

## Variation in the shape of the mouse mandible

### 1. Effect of age and sex on the results obtained from the discriminant functions used for genetic monitoring

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#### SUMMARY

The shape of the mandible of the mouse can be described by a series of discriminant functions which have been used to discriminate between and investigate relationships among strains of mice. The effect of sex and variation in age of the animal on the results obtained from these functions has been investigated. Significant sex differences in mandible shape were detected, but these were considerably smaller than the differences found between two inbred strains. A simple correction for expressing each measure as a proportion of the sum of the measures on each bone removes the effects of overall size. Significant age effects were found, but these were only large in animals under seven weeks of age where considerable changes are taking place in the relative lengths of bone measurements. Routine testing for genetic authenticity using the shape of the mandible is possible over a wide age range and may be an efficient method for monitoring genotypes at the end of long-term experiments.

#### 1. INTRODUCTION

Genetic contaminations of mouse and rat strains are not uncommon (Festing & Lovell, 1980; Kahan *et al.* 1982) and may result in a considerable waste of resources. There are several methods of genetic monitoring, including a comparison of the shape of the mandible of suspect animals with mandibles of authentic animals (Festing, 1972; Lovell & Festing, 1982). Festing (1973) made a preliminary study of the growth of the mandible and suggested that the shape (i.e. the relative length of different measurements of the mandible) does not change much as the animal ages.

The shape of the mandible can be described by a series of discriminant functions

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(Festing, 1979) which have been used for genetic monitoring (Festing, 1974), and for exploring the relationship between inbred strains (Festing & Lovell, 1980). The aim of this study was to investigate the effect of the animal's age and sex on the results obtained using this particular set of functions. Mandibles from animals outside the age range used in routine genetic monitoring were included in some of the samples.

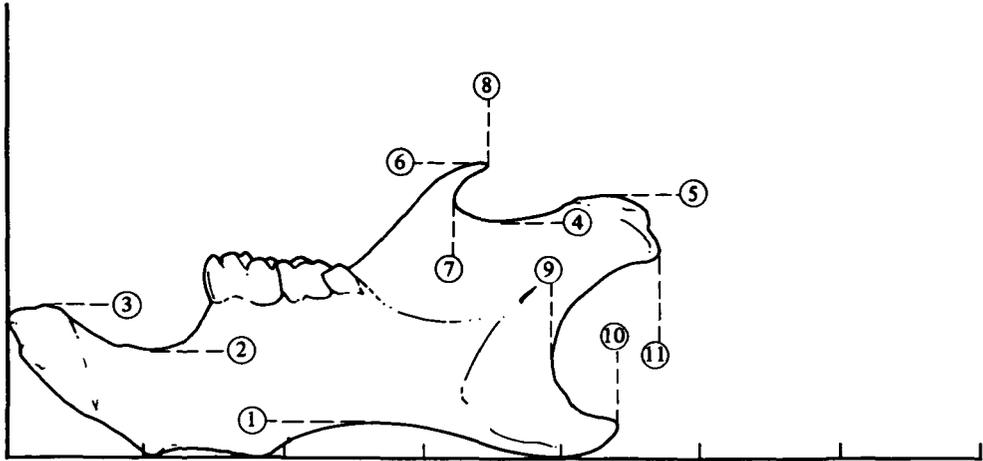


Fig. 1. Diagram of right mandible of a C57BL/6J mouse showing the eleven measurements made on each mandible in this study.

## 2. MATERIALS AND METHODS

### (i) *Animals*

The mice used in this study were from two inbred strains and two  $F_1$  hybrids of various ages. Eight- to ten-week-old male and female C57BL/6J and DBA/2J were obtained from the Jackson Laboratory, Bar Harbor, Maine. They were maintained up to eighteen weeks of age in the NIEHS animal facility under conditions conforming to the AALAS guidelines. NIH-31 diet (Ziegler Bros., Inc., P.O. Box 95, Garner, PA 17324) and water were offered *ad libitum*. Male NZW  $\times$  NZBF $_1$  (NZB  $\text{♀}$   $\times$  NZB  $\text{♂}$ ) and B6D2F $_1$  (C57BL/6J  $\text{♀}$   $\times$  DBA/2J  $\text{♂}$ ) hybrids were bred at the MRC Laboratory Animals Centre under conditions conforming to category \* under the MRC Laboratory Accreditation Scheme (Townsend, 1969). They were offered BP diet No. 3 (BP Nutrition (UK) Ltd, Witham, UK) and water *ad libitum*. The mice were humanely killed and the mandibles prepared by papain digestion (Festing, 1972).

