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Correlation between genetic distances based on single loci and on skeletal morphology in inbred mice

MICHAEL F. W. FESTING[1]* AND THOMAS H. RODERICK[2]
[1] *MRC Experimental Embryology and Teratology Unit, Carshalton, Surrey, England*
[2] *The Jackson Laboratory Bar Harbor, Maine, USA*

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Summary

Genetic and morphometric distances between 12 inbred strains of mice ranging from closely related substrains to a sub-species were estimated using published data on single locus polymorphisms, and on the basis of up to 44 measurements on seven different bones, respectively. Simulation was also used to investigate sampling effects for the single loci. There were strong and statistically highly significant correlations among all measures of genetic distance ranging from 0·58 for the comparison of single loci with the logarithm of the Mahalanobis distance based on 24 measurements on four bones, to 0·72 for estimates of genetic distance based on single loci and the morphology of the mandible. These findings are in sharp contrast with those of Wayne & O'Brien (1986) who claimed that 'structural gene and morphometric variation of mandible traits are uncoupled between mouse strains'. Their failure to find such a correlation is probably because their sample of inbred strains included only a single pair of closely related substrains, and no substrains separated for less than 40 years, and because they failed to correct for non-linearity between morphometric and single-locus measurement scales. Simulations and regression analysis suggested that genetic distances could be estimated with approximately equal precision using morphological data on bone measurements or about 10 cladistically informative single loci, which would usually involve sampling about 50 loci. Data based on single-gene markers is usually more informative than morphometric data for studying the similarity of independently-derived strains. However, similarities among closely related populations such as sublines of an inbred strain can usually be studied more efficiently using morphometry.

1. Introduction

On an evolutionary scale, reproductively isolated groups of organisms will gradually diverge, initially as a result of genetic segregation of existing polymorphic loci, and later as a result of the accumulation of new mutations. The extent of the genetic divergence can be measured by direct analysis of DNA sequences (Field *et al.* 1988), by sampling individual genetic loci using biochemical (e.g. electrophoretic) or immunological techniques ('genetic distances' in this paper), or by studying morphological features (morphological distances) such as skeletal measurements in vertebrates (Festing, 1972, 1973, Lovell & Johnson, 1983). Morphological characters have the disadvantage of being a complex phenotype in which individual loci may be expressed to varying degrees, but the data are usually easy to collect, and may sample many loci simultaneously. Data on individual loci is easier to interpret, and is usually more informative with each locus being weighted equally for distance estimates. However, the collection of such data can be laborious, expensive, and in some cases impossible when suitable tissues are not available as in the case of archaeological or fossil specimens. Moreover, in studies of very closely related groups such as sublines of inbred mouse strains, morphological characters have been shown to be effective in differentiating between such groups, whereas single locus markers have not (Festing, 1973).

There have been few attempts to compare results obtained using genetic and morphological distances across many populations of mice, though Wayne & O'Brien (1986) compared the two methods using fifteen inbred mouse strains. They found a 'non-significant' correlation of $r = 0{\cdot}24 \pm 0{\cdot}1$ between distances estimated by the two methods, using size uncorrected morphological data and a 'significant' correlation of $r = 0{\cdot}26 \pm 0{\cdot}1$ ($P < 0{\cdot}05$) using size-corrected data, and concluded that 'structural gene

