

## Footpad bristles: a convenient metric character in mice

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### SUMMARY

A new metric character in mice, 'footpad bristle number' is described. The character is easily measured under a stereo-microscope, is very highly inherited, and is independent of age, sex and the environment common to littermates. Such a character might prove useful in a number of quantitative genetic studies in mice.

### 1. INTRODUCTION

In certain types of genetic investigation metric characters are used as a means of differentiating between populations or in order to investigate some quantitative genetic hypothesis, rather than because of their own intrinsic interest. Most metric characters in mice are either difficult or impossible to measure in the live animal (e.g. skeletal measurements, Festing, 1972), or are closely related to body weight. This can be a disadvantage, as body weight is dependent on both age and the environment, and in some cases (e.g. in wild populations) age is unknown and the immediate environment cannot be specified. Moreover, even in laboratory populations, it is often inconvenient either to measure the metric character at a given age, or to have to make a correction for body weight.

The ideal metric character in mice might be akin to bristle number in *Drosophila*. It would be highly heritable, easily measured in the dead or live animal (anaesthetized if necessary), and independent of age or weight over a large proportion of the total life-span. Ideally, the character should be anatomical, and should be relatively independent of the animals' postnatal environment. The only character described so far that approaches this specification is the number of tail rings (Grüneberg, 1952), but with a mean number of about 180 rings these are tedious to count. A search for a more convenient character shows that the number of bristles on the hind footpad of mice appears to be a potentially useful metric character.

### 2. MATERIALS AND METHODS

#### (i) *Counting the bristles*

The six walking pads on the ventral surface of the hind feet of mice (Cook, 1965) are entirely free from hair and surround an elliptical area containing a number of

bristles. These bristles can be counted in the dead or anaesthetized animal under a stereo-microscope (30-80 × magnification) with good side illumination. The most convenient method of counting was found to be by using the higher levels of magnification and focusing only on the base of the bristles. Some practice and experimentation with the angle and intensity of the illumination was necessary before repeatable results could be obtained. A zoom stereo-microscope was found to be most convenient as all intermediate levels of magnification could be obtained. In some strains (particularly NZW) a proportion of double hair follicles were noted; these were counted as one. Thus the counts given in this paper strictly refer to hair follicles, though the term 'bristles' is used for convenience. All counts given in this paper were completed on dead mice and depending on the mean number of bristles in the strains being counted, it proved possible to count about 30-40 mice per hour.

(ii) *Experiment 1*

Five female mice from each of six inbred strains (B10.A/Lac, B10.LP-a/Lac, NZW/Lac, C3H/HeLac, NMRI/Lac, and CBA/H-T6/Lac), aged approximately 6 months old were used in the first experiment, and duplicate counts were made on the 30 randomized individuals. The origin and characteristics of all strains used are described by Parrott & Festing (1971). The mice were reared in Specified Pathogen Free (SPF) conditions, but were transferred to a conventional animal room at about 4 weeks of age. To ensure that each strain was fully inbred confirmatory histo-compatibility tests were conducted. Counts on two additional strains, B10.BR/Lac and A2G/Lac were made at a later date. Strain means and standard deviations were examined to determine whether the raw data should be transformed. An hierarchical analysis of variance was used to estimate the proportion of the observed variation that could be accounted for by differences between the six strains, differences between individuals within strains and differences between counts on individuals.

(iii) *Experiment 2*

Five litters totalling 28 mice of strain CE/Lac, ranging in age from 25 to 48 days, were analysed for heterogeneity among the litter by sex subclasses in order to determine the importance of sex, age and maternal influences due to variation in litter size and mothering ability. Small numbers of young (21-40 days) and old (6 months) mice of strains A2G and B10.BR were also compared to examine the effects of age and size over a wider range of ages. The effect of sex on mean bristle count was also estimated from an analysis of counts on 22 male and 18 female C57BL/10ScSn and 11 male and 15 female NMRI mice, all of comparable ages. Finally, small numbers of homozygous hairless (hr hr) mice were examined.