### (ii) *Statistical procedures*

Eleven measurements were made on the right mandible of each mouse (Fig. 1). Each measurement was then expressed as a percentage of the sum of all 11 measurements in order to correct for gross differences in overall size. Four canonical variates (CV I–CV IV) were obtained from them using the discriminant

functions described by Festing (1979, p. 50). These canonical variates can be regarded as a numerical index of mandible shape after correction for gross differences in size. Overall size was estimated from the sum of the overall measurements.

The data from the two inbred strains were analysed by a series of factorial analyses of variance and by joint regression analysis (Snedecor & Cochran, 1968). The variation in the shape of the mandible within each genotype was also investigated by regressing the canonical variates on age and the overall size of the mandible (calculated as the sum of the measurements on each bone).

Table 1. *Factorial analysis of variance of four measures of mandible shape of C57BL/6J and DBA/2J mice<sup>a</sup>*

Source	df	CV I	CV II	CV III	CV IV
Age (A)	6	NS <sup>b</sup> (1)	NS (1)	***	5 NS (1)
Genotype (G)	1	*** 26	*** 41	***	37 *** 6
Sex (S)	1	NS (0)	*** 22	NS (0)	*** 25
A × G	6	** 7	* 2	NS (0)	NS (1)
A × S	6	NS (0)	NS (-1)	* 4	* 3
G × S	1	* 3	*** 4	NS (0)	NS (0)
A × G × S	6	* 6	NS (0)	NS (2)	** 11
Within-cell mean square	356	0.139 57	0.135 31	0.149 51	0.132 53

NS = not significant; \* =  $P < 0.05$ ; \*\* =  $P < 0.01$ ; \*\*\* =  $P < 0.001$ .

<sup>a</sup> Analysis carried out on age/genotype/sex means. Error term calculated using harmonic mean (Snedecor & Cochran, 1968). Weighted least-squares analysis including ages where not all genotypes were available were carried out using the statistical program BMDP 2V (Dixon & Brown, 1979), results not shown. All analyses gave similar results with only slight differences in the probability levels for some items.

<sup>b</sup> Proportion of total variation in sample accounted for by factor (non-significant items in parentheses).

### 3. RESULTS

#### (i) C57BL/6J and DBA/2J

Factorial analysis (Table 1) showed highly significant differences between the two strains for all four canonical variates ( $P < 0.001$ ) and between the sexes of the same strain. The relative lengths of measurements 7, 8 and 11 were smaller in the female mice while measurements 1 and 10 were larger. These differences resulted in females having significantly larger negative CV II and larger positive CV IV values ( $P < 0.001$ , Table 2).

The only canonical variate which showed a significant age effect was CV III, the values decreasing in older animals. A number of first- and second-order interactions were also significant. Only one of these items contributed more than 10% of the variation in the material. None of the interactions involving age had a simple interpretation.

The linear regression of overall size on age had been highly significant in each group. The simple correction of expressing each measure as a proportion of the sum of measures, however, removes most of the effect of overall size from the measures of mandible shape.

