GENETIC VARIATION IN THE SHAPE OF THE MOUSE MANDIBLE AND ITS RELATIONSHIP TO GLUCOCORTICOID-INDUCED CLEFT PALATE ANALYZED BY USING RECOMBINANT INBRED LINES

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ABSTRACT

Variation in mandible shape has been investigated in a set of recombinant inbred (RI) lines of mice, the C57BL/6J × A/J (BXA;AXB) RI lines. Considerable genetic variation was detected between the RI lines, but most lines were intermediate in shape when compared with the parent lines. Variation in mandible shape could not be explained by any single gene differences known between the parent lines including the *H-2* locus. Some RI lines had mandible shapes unlike either parent, and one in particular, line BXA1, had an unusual shape with a pronounced condyloid process. It was concluded that mandible shape has a complex inheritance involving a number of genes, each with small effects. In some cases, recombination of the genes can produce bone shapes quite different from those of the original parent line.—There was no evidence that the variability in steroid-induced cleft palate incidence in the BXA;AXB RI lines is related to the variation in adult mandible shape as detected in this study.

THERE is considerable variation in the shape of the mandible between different strains of mice (FESTING 1972). This variation has been used to investigate whether colonies of mice have become genetically contaminated (FESTING and LOVELL 1980) and to distinguish between wild mouse populations (THORPE, CORTI and CAPANNA 1982). In a previous study we showed that although an animal's age and sex affected mandible shape, these factors had small effects when compared with strain variation (LOVELL, TOTMAN and JOHNSON 1984). In this paper we investigate the variation in mandible shape in a set of recombinant inbred (RI) lines of mice and the relationship of mandible shape to glucocorticoid-induced cleft palate in these RI lines.

Recombinant inbred lines are inbred strains derived by brother × sister matings of the progeny from an F_2 cross between two inbred strains. Each individual within a line is genetically identical, and each line carries one of the alleles at any locus where there is a difference between the two parental lines. The strain distribution pattern (SDP) of these allelic differences can be used

to identify linkage with and pleiotropic effects of polymorphic loci. RI lines are particularly useful for studying measurements which can only be made after the animal has been killed. The set we have used were developed by M. NESBITT from crosses between A/J and C57BL/6J mice (NESBITT and SKAMENE 1984). These two strains differ in the incidence of steroid-induced cleft palate in fetuses following the injection of cortisone on days 11–14 of pregnancy (FRASER and FAINSTAT 1951). A/J has a high incidence (24%), whereas C57BL/6J has a low incidence (<1%) (LIU and ERICKSON 1986b).

There was evidence in this set of RI lines of a relationship between the incidence of steroid-induced cleft palate and the response of glucocorticoid binding to dithiothreitol and a higher incidence associated with the *b* allele of β_2-microglobulin and β-glucuronidase (LIU and ERICKSON 1986b). It has been suggested that retardation of the growth of the mandible is implicated in drug-induced cleft palate (BURDI *et al.* 1973). The C57BL/6J × A/J lines have been investigated to see if the variation in mandible shape seen in adult mice can be related to the level of steroid-induced cleft palate in fetuses or to variables known to be related to the incidence of cleft palate, such as *H-2* (BONNER and SLAVKIN 1975; ERICKSON, BUTLEY and SING 1979).

MATERIALS AND METHODS

Animals: BXA, AXB RI lines and A,B *H-2* congenics: The inbred and congenic strains were obtained from the Jackson Laboratory, Bar Harbor, Maine. C57BL/10J were used as they were congenic partners of the B10.A strain used in previous studies (ERICKSON, BUTLEY and SING 1979). Studies have shown that C57BL/10J and C57BL/6J have similar mandible shapes (FESTING and LOVELL 1981, figure 2). The RI lines were descendants of pairs supplied by MURIEL NESBITT of the University of California. The *H-2* congenic lines B10.A and A.BY/Sn were obtained from the Jackson Laboratory and were maintained by brother-sister mating. The mouse colony at the University of Michigan was kept at 20–22°, with 12-hr light and 12-hr dark periods. All mice were offered Purina mixture 1020 and tap water *ad libitum*.

