogenetic factors. *Am. Zool.* **12**, 125–35.
- Lyon, M. F. (1972). *X*-chromosome inactivation and developmental patterns in mammals. *Biol. Rev. Cambridge Philos. Soc.* **47**, 1–35.
- McLaren, A., Gauld, I. K., and Bowman, P. (1973). Comparison between mice chimaeric and heterozygous for the *X*-linked gene *Tabby*. *Nature (London)* **241**, 180–3.
- Rendel, J. M. (1965). Bristle pattern in *scute* stocks of *Drosophila melanogaster*. *Am. Nat.* **99**, 25–32.
- Tagaki, N., and Sasaki, M. (1975). Preferential inactivation of paternally derived *X* chromosome in the extraembryonic membranes of the mouse. *Nature (London)* **256**, 640–2.

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