Table 2. Average values of the first four canonical variates of the mandible of genotypes used in this study ( $\pm$  standard deviation) overall size is based on the sum of the eleven values taken on each mandible

Genotype and sex	Age range (days)	n	CV I	CV II	CV III	CV IV	Overall size
C57BL/6J ♂♂	58-126	116	3.91 $\pm$ 1.38	-0.19 $\pm$ 1.36	0.19 $\pm$ 1.51	2.18 $\pm$ 1.18	573.9 $\pm$ 10.1
C57BL/6J ♀♀	57-126	147	3.94 $\pm$ 1.45	-1.98 $\pm$ 1.36	0.33 $\pm$ 1.29	3.57 $\pm$ 1.41	562.2 $\pm$ 12.9
DBA/2J ♂♂	61-125	159	2.47 $\pm$ 1.32	-2.80 $\pm$ 1.19	2.18 $\pm$ 1.45	1.78 $\pm$ 1.32	547.8 $\pm$ 11.2
DBA/2J ♀♀	74-128	91	2.81 $\pm$ 1.26	-3.72 $\pm$ 1.23	1.80 $\pm$ 1.46	2.80 $\pm$ 1.31	545.0 $\pm$ 10.8
NZW $\times$ NZBF <sub>1</sub> ♂♂	11-183	180	-0.30 $\pm$ 1.36	-0.52 $\pm$ 1.28	1.56 $\pm$ 1.51	-0.07 $\pm$ 1.61	601.5 $\pm$ 35.5
B6D2F <sub>1</sub> ♂♂	36-145	69	2.66 $\pm$ 1.21	-2.90 $\pm$ 1.31	2.40 $\pm$ 1.08	1.34 $\pm$ 1.28	594.3 $\pm$ 11.9

The original analysis used to obtain the discriminant function produced a set of canonical variates with an overall mean of zero and a within-group standard deviation of one. Table 2 shows that the within-group standard deviations are greater than one for the inbred strains, reflecting to some extent the age effects described earlier. Other environmental effects over the collection period probably account for some of the increased variance.

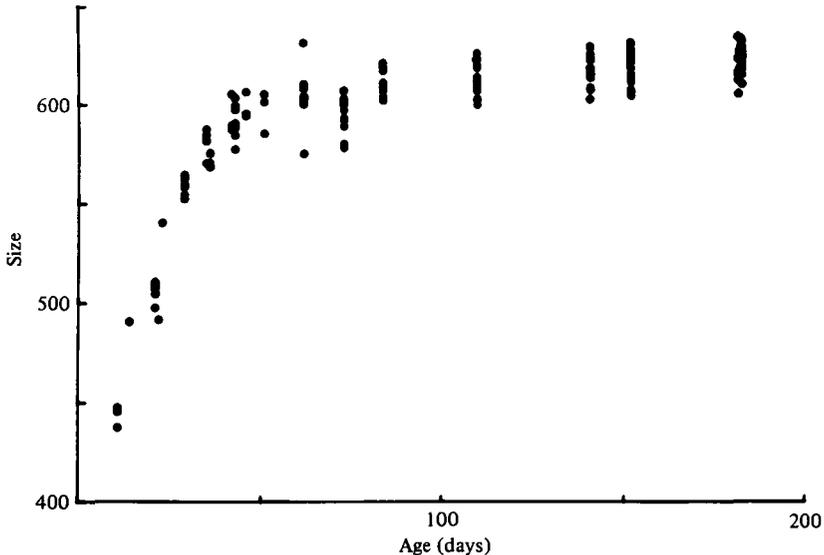


Fig. 2. Graph of mandible size against age of individual NZW  $\times$  NZBF<sub>1</sub> hybrid males.

### (ii) $F_1$ hybrids

The mean values from the canonical variates of the two  $F_1$  hybrids are shown in Table 2. In all cases the associated standard deviations are larger than one, showing that bones from mice of different ages may increase the variation. In general the B6D2 $F_1$  hybrids (which had a smaller age range) were less variable than the NZW  $\times$  NZBF<sub>1</sub>. Both hybrids showed a significant regression of overall mandible size on age (Fig. 2); however, most of the increase in size occurred in mice younger than 70 days. Although the B6D2 $F_1$  hybrids showed significant regressions of three canonical variates on age, the regression coefficients were small and the linear trends difficult to detect in plots of CVs against age.

The results of the regression analysis with NZW  $\times$  NZBF<sub>1</sub> hybrids were more complex, with significant linear, quadratic effects or deviation from the regression lines being found for all of the CVs. Plots of the CVs against age showed that the values of the CVs were nearly constant in 50- to 185-day-old mice. The values of three of the CVs from the youngest mice differed appreciably from those of older mice. Fig. 3 shows the plot of CV IV; similar plots were produced with CV II and CV III.