Preparation of mandibles and statistical analysis: All the mice were humanely killed, and the mandibles were prepared by papain digestion (FESTING 1972). Eleven measurements were made on the right mandible of each mouse. Figure 1 shows these measurements. Each value was then expressed as a percentage of the sum of all 11 measurements to correct for overall size. A series of four canonical variates were obtained by applying the discriminant functions described by FESTING (1979, p. 50) to the corrected values. These discriminant functions were developed in an analysis of samples of young male mice from a number of commercial stocks of mice, using a canonical variate analysis (FESTING 1974). They have subsequently been used to investigate the effects of various factors on the shape of the mandible. The canonical variate analysis reduces the correlated set of 11 measurements on each mandible into a smaller series of uncorrelated measures which "explain" a large proportion of the variation in the material. The discriminant functions are those linear combinations of the original 11 measurements which maximize the ratio of between-group variation to the within-group variation.

Variability in mandible shape in the present study was investigated by plotting scores on one canonical variate against another. Relationships between genotypes were investigated by cluster analysis, using the Genstat statistical package. A measure of the multivariate distance between each pair of samples was calculated from the sum of the squared differences between their mean canonical variate values. This set of measures, similar to a set of generalized distances or Mahalonobis d^2 distances, was used to create

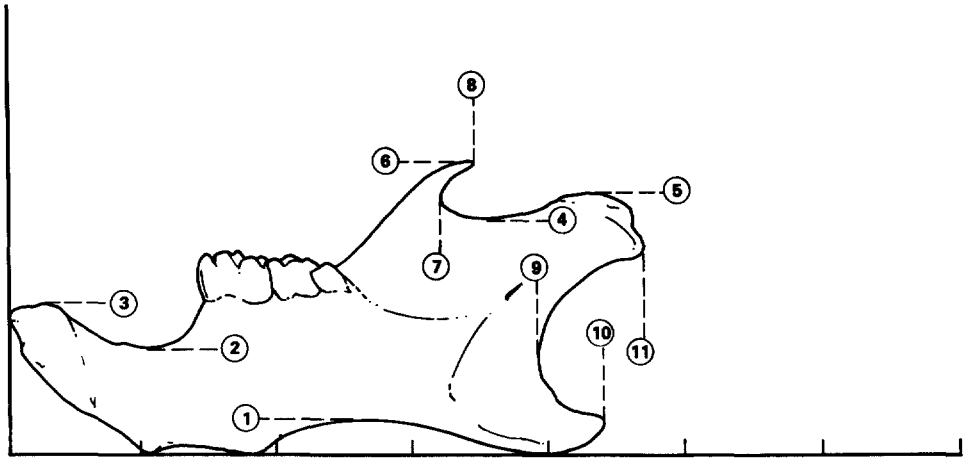

FIGURE 1.—Diagram of the right mandible of a C57BL/6J mouse showing the 11 measurements made on each mandible in this study.

a similarity matrix. Cluster analysis was carried out on this similarity matrix using nearest neighbor criteria (EVERITT 1974). Groups initially consist of single members and are merged according to their nearest members. The groups with the smallest distance are joined together. Cluster analysis should not be considered a tool for formal statistical analysis but, rather, as an aid to investigating complex multivariate problems. Comparisons between sets of RI lines carrying one or other of the alleles at a given locus were carried out using Scheffé's test. This provides a conservative statistical test minimizing the risk of false positives in *a posteriori* comparisons (SNEDECOR and COCHRAN 1967).

Glucocorticoid-induced cleft palate: The methods for obtaining the data on glucocorticoid-induced cleft palate in the AXB and BXA recombinant inbred lines are described in the accompanying papers (LIU and ERICKSON 1986a,b).