(iv) *Experiment 3*

F1, F2 and backcrosses to both parents were made between strains C57BL/10ScSn × NMRI and C57BL/10ScSn × CE, except that no F2 progeny were

obtained in the latter case. Unfortunately, only small numbers of some of these crosses were available. Data on these crosses were used in scaling tests (Mather & Jinks, 1971) to determine the adequacy of fit to an additive-dominance mode of inheritance. According to this model the following statistics should not be significantly different from zero:

$$\begin{aligned} A &= 2B_1 - P_1 - F_1 & V_A &= 4V_{B_1} + V_{P_1} + V_{F_1} \\ B &= 2B_2 - P_2 - F_1 & V_B &= 4V_{B_2} + V_{P_2} + V_{F_1} \\ C &= 4F_2 - 2F_1 - P_1 - P_2 & V_C &= 16V_{F_2} + 4V_{F_1} + V_{P_1} + V_{P_2} \end{aligned}$$

where  $P_1$  and  $P_2$  are the parental means, and  $F_1$ ,  $F_2$ ,  $B_1$  and  $B_2$  are the means of the first- and second-generation crosses and the backcrosses to the two parental strains, respectively and  $V_x$  stands for the variance of the respective group. In the case of the crosses involving C57BL/10ScSn and NMRI all three statistics could be calculated, but in the crosses with CE only A and B (designated A\* and B\*) could be calculated as no  $F_2$  crosses were available.

Further analysis to determine the average degree of dominance, the number of gene loci controlling foot bristle number, and the heritability of bristle number were not carried out because such analyses depend on precise estimates of the variances within each of the segregating generations, and the numbers were too small to provide such data.

### 3. RESULTS

#### (i) Experiment 1

The mean bristle count in eight strains of mice is given in Table 1. There was a high positive correlation between the mean and the standard deviation of the raw bristle count, as would be expected with a Poisson distribution. A square-root transformation of the raw data substantially reduced, though it did not entirely eliminate this correlation, and all subsequent analyses were carried out on the square-root of the raw data.

Table 1. *Strain means in Experiment 1*

Strain	Raw data		Range 1st count	Transformed $\sqrt{x}$	
	Mean	s.d.†		Mean*	s.d.†
B10.A‡	77.4	9.8	62-89	8.78	0.55
B10.LP-a‡	68.4	9.7	54-74	8.25	0.60
B10.BR	60.2	7.6	51-67	7.74	0.50
NZW‡	56.4	5.7	47-56	7.50	0.39
C3H/He‡	37.4	2.9	30-42	6.11	0.24
A2G	26.8	4.0	23-32	5.16	0.39
NMRI‡	22.0	4.0	16-26	4.67	0.44
CBA-T6‡	20.6	4.4	16-26	4.52	0.48

(All samples consist of 5 female mice approximately 6 months old.)

\* Least significant difference = 0.26.

† Standard deviation.

‡ Means of duplicate counts.

Mean transformed bristle count ranged from 4.52 to 8.78, a range of over nine phenotypic standard deviations (using the average of the standard deviations given in Table 1), indicating a substantial degree of inheritance. Examination of the observed ranges showed that there was no overlap between strains with a high and a low bristle count. All means except B10.BR and NZW and NMRI and CBA-T6 were significantly different from one another, as judged approximately by the least significant difference (Snedecor, 1956). Thus among eight pure lines at least six different mean counts were observed.

Table 2. *Analysis of variance of duplicate counts and strain means (data  $\times 10 \sqrt{x}$ )*

Source	Degrees of freedom	Mean square	Expected mean square
Strains	5	3052.64	$\sigma_0^2 + 2 \sigma_1^2 + 10 \sigma_8^2$
Individual within strains	24	38.55	$\sigma_0^2 + 2 \sigma_1^2$
Counts within individuals	30	4.10	$\sigma_0^2$
Total	59		

Table 3. *Variance components from Experiment 1*

Item	Component	Value	Percentage
Counts	$\sigma_0^2$	4.10	1.3
Individuals	$\sigma_1^2$	17.23	5.3
Strains	$\sigma_8^2$	301.41	93.4
			100.0

Duplicate counts made on six of the strains were analysed to determine the contribution of counting error, variation within strains and variation between strains to the total variation. The mean difference between the first and second count was three bristles, with the most serious discrepancy amounting to 13 bristles (69 on the first count and 82 on the second). In general the second count was higher than the first, indicating one possible source of bias as counting skill improves, though as noted below such a bias is negligible in comparison with the difference between strains.

Analysis of variance of the six strains in which duplicate counts were made is given in Table 2, and the estimated variance components are given in Table 3. These components show that 93.4% of the observed variation could be accounted for by differences between strains, 5.3% by variation between individuals within strains, and only 1.3% by variation between counts. Thus in most studies duplicate counts would probably not be worthwhile, as counting error is minimal, apart from the improvement from first to second count noted above.