Table 3 shows the average relative lengths of the eleven measurements made on the mandibles from the NZW  $\times$  NZBF<sub>1</sub>s of different ages. A principal component

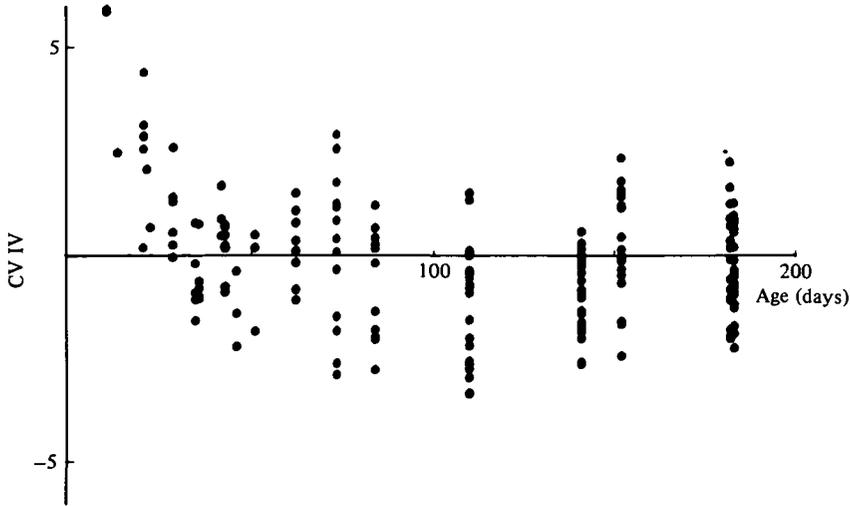


Fig. 3. Graph of CV IV against age of individual NZW  $\times$  NZBF<sub>1</sub> males.

Table 3. Mean lengths as a percentage of overall size of eleven measurements made on NZW  $\times$  NZBF<sub>1</sub> male mice of different ages

Age range (days)	n	1	2	3	4	5	6	7	8	9	10	11	Overall size <sup>a</sup>
182-183	39	1.04	2.57	3.62	6.50	7.57	8.17	11.34	11.96	14.23	16.44	16.57	623.0
152	22	0.97	2.63	3.72	6.37	7.44	8.07	11.44	12.01	14.28	16.49	16.58	621.2
141	22	0.98	2.59	3.67	6.50	7.44	8.21	11.35	11.93	14.29	16.49	16.55	618.6
110	20	1.02	2.57	3.60	6.52	7.52	8.27	11.38	11.91	14.22	16.46	16.55	614.0
84	11	1.00	2.60	3.65	6.46	7.44	8.20	11.38	11.97	14.30	16.52	16.47	612.0
73	13	1.01	2.62	3.70	6.46	7.34	8.17	11.42	12.16	14.25	16.38	16.50	596.7
62	8	0.99	2.60	3.62	6.39	7.38	8.14	11.40	12.11	14.36	16.55	16.47	605.0
46-51	6	1.00	2.68	3.76	6.47	7.36	8.23	11.29	12.05	14.28	16.51	16.37	597.7
42-43	11	1.01	2.65	3.73	6.37	7.32	8.12	11.37	12.12	14.38	16.51	16.43	592.6
35-36	10	1.02	2.64	3.78	6.44	7.32	8.21	11.32	12.06	14.42	16.49	16.30	576.2
29	6	1.02	2.75	3.88	6.39	7.20	8.15	11.41	12.18	14.33	16.21	16.48	558.2
21-23	8	0.84	2.68	4.01	6.20	6.96	7.95	11.67	12.38	14.49	16.12	16.71	508.0
11-14	4	0.71	2.70	4.35	5.88	6.49	7.58	12.21	12.87	14.52	15.78	16.93	454.8
Pooled standard deviation		0.06	0.08	0.13	0.10	0.14	0.12	0.13	0.17	0.10	0.13	0.12	8.9

<sup>a</sup> Overall size is the sum of eleven measures made on each mandible.

analysis of the data produced two components which explained 66% of the variation in the shape of the mandible. The first component, which accounted for 54%, gave positive weightings to those measures (1, 4, 5, 6 and 10) which were relatively shorter in mice under four weeks of age and negative weighting to those (2, 3, 7, 8, 9 and 11) which were relatively longer. In these young mice the size of the processus angularis is reduced disproportionately.