* Corresponding author

and morphometric variation of mandible traits are uncoupled between mouse strains.' Unfortunately, their data were inadequate in that they included only a single pair of closely related substrains, with no strains having been separated for less than 40 years, even though numerous studies have shown that closely related substrains tend to be very similar both morphologically and genetically. Indeed, Festing (1972) suggested using the shape of the mandible as a method of genetic quality control. If their size-uncorrected data are re-analysed with the inclusion of an extra 10 points (in addition to 105 data points they used) of morphological and genetic distances identical to the distances between C57BL/6 and C57BL/10 (a pair of substrains known to be genetically very similar) in order to compensate for the under representation of closely related strains, then the correlation increases to 0·45. Also, in comparing Nei's genetic distance as the genetic measure of similarity with the Mahalanobis distance for the morphometric characters they used the coefficient of linear correlation to compare two variables which were clearly related in a non-linear manner. Re-analysis of their published data using the logarithm of the Mahalanobis distance increases the correlations from 0·24 to 0·34 for the size uncorrected data. If both corrections are made (an additional 10 points and the logarithmic transformation), then the correlation rises to 0·63. These correlations are highly significantly different from zero ($P < 0{\cdot}01$) using the same modified Mantel's test that they used (Sokal, 1979). It is surprising that they did not report the increase in the correlation following a logarithmic transformation, as they used such a transformation in an earlier draft of their paper (O'Brien, personal communication 2nd. Jan. 1985), and should be aware that correlations are sensitive to the scale of measurement. The aim of this paper is to present new data with mouse strains selected to cover a wide range of similarity, and more morphological measurements in order to clarify the relationships between genetic and morphological distances.

2. Materials and Methods

(i) *Mice*

Data was collected from males of 12 strains of inbred mice maintained at the Jackson Laboratory, Bar Harbor, Maine. These strains were chosen to include a number of closely related substrains, less closely related strains of standard laboratory mice, and a distinct sub-species inbred from wild mice. Strains C57BL/6J (B6J in this report), CBA/CaJ (CBAC), SWR/J (SWR), NZB/BlJ (NZB), CBA/J (CBAJ), AEJ, SJL/J (SJL), BALB/cJ (BALB), C57BL/Ks (BKS) and C57BL/10J (B10) are standard inbred strains described by Festing (1979). Strain C57BL/6Rk (B6RK) is a closely-related subline of C57BL/6J. Strain MOLD is an inbred strain developed from wild *Mus musculus molussinus*, without any intercrossing with laboratory mice. All mice were maintained in standard laboratory conditions, and were at least six weeks of age at the time they were killed.

(ii) *Bones and morphological data*

Skeletons were prepared by standard methods (Festing, 1972). A total of 44 measurements were made on seven bones using methods previously described (Festing 1972, 1973, 1976). The bones used and the measurements taken are shown in Fig. 1*a–g*. Each measurement (except no 2 of the ulna) represents the distance to a tangent to a curve measured from the base line in arbitrary units (approximately 0·125 mm).

(iii) *Single-gene markers*

Data on single-gene markers were taken from a data bank compiled over a number of years from many different sources (Roderick *et al.* 1981). Only cladistically informative loci were included (i.e. loci at which there were differences among this sample of strains). The data included protein polymorphisms detected electrophoretically and immunologically, as well as a few other miscellaneous loci such as retinal degeneration (*rd*). Data collected in this way as a result of the work of many investigators over a number of years may contain some biasses and inaccuracies, particularly in the treatment of closely related strains. In some cases it was not possible to identify all sublines and substrains separately. Published differences between substrains may be real, or a result of errors and misprints. Where such discrepancies have been found, the data must be discarded, but this may lead to bias, with over-estimation of the genetic similarity between substrains such as CBA/Ca and CBA/J. Similarly, although there are no known differences between C57BL/6J and C57BL/6Rk, it is not always clear how many loci have been tested, since in many cases only discrepancies would be reported. In this case C57BL/6Rk has been identified with all C57BL/6 mice which are not C67BL/6J, but this probably over-estimates the number of loci tested. However, in spite of its limitations, these data probably represent the best available estimate of the genetic similarity of the strains used in this study.