(ii) *Experiment 2*

An analysis of variance was used to determine the degree of heterogeneity among ten litter by sex subclasses (5 litters) in CE mice. These subclasses varied in size from one to six individuals, and although the age range was only about 4 weeks, it covered the period of maximum growth. There was no evidence of heterogeneity ( $F = 1.42$ , 9 and 18 degrees of freedom), suggesting that neither age, sex, nor the common environment among littermates is of any great importance in determining foot bristle number.

Mean bristle counts in seven 21–40-day A2G and 4 B10.BR mice were compared with the mean bristle count on 6-month-old mice given in Table 1. Although a slightly lower bristle count was observed in the older mice, the difference was not statistically significant. However, in view of the low numbers of animals, this test lacked statistical power and it is probably safer to conclude that there may have been some loss of bristles in the older mice, though this loss was not substantial.

Statistical analysis of the bristle counts in 22 male and 18 female C57BL/10ScSn and 11 male and 15 female NMRI supported the finding on CE mice of no difference between the sexes.

No foot bristles were observed in 3- to 6-week-old homozygous hairless mice.

(iii) *Experiment 3*

Mean (transformed) bristle count pooled across sexes in three pure lines and various types of cross are given in Table 4. Mean bristle count in the cross designated  $F_1$  was slightly lower than the mean of parental strains, whereas in  $F_1^*$  the mean count was slightly higher than the mid-parental count. Thus there was no evidence of heterosis. Means of the backcrosses were in all cases intermediate between those of the pure lines and the  $F_1$  hybrids.

The five scaling statistics A, B, C,  $A^*$  and  $B^*$  are given at the bottom of Table 4. None of these appears to be significantly different from zero, with the possible exception of B. Thus the data would appear to fit an additive-dominance model as described by Mather & Jinks (1971).

Further analysis of the data in Table 4 was prevented by the extremely high variance observed in the  $F_2$  cross. When substituted in Wright's (1934) formula to estimate the number of effective factors controlling a quantitative character, this led to an estimate of less than one locus. The very high variance in the  $F_2$  cross may be attributed to sampling variance with such a small sample size.

## 4. DISCUSSION

The number of bristles on the hind footpad appears to be a useful new metric character in mice. It is easy to measure in either dead or alive anaesthetized mice, is independent of age at least during the period of most active growth, and is very highly heritable. The exact mode of inheritance has not been determined, but the

Table 4. Mean bristle counts in inbred strains and crosses (transformed  $\sqrt{x}$ )

Designation	Strain or cross†	N	Mean *	S.D.‡
P <sub>1</sub>	C57BL/10ScSn(B)	46	8.215	0.539
P <sub>2</sub>	NMRI (N)	34	4.790	0.610
F <sub>1</sub>	N × B F1	5	6.042	0.343
F <sub>2</sub>	N × B F2	20	6.260	1.489
B2	(N × B F1) × N	12	5.992	0.962
B1	(N × B F1) × B	21	7.243	0.901
P* <sub>2</sub>	CE	28	6.391	0.618
F* <sub>1</sub>	CE × B F1	13	7.599	0.831
B* <sub>1</sub>	(CE × B F1) × B	13	7.487	0.720
B* <sub>2</sub>	(CE × B F1) × CE	13	7.108	0.593

## Scaling tests

A	0.229 ± 0.459
B	1.152 ± 0.586
C	-0.049 ± 1.137
A*	-0.840 ± 0.468
B*	0.370 ± 0.418

† Female parent recorded first. There were no reciprocal crosses.

‡ Standard deviation.

observation that many inbred strains differ significantly from each other rules out a single-locus two-allele Mendelian mode of inheritance, and it seems probable that many loci are involved. Scaling tests show that after a square-root transformation of the raw data, an additive-dominance model of inheritance can be assumed, indicating a relatively simple mode of inheritance. However, there were insufficient data to obtain reliable estimates of the main genetic parameters. The lack of a difference between sexes and the absence of a correlation between littermates are added advantages, since these are two more factors that do not need to be taken into account when studying different populations.

A character of this type might prove useful in studying natural mouse populations, where age, number of siblings and the proximal environmental conditions experienced by individual mice are not known. The character might also be useful in distinguishing between laboratory populations in much the same way as mandible shape is now being used (Festing, 1974). If the mode of inheritance can be established more satisfactorily, bristle number in mice might become analogous to bristle number in *Drosophila*, which has been used in a very wide range of genetic studies.

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