RESULTS

Duplicate samples from the C57BL/6J × A/J (and reciprocal cross) RI lines were similar in shape, although the two BXA8 samples were somewhat variable. Samples from males and females of the same strain were similar in shape. There was evidence of considerable genetic variation between the RI lines, as shown by the mean canonical variate (CV) values (Table 1; Figure 2) and the cluster analysis (Figure 3). The original analysis used to produce the discriminant function results in a series of canonical variates with an overall mean of zero and within-group standard deviations of one. The within-group standard deviations here are, in general, greater than one. This increased variability is probably due to the small sample sizes and the wide age ranges. Some variability may be due to genetic variation within the RI lines, as some of the animals were derived from the 15th generation of inbreeding, at which point only 95% homozygosity would be expected.

Two RI lines, BXA2 and BXA11, had shapes similar to the inbred strain C57BL/10J, which is a substrain of C57BL/6J, and to its *H-2* congenic strain B10.A (LOVELL, TOTMAN and JOHNSON [1984] reported values of 3.91, −0.19, 0.19 and 2.18 for CV I to CV IV for a large sample of 116 male C57BL/6J

TABLE 1

Mean canonical variate values for A/J, C57BL/10J, two congenic strains and 19 recombinant inbred lines

Group no.	Strain	Sex	*n*	CV I	CV II	CV III	CV IV	% Steroid-induced cleft palate[a]	*H-2*[a]	β2m[a]	*Gus*[a]
1	A/J	M	5	−0.22 ± 1.76	1.23 ± 1.06	2.81 ± 0.86	1.44 ± 1.58	24	A[b]	A	A
2	A.BY/Sn	M	3	0.80 ± 2.56	−0.32 ± 1.71	1.60 ± 2.03	−0.63 ± 1.67	22[c]	B	A	A
3	A×B1	F	3	0.35 ± 2.04	−1.87 ± 1.54	3.93 ± 0.77	3.98 ± 1.13	9	A	A	B
4	A×B2	M	4	3.53 ± 0.66	−2.40 ± 0.50	4.10 ± 1.33	1.35 ± 2.26	6	A	B	A
5	A×B5	M	4	1.94 ± 0.61	−0.39 ± 1.13	2.72 ± 1.36	1.45 ± 1.61	12	A	A	A
6	A×B5	M	3	0.65 ± 1.18	0.27 ± 0.26	2.45 ± 1.20	1.74 ± 2.94				
7	A×B6	M	3	5.34 ± 1.99	−2.05 ± 1.36	3.76 ± 0.48	2.20 ± 1.32	1	B	A	A
8	A×B7	M	3	0.38 ± 1.98	−0.17 ± 1.40	2.77 ± 0.48	2.76 ± 2.44	—[d]	A	B	B
9	A×B9	F	3	−1.76 ± 1.70	1.82 ± 2.44	−0.54 ± 2.51	4.02 ± 0.55	24	A	A	A
10	A×B12	M	3	2.02 ± 0.65	−0.71 ± 0.79	−1.03 ± 1.19	2.41 ± 2.14	13	A	A	A
11	A×B13	M	3	1.26 ± 1.47	0.04 ± 1.44	4.80 ± 1.69	1.40 ± 0.38	8	B	B	B
12	A×B15	M	4	−1.24 ± 0.59	1.12 ± 2.18	1.86 ± 1.70	4.29 ± 0.45	0	A	A	—
13	A×B17	M	4	2.67 ± 1.32	−0.41 ± 4.98	3.08 ± 1.75	3.04 ± 1.55	0	A	A	A
14	B×A1	M and F	3	5.48 ± 2.58	−6.14 ± 3.39	5.20 ± 2.09	0.54 ± 2.84	—	B	A	A
15	B×A2	M	4	3.24 ± 2.39	−3.56 ± 3.23	2.50 ± 2.69	3.78 ± 1.16	1	B	A	A
16	B×A4	M	3	3.33 ± 2.20	1.46 ± 2.12	3.07 ± 1.29	−1.17 ± 3.72	21	A	B	A
17	B×A6	M	5	−1.16 ± 1.40	−2.05 ± 1.35	5.46 ± 0.96	1.88 ± 2.64	6	A	A	B
18	B×A8	M	4	1.52 ± 1.01	−0.74 ± 1.47	4.66 ± 1.61	1.52 ± 2.00	0	A	B	—
19	B×A8	M	4	0.80 ± 0.69	−1.19 ± 1.08	6.11 ± 0.30	−0.33 ± 1.06				
20	B×A10	M	3	−0.33 ± 1.26	2.17 ± 0.43	5.07 ± 0.93	−0.13 ± 2.69	0	B	A	A
21	B×A11	M	3	3.28 ± 0.54	−1.45 ± 0.73	0.79 ± 2.22	3.04 ± 0.55	0	B	B	A
22	B×A11	F	3	4.27 ± 0.71	−3.94 ± 0.16	3.41 ± 0.37	5.28 ± 1.00				
23	B×A14	M	3	0.85 ± 0.55	0.21 ± 3.74	3.08 ± 0.53	1.30 ± 3.01	35	B	B	B
24	B×A15	M	3	−1.84 ± 1.23	2.82 ± 0.56	2.40 ± 0.92	2.09 ± 1.00	5	B	A	A
25	B×A15	F	4	−1.02 ± 1.37	−0.53 ± 2.65	4.84 ± 1.77	4.51 ± 2.44				
26	B10.A	M	5	3.48 ± 1.12	−3.49 ± 0.70	1.56 ± 1.43	3.66 ± 1.89	8[c]	A	B	B
27	C57BL/10J	M	3	3.30 ± 0.49	−2.54 ± 2.03	0.84 ± 0.93	4.49 ± 1.22	1	B	B	B