(iv) *Statistical methods*

The morphometric data were analysed using the BMDP, GENSTAT, and MINITAB statistical packages. Mahalanobis distances were used to compare different strains. Preliminary analyses showed that similar results were obtained with raw data uncorrected for size, (i.e. discriminating on both size and shape) and data corrected for size by dividing through by the sum of all the measurements, a method shown

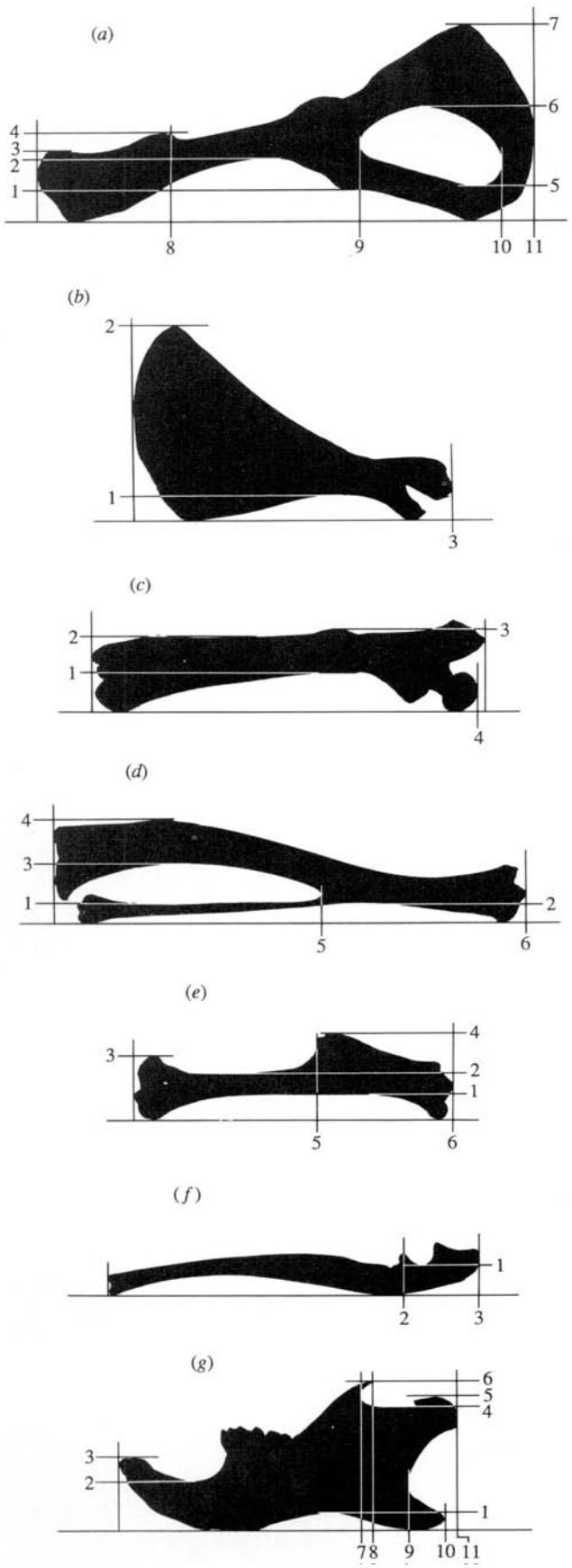

Fig. 1. (*a*) Right pelvis, measurements X(1)–X(11). (*b*) Left scapula, X(12)–X(14). (*c*) Right femur (without ephyses), X(15)–X(18). (*d*) Left tibio-fibula, X(19)–X(24). (*e*) Right humerus (without head), X(25)–X(30). (*f*) Left ulna X(31)–X(33). (*g*) Right Mandible, X(40)–X(50).

by Lovell *et al.* (1984) to correct accurately for size, giving discrimination only on shape. The same method was used by Wayne & O'Brien (1986) who also found similar results. Accordingly, only size-uncorrected measurements were used so that discrimination between strains is based on both size and shape. Separate analyses were made on the 11 measurements of the right mandible (for comparison with other studies), 20 variables on three bones (mandible, humerus and ulna), 24 variables on four different bones (pelvis, scapula, femur, and tibio-fibula), and all 44 variables in order to explore the effects of selection of individual bones. Average-linkage cluster analyses were based on the first four canonical variates (discriminant functions) following a canonical variate analysis.