## 4. DISCUSSION

The discriminant functions used in this study were obtained from an analysis of a series of samples of young male mice from a number of commercial stocks of mice (Festing, 1974). Despite this narrow range of material the discriminant functions can detect considerable variation in the shape of the mandible. They are able to discriminate between inbred strains, provide information on their relationships (Festing & Lovell, 1981) and detect contamination in mouse colonies (Festing & Lovell, 1980). In this study we were able to show differences in mandible shape between the two inbred strains, C57BL/6J and DBA/2J, and between males and females within a strain.

A sex difference in mandible shape has been observed before (Bailey, 1981; Lovell, unpublished data). The female mandible is smaller, with a disproportionate reduction in the size of the processus angularis.

Only young adult male mice have been routinely used in the MRC Genetic Monitoring Scheme (Festing & Lovell, 1980), but the results of this study show that samples of either sex could be used provided they were compared with samples of the same sex.

The within-genotype variation was larger than was found in a survey by Festing (1976) and is probably a result of the age range of the material. However, the significant age effects in the analyses of the inbred strains were only a small proportion of the total variation in the sample, reflecting the power of the statistical tests used. Some misclassification may result from the use of samples with a wide age range, but this is unlikely to lead to confusion with a gross genetic contamination.

None of the mandibles from the animals in this study had unusual shapes or malformations. Analysis of mandible shape could therefore provide a convenient check for genetic authenticity at the end of long-term experiments using the controls or animals exposed to treatments that do not affect bone shape. Older animals could be used if subsequent studies show no increase in deformities from ageing, injury or disease.

A number of methods are available for correcting the effect of overall size on shape: Mossiman & Jones (1979) suggest, for instance, that log transformation may be suitable. The simple correction used here is both effective and sufficient except for the mandibles from the youngest mice in the NZW  $\times$  NZBF<sub>1</sub> group.

The relative lengths of bone measurements change rapidly during the first few weeks of the mouse's life. After seven weeks of age relative lengths are more nearly constant although there is some increase in the mandible's overall size. The use of younger animals may result in misleading results in routine genetic monitoring. Although not primarily a study of the changes in bone shape as a function of growth in young mice, the morphometric methods used here might be a suitable approach for this type of analysis. There was no difficulty in preparing mandibles from mice as young as 11 days old.

Other factors probably influence mandible shape. Fluctuation in some environmental factors over time may have caused the increase in variation appearing in the analysis as complex age effects. Grüneberg (1963) has reviewed a large set of

experiments on the effects of various factors on the manifestation of a series of skeletal abnormalities. The quality of the diet fed to mothers affects the frequency of qualitative skeletal abnormalities in their offspring (Searle, 1954; Deol & Truslove, 1957). Age, weight, and parity of the mother also influence the incidence of variants (Howe & Parsons, 1967). The results of investigations on variation in the shape of the mandible caused by differences in diet and husbandry will be reported elsewhere.

Improved discrimination might be possible between mice of different ages if the discriminant functions were obtained from an analysis of mandibles from mice of the same strain but different ages. The principal component analysis of the NZW  $\times$  NZBF<sub>1</sub> mice is an example of one such analysis. Discrimination would be improved further if no correction was made for overall size. The discriminant functions used in this study were, however, developed to distinguish between mice of different genotypes. They have been successfully applied to a wide range of genotypes and are capable of detecting subtle changes in mandible shape. The results here show that the sex differences in mandible shape are smaller than strain differences and that the shape does not change appreciably over a wide age range. Genetic monitoring using the mandible could therefore be carried out on mice of varying age.

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