[a] Data from LIU and ERICKSON (1986b) unless otherwise indicated.
[b] A = A/J-like, B = C57BL/6J-like.
[c] Data taken from ERICKSON, BUTLEY, and SING (1979).
[d] Not available.

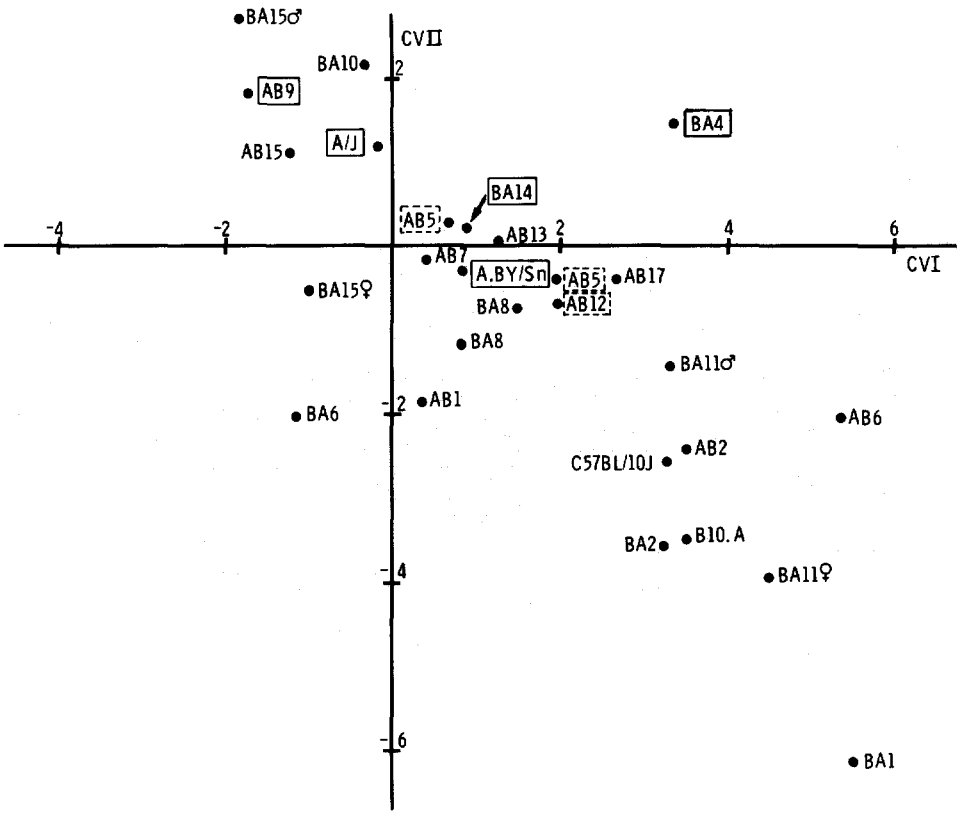

FIGURE 2.—Graph of CV I against CV II of the mandibles from C57BL/6J × A/J recombinant inbred lines. Solid box around strain name indicates strains with steroid-induced cleft palate incidence >20%. Dotted lines around strain name indicates strains with steroid-induced cleft palate incidence (10–20%).

mice). Three RI lines, AXB5, AXB7 and BXA14, clustered near the A/J sample. The other RI lines differed from both parental lines, but in general, most had scores on the first two canonical variates similar or intermediate to the parent lines.

Two lines, AXB6 and, in particular, BXA1, had scores on the first canonical variate which were larger than those for C57BL/10J. BXA1 had a very distinct and unusual shape (Figure 4) which resulted in high positive values for CV I and CV III and in negative values for CV II. The three mandibles from this line (from two males and one