Similarities between strains for the single-gene characters were estimated from the percentage of genes similar in each pair of strains (Dunn & Everitt, 1982), though for the correlations the genetic dissimilarities (100-similarity) were used for comparisons with the Mahalanobis distances.

Nei's genetic distance (Nei, 1978) was also calculated for comparison with previous studies (Wayne & O'Brien, 1986). It is debatable whether this is an appropriate distance measure for this type of data, where only loci which are polymorphic between strains have been included. One result is that in absolute terms, the apparent genetic distances between these inbred strains are larger than would normally be expected between species.

The Mahalanobis distance D^2 is a squared distance, which would tend to emphasise distant relationships, whereas Nei's genetic distance is the logarithm of a proportion, which would tend to minimize distant relationships; the relationship between the two is unlikely to be linear. Accordingly, the logarithm of the Mahalanobis distance was used in estimating the coefficient of linear correlation between morphometric and genetic distances as this improved the linearity of the relationship between the two variables. However, correlations using the untransformed data are also presented for the percent dissimilarity and Nei's genetic distance.

The similarities between the two distance matrices were compared using the product-moment correlations with Nei's genetic distance and the proportion of loci dissimilar between the two strains on the one hand, and the Mahalanobis distance (with or without a log transformation) on the other, for a range of morphological measurements. Spearman's rank correlation, rho, was also used as this has the useful property of being invariant under monotone transformations of the X and Y variables (Dietz, 1983). Tests of the hypothesis that the correlations were zero against the alternative hypothesis that they were different from zero (i.e. two-tailed tests) were made using Mantel's permutation test (Dietz, 1983; Sokal, 1979), which can easily be programmed using a

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MINITAB macro. This is necessary because not all pair-wise comparisons in a distance matrix are independent.

Average-linkage cluster analysis used the GENSTAT statistical package.

Sampling variation for the genetic distances (percent dissimilarity) was simulated using MINITAB. It was assumed that a pair of isogenic strains are identical at a certain proportion of loci (the true similarity) of the type used in this investigation (i.e. considering only loci polymorphic in this sample of strains), and that this may be estimated from a sample of loci. If only a few loci are sampled, then there may be substantial sampling errors, which will decrease as the number of loci sampled increases. In this study, the observed genetic similarity p between each pair of strains was assumed to be the true genetic similarity. For example, it was assumed to be 0·38 for B6J and CBAC. Two random Bernoulli samples of size 10, 20 or 30 'loci' were then drawn with probability P (i.e. 0·38 for B6J and CBAC) for each of the 66 comparisons, and the correlations between the two samples was computed. Each simulation was run 10 times. This may be regarded as the correlation between estimated relationships in this sample of strains estimated from two independent sets of loci of size 10, 20 or 30.

Linear regression was used to estimate the percent dissimilarity from the $\log_{10}$ of the Mahalanobis distance, and from a simulated sample (as above) based on 10 loci in order to compare the accuracy of morphometric estimates of genetic dissimilarity with those based on only a few cladistically informative loci.

3. Results

The total number of loci used to compare strains ranged from 19 (CBAC vs. MOLD) to 144 (BALB vs. B6RK), and the percent similarity ranged from 100% (B6J vs. B6RK) to 16·7 (MOLD vs. various substrains of C57BL) (Table 1).

The means and sample sizes for each of the 44 morphometric characters for each strain are given in Table 2. Multivariate analysis showed large and highly significant differences between strains for all groups of characters (e.g. mandible, 20-variables, 24-variables and 44 variables). Typically, even closely related substrains such as C57BL/6 and C57BL/10 were significantly different from one another, and in most analyses individuals could be assigned to the correct strain with more than 95% accuracy using a jack-knifed classification analysis. Any miss-classification was usually between closely related strains. These results are in accordance with previous studies (e.g. Festing, 1972; Wayne & O'Brien, 1986).