female) had the highest relative values for the position of the condyloid process, but had the shortest relative length for the position of the inferior notch and angular process. Unfortunately, the breeding performance of this line was extremely poor, so no further samples were available and no data were obtained on the incidence of steroid-induced cleft palate.

A further series of lines, BXA4, BXA6, BXA10, AXB9 and AXB15, were distinct from other lines based on the cluster analysis. The other *H-2* congenic

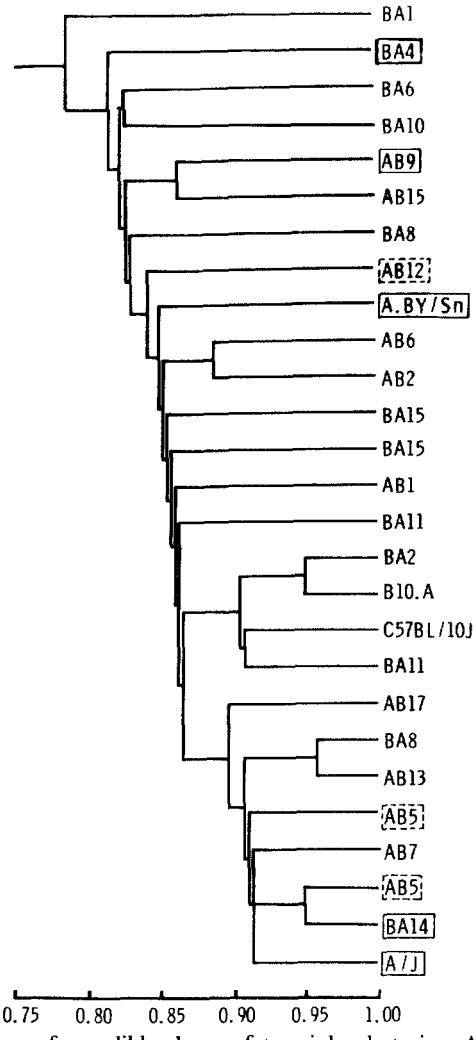

FIGURE 3.—Dendogram of mandible shape of two inbred strains, A/J and C57BL/10J, two congenic strains, A.BY/Sn and B10.A and 19 recombinant inbred lines derived from crosses between A/J and C57BL/6J.

strain A.BY/Sn was also different from A/J. A.BY/Sn is congenic with the A/WySn substrain that branched from A/J in the 1930s before 20 generations of brother × sister matings had been completed. Differences in shape between the two A strains might result from different *H-2* alleles, but are probably due to different genetic backgrounds.

There was no evidence that allelic differences at the *H-2*, *B2m* or *Gus* loci affected mandible shape. (These three loci were chosen because of apparent effects on glucocorticoid-induced cleft palate incidence; LIU and ERICKSON 1986b). None of the comparisons between sets of lines, with one allele compared with the other, were significant using Scheffé's test on the indices of mandible shape, CV I–CV IV. There was no evidence of an association be-

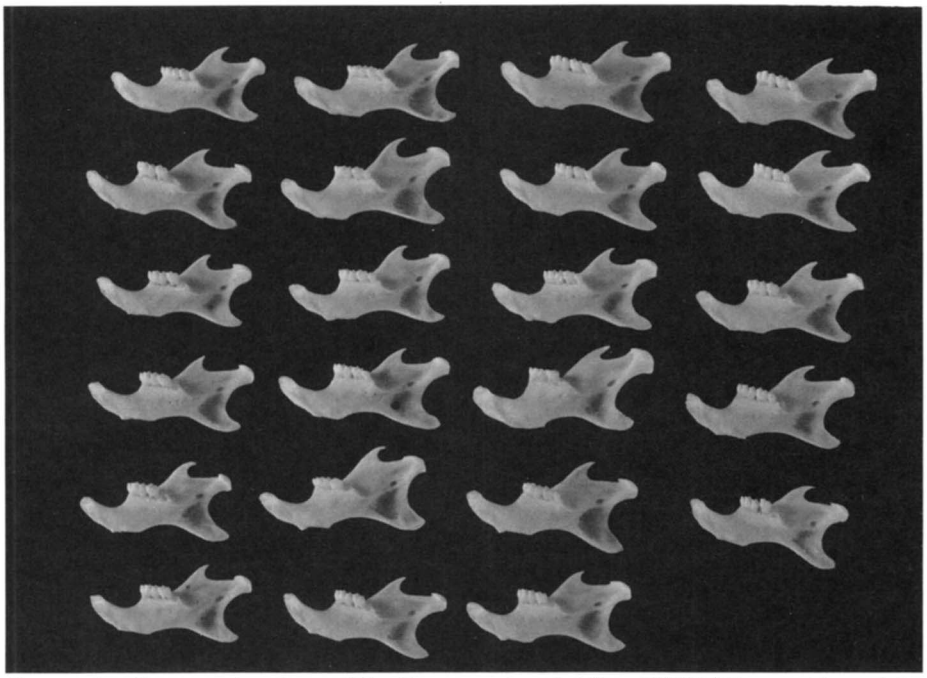

FIGURE 4.—Photographs of mandibles from a series of A/J × C57BL/6J recombinant inbred lines. *Top row* (*left* to *right*): A/J, A.BY/Sn, B10.A, C57BL/10J; *second row*: AXB1, AXB2, AXB5, AXB6; *third row*: AXB9, AXB7, AXB12, AXB13; *fourth row*: AXB15, AXB17, BXA1, BXA2; and *bottom row*: BXA11, BXA14, BXA15.

tween mandible shape and the incidence of hydrocortisone-induced cleft palate. (Spearman's rank correlation between the incidence and CV I–CV IV values was nonsignificant in each case.)

Two strains with a high incidence, BXA14 and AXB5, had mandible shapes somewhat similar to A/J, the high-incidence strain, but two other lines, AXB13 and AXB17, had similar shapes and low or zero incidences of steroid-induced cleft palate. Other strains with mandible shapes distinct from either parent had low incidences of cleft palate.

None of the strains with high or intermediate incidences of cleft palate had unusually large or small mandible bodies or mental symphyses. These lines showed no distinctive features aside from line AXB9 having, on average, the smallest overall size and relative height of the coronoid process.