The Mahalanobis distances based on the mandible, 20 variables, 24 variables and all 44 variables are given in Table 3. Cluster analysis of the 12 strains based on the single locus data are shown in Fig. 2. This

Table 1. *Percentage similarity (below diagonal) and total number of loci (above diagonal) for the single loci*

	B6J	CBAC	SWR	NZB	CBAJ	MOLD	AEJ	B6RK	SJL	BALB	BKS	B10
B6J	—	108	118	77	108	24	33	133	110	131	82	97
CBAC	38·0	—	104	66	105	19	26	119	99	120	72	88
SWR	44·1	51·9	—	77	105	24	33	125	108	123	79	95
NZB	42·9	62·1	59·7	—	71	19	28	78	74	78	58	64
CBAJ	35·2	99·0	51·4	56·3	—	24	33	116	102	116	75	91
MOLD	16·7	26·3	37·5	42·1	29·2	—	23	24	23	24	22	24
AEJ	66·7	50·0	84·8	46·4	51·5	26·1	—	33	32	33	30	33
B6RK	100·0	39·5	42·4	42·3	34·5	16·7	66·7	—	119	144	82	108
SJL	44·5	54·5	72·2	59·5	52·0	43·5	68·8	43·7	—	120	72	91
BALB	52·7	59·2	57·7	53·8	57·8	25·0	63·6	52·1	50·8	—	82	107
BKS	86·6	50·0	51·9	46·6	46·7	18·2	63·3	86·6	47·2	52·4	—	71
B10	96·9	37·5	43·2	42·2	34·1	16·7	66·7	97·2	40·7	56·1	85·9	—

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Table 2. *Strain means, pooled within-group standard deviations and sample size (N) for the 44 morphometric variables X(1)–X(44) (See Fig. 1)*

Strain	X(1)	X(2)	X(3)	X(4)	X(5)	X(6)	X(7)	X(8)	X(9)	X(10)	X(11)
B6J	6·67	16·80	18·20	24·65	12·20	32·92	53·75	41·92	90·60	126·97	134·70
CBAC	6·12	14·37	19·12	22·00	12·37	30·12	51·75	39·62	85·75	119·50	129·62
SWR	7·90	16·70	19·70	23·20	9·50	30·60	53·10	39·10	87·90	125·10	134·70
NZB	13·30	24·10	19·10	30·90	12·00	37·40	60·90	45·80	96·70	137·30	147·80
CBAJ	6·56	14·22	21·33	22·44	11·78	29·89	50·78	39·56	89·11	125·22	135·89
MOLD	7·90	16·30	17·60	21·20	9·30	30·40	48·20	38·80	78·80	116·00	124·30
AEJ	7·00	16·50	21·17	24·00	12·33	34·17	51·67	40·67	94·33	135·33	140·17
B6RK	7·00	17·80	20·80	26·40	12·40	34·60	55·80	43·60	95·60	134·00	142·60
SJL	7·60	16·40	21·00	25·40	10·20	30·10	52·80	38·20	85·20	121·40	129·20
BALB	8·30	17·10	20·20	23·50	10·80	33·60	54·70	42·60	86·90	124·90	132·40
BKS	8·73	17·00	18·45	25·55	13·36	32·00	51·09	42·64	87·73	124·00	133·45
B10	7·10	17·00	18·00	24·70	11·40	33·40	54·00	43·70	93·60	131·30	138·10
Mean	7·69	17·02	19·16	24·53	11·57	32·46	53·30	41·46	89·29	126·39	134·78
S.D.	0·84	1·01	1·15	1·15	0·65	1·13	1·71	2·71	3·26	4·35	4·93