DISCUSSION

The discriminant functions used in this study were those developed by M. F. W. FESTING for the routine checking of mouse colonies for genetic contamination. They are capable of detecting considerable genetic variation, as well as providing estimates of the effect of sex differences and variability in age on mandible shape (LOVELL, TOTMAN and JOHNSON 1984). These functions were able to show considerable variation in mandible shape between the genotype

of two sets of recombinant inbred lines. The mandible shapes of most of the lines were intermediate to the parental lines, and no "major" genes affecting mandible shape were detected. BAILEY (1984) has investigated variation in the shape of the mandible using a series of congenic strains differing from C57BL/6By by only a chromosomal segment bearing a histocompatability gene introduced from BALB/cBy. He showed that 8 of 25 such strains had significantly different mandible shapes and estimated that at least 30 genes affect the shape of the mandible.

Mandible shape is therefore unlikely to be determined by the effects of just one or two major genes, but, rather, is the result of a series of genes, each with small effects. These genes probably act during development by varying the growth of various parts of the skeleton. ATCHLEY, PLUMMER and RISKA (1985a,b) have recently reported the results of the analysis of variation in mandible shape in a large sample of an outbred stock of mice. They reviewed the factors acting during development, whether intrinsically (by varying the timing and degree of growth of the embryonic skeletal tissue) or extrinsically (by the action of such factors as other bones or muscles affecting the shape by mechanical loadings on various muscle attachments and joints). ATCHLEY and his co-workers used principal components analysis, another multivariate analysis, to investigate variation in a set of similar measurements to those used by us. They attempted to relate the pattern of variation they found to these underlying processes of embryonic development of the mandible. They produced a set of functions of the original measurements that appear to relate to some of the developmental history of the functional units making up the mandible. The discriminant functions used here are, in all probability, measuring similar patterns. The first discriminant function, for instance, gives high positive scores to strains such as AXB6 that have a high length/height ratio. The second discriminant function gives high negative scores to strains such as AXB6 and BXA1 that have pronounced condyloid processes. Figures 2, 3 and 4 show that there is considerable variation in the shape of the mandible between the RI lines from two inbred strains. It is not possible to compare the variations found here with that reported by ATCHLEY, PLUMMER and RISKA, because they do not illustrate the variation they investigated, nor do they provide summary statistics of the individual measurements they made.

In a few cases, such as line BXA1, the shape of the mandible was unlike either parental line. The genes determining mandible shape have recombined to produce a new gene combination resulting in a different and distinct bone shape. Unfortunately, the breeding performance of line BXA1 was extremely poor, and few animals were available for investigation. It would be interesting to see if the musculature associated with the coronoid process in this line is reduced or abnormal and to note how the mandible shape is produced during development.

Several polymorphisms existing in the RI lines sets could not be related to large differences in mandible shape. Although alleles at the *H-2* locus have been shown to affect mandible shape, these effects are small when compared with the differences between two inbred strains (FESTING 1972). Variation

between a congenic strain and an inbred strain such as that found here between A/J and A.BY/Sn is probably due to differences at a number of other gene loci, especially considering the long divergence time between A/J and A/WySn, as well as at the *H-2* locus. JURILOFF (1982) has shown considerable differences in the incidence of spontaneous cleft lip between the A/J and A/WySn inbred strains. These findings suggest that A/J is not the ideal congenic partner for A.BY/Sn.

It has been suggested that variation in the time and degree of growth of a facial structure may be important in the etiology of steroid-induced cleft palate. The aspects of bone shape identified by the morphometric methods in this study are the results of such processes during development. None of the mandibles studied are, of course, from mice which had a cleft palate. However, there was no indication of unusual shapes or aberrant growth in those genotypes which had a high predisposition to cortisone-induced cleft palate. On the contrary, a range of mandible shapes were found, none of which seemed to have a correlation with cleft palate incidence. In fact, strains with similar mandible shapes may have very different incidences. Thus, adult mandible shape is not a useful indicator of a genotype's susceptibility to glucocorticoid-induced cleft palate.

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LITERATURE CITED

ATCHLEY, W. R., A. A. PLUMMER and B. RISKA, 1985a Genetics of mandible form in the mouse. Genetics **111:** 555–577.

ATCHLEY, W. R., A. A. PLUMMER and B. RISKA. 1985b Genetic analysis of size-scaling patterns in the mouse mandible. Genetics **111:** 579–595.

BAILEY, D. A., 1984 Genes that shape the mandible. Mouse Newsl. **71:** 30.

BONNER, J. J. and H. C. SLAVKIN, 1975 Cleft palate susceptibility linked to histocompatability-2 (H-2) in the mouse. Immunogenetics **2:** 213.

BURDI, A., M. FEINGOLD, K. S. LARSSON, I. LECK, F. F. ZIMMERMAN and F. C. FRASER, 1973 Etiology and pathogenesis of congenital cleft lip and cleft palate: an N.I.D.R. State-of-the-art report. Teratology **6:** 255–270.

ERICKSON, R. P., M. S. BUTLEY and C. F. SING, 1979 H-2 and non-H-2 determined strain variation in palatal shelf and tongue adenosine 3′:5′ cyclic monophosphate. J. Immunogenet. **6:** 253–262.

EVERITT, B. S., 1974 *Cluster Analysis.* Heinemann, London.

FESTING, M. F. W., 1972 Mouse strain identification. Nature **238:** 351–352.

FESTING, M. F. W., 1974 Genetic monitoring of laboratory mouse colonies in the Medical Research Council Accreditation Scheme for the suppliers of laboratory animals. Lab. Anim. **8:** 291–299.

FESTING, M. F. W., 1979 *Inbred Strains in Biomedical Research.* Macmillan, London.

FESTING, M. F. W. and D. P. LOVELL, 1980 Routine genetic monitoring of commercial and other mouse colonies in the U.K. using mandible shape; five years of experience. pp. 341–348. In: *The Proceedings of the 7th ICLAS Symposium (Utrecht, 1979),* Edited by A. SPIEGEL, S. ERICHSEN and H. A. SOLLEVELD. Gustav Fischer Verlag, Stuttgart.

Festing, M. F. W. and D. P. Lovell, 1981 Domestication and development of the mouse as a laboratory animal. Symp. Zool. Soc. Lond. **47:** 43–62.

Fraser, F. C. and T. D. Fainstat, 1951 The production of congenital defects in the offspring of pregnant mice treated with cortisone. Pediatrics **8:** 527–533.

Juriloff, D. M., 1982 Differences in frequency of the cleft lip among the A strains of mice. Teratology **25:** 361–368.

Liu, S. L. and R. P. Erickson, 1986a Genetics of glucocorticoid receptor levels in recombinant inbred lines of mice. Genetics **113:** 735–744.

Liu, S. L. and R. P. Erickson, 1986b Genetic differences among the A/J × C57BL/6J recombinant inbred mouse lines and their degree of association with glucocorticoid induced cleft palate. Genetics **113:** 745–754.

Lovell, D. P., P. Totman and F. M. Johnson, 1984 Variation in the shape of the mouse mandible. I. Effect of age and sex on the results obtained from the discriminant functions used for genetic monitoring. Genet. Res. **43:** 65–73.

Nesbitt, M. N. and E. Skamene, 1984 Recombinant inbred mouse strains derived from A/J and C57BL/6J: a tool for the study of genetic mechanisms in host resistance to infection and malignancy. J. Leukocyte Biol. **36:** 357–364.

Snedecor, G. W. and W. G. Cochran, 1967 *Statistical Methods* Ed. 6. Iowa State University Press, Ames, Iowa.

Thorpe, R. S., M. Corti and E. Capanna, 1982 Morphometric divergence of Robertsonian populations/species of *Mus*: a multivariate analysis of size and shape. Experientia **38:** 920–923.

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