Strain	X(12)	X(13)	X(14)	X(15)	X(16)	X(17)	X(18)	X(19)	X(20)	X(21)	X(22)
B6J	7·35	55·45	82·90	9·72	20·72	27·17	105·27	3·37	5·45	16·65	29·40
CBAC	6·50	53·00	78·25	9·12	19·37	23·62	96·87	2·62	4·50	15·25	28·25
SWR	7·00	53·60	87·30	10·70	21·00	22·60	106·00	4·20	5·30	16·30	29·30
NZB	7·00	59·00	91·30	12·70	20·50	28·10	117·10	3·90	4·10	18·60	32·50
CBAJ	6·11	54·22	84·11	9·67	19·44	22·89	100·56	4·33	5·44	16·89	30·00
MOLD	6·50	50·00	76·00	10·20	17·40	22·00	98·50	2·90	6·40	16·40	25·70
AEJ	7·67	58·67	86·33	10·83	24·17	28·00	107·83	4·83	6·67	17·67	30·67
B6RK	7·80	58·60	87·00	10·00	22·00	29·40	112·00	3·40	5·80	17·60	31·60
SJL	7·00	55·10	81·70	11·20	22·60	26·80	100·60	3·90	5·80	16·60	29·10
BALB	6·60	55·90	82·80	10·40	20·30	25·60	100·50	7·70	8·50	19·60	30·60
BKS	7·82	54·82	82·73	10·27	20·55	25·36	100·55	4·09	6·00	15·64	28·00
B10	7·70	56·50	84·90	10·30	20·70	26·40	104·40	3·30	4·60	16·20	29·40
Mean	7·12	55·24	83·47	10·30	20·61	25·83	104·14	3·92	5·65	16·86	29·41
S.D.	0·62	3·03	3·21	0·78	0·79	1·52	4·51	0·82	0·77	1·00	1·26

Strain)	X(23)	X(24)	X(25)	X(26)	X(27)	X(28)	X(29)	X(30))	X(31)	X(32)	X(33)
B6J	73·02	129·32	7·07	13·77	21·17	23·22	46·52	84·20	7·20	78·55	98·22
CBAC	69·87	120·12	6·62	13·50	19·00	22·75	45·25	80·87	6·00	74·75	94·62
SWR	73·20	129·40	7·40	13·10	18·30	24·30	48·10	87·90	6·70	81·10	101·40
NZB	84·10	140·40	7·50	15·40	21·40	25·80	51·70	97·70	7·80	84·70	108·30
CBAJ	73·56	126·56	6·89	14·00	20·56	22·56	47·44	82·56	6·56	77·67	98·56
MOLD	71·60	117·20	6·00	12·70	18·20	20·70	45·10	79·20	4·50	72·20	90·30
AEJ	74·33	131·83	7·83	14·00	19·67	26·00	48·67	88·67	6·83	81·17	101·50
B6RK	76·00	132·80	7·20	14·20	21·20	24·80	48·40	88·80	7·80	80·60	100·80
SJL	75·80	125·00	7·50	14·30	20·70	23·40	42·20	80·00	6·10	75·80	95·80
BALB	71·60	128·10	8·50	15·00	20·40	26·00	46·10	83·00	6·30	77·60	98·30
BKS	72·73	128·00	7·64	14·00	21·45	21·36	45·55	81·18	7·09	78·82	99·09
B10	72·40	129·00	7·60	14·50	22·30	23·10	45·90	85·90	7·20	80·30	100·10
Mean	73·78	128·25	7·27	13·99	20·55	23·47	46·59	84·63	6·76	78·51	98·69
S.D.	2·98	3·66	0·65	0·66	0·80	1·17	1·62	3·37	0·61	2·27	2·76

Strain)	X(34)	X(35)	X(36)	X(37)	X(38)	X(39)	X(41)	X(41)	X(42)	X(43)	X(44)	N
B6J	6·00	15·05	20·75	34·85	38·85	44·67	64·35	68·70	78·67	90·47	93·60	40
CBAC	5·37	13·50	19·12	34·25	37·62	42·50	59·25	62·12	73·37	85·62	85·75	8
SWR	4·20	13·80	20·30	33·70	36·50	41·20	65·50	68·60	79·00	89·50	93·00	10
NZB	6·20	15·10	21·60	36·50	41·40	46·20	68·00	72·70	86·40	99·00	100·10	10
CBAJ	5·67	14·11	19·78	35·67	38·33	44·22	61·78	64·44	76·11	88·44	88·78	9
MOLD	5·30	13·20	18·40	32·30	34·30	42·00	61·60	67·10	73·60	80·10	84·30	10
AEJ	6·50	15·83	21·83	37·00	41·50	47·33	67·67	72·50	83·17	93·00	98·83	6
B6RK	5·80	15·80	21·60	36·20	41·20	45·60	65·60	69·20	79·80	93·00	96·20	5
SJL	4·10	13·60	19·10	34·10	38·30	42·00	61·60	65·70	77·50	88·20	90·00	10
BALB	5·00	14·30	20·70	36·20	41·10	45·00	63·50	65·30	81·00	90·50	94·90	10
BKS	6·27	14·55	20·73	35·09	39·27	44·91	66·73	72·73	79·09	91·91	95·55	11
B10	6·00	15·60	21·40	35·40	39·80	45·20	65·30	69·70	79·50	89·60	93·80	10
Mean	5·60	14·58	20·45	34·96	38·86	44·22	64·22	68·32	78·83	89·95	92·91	
S.D.	0·54	0·65	0·90	1·03	1·25	1·15	1·31	1·72	1·58	1·97	1·85	

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Table 3(*a*). *Mahalanobis distances based on 24 measurements of the pelvis, scapula, femur and tibio-fibula (above diagonal), and on 20 measurements of the humerus, ulna and mandible (below diagonal)*

	B6J	CBAC	SWR	NZB	CBAJ	MOLD	AEJ	B6RK	SJL	BALB	BKS	B10
B6J	—	8·71	13·61	15·78	10·73	13·67	9·94	4·13	11·36	11·08	6·12	5·11
CBAC	7·02	—	14·12	18·23	5·94	12·44	14·22	9·15	12·35	13·65	9·30	9·72
SWR	8·26	8·18	—	14·41	11·72	14·88	16·58	14·82	12·08	12·80	13·15	13·24
NZB	8·01	10·21	8·86	—	16·85	18·08	20·65	16·35	17·79	16·92	14·44	14·99
CBAJ	6·00	4·04	8·22	9·09	—	12·97	14·30	11·03	11·98	12·98	9·63	11·01
MOLD	10·57	9·48	11·02	13·82	9·95	—	15·50	13·98	12·07	11·76	13·26	14·33
AEJ	7·00	10·66	9·92	9·23	10·39	11·40	—	8·95	12·64	13·78	11·58	10·16
B6RK	4·12	7·99	9·12	8·36	7·59	13·30	7·66	—	11·70	12·41	7·97	6·33
SJL	7·42	7·15	8·50	9·82	7·85	12·06	10·55	8·61	—	10·30	11·71	12·92
BALB	8·24	9·27	9·96	9·54	9·50	13·67	7·13	7·98	7·77	—	11·73	12·27
BKS	4·86	10·21	9·63	9·08	8·59	11·92	8·39	6·92	9·73	10·77	—	7·23
B10	3·10	8·09	9·04	8·34	6·69	10·88	8·18	5·55	7·46	9·31	5·01	—

Table 3(*b*). *Mahalanobis distances based on all 44 measurements (7 bones) above diagonal, and on 11 measurements of the mandible below diagonal*

	B6J	CBAC	SWR	NZB	CBAJ	MOLD	AEJ	B6RK	SJL	BALB	BKS	B10
B6J	—	13·89	18·38	20·05	16·32	17·90	14·31	6·67	15·73	16·26	7·94	7·15
CBAC	5·71	—	17·09	23·27	8·32	17·51	18·08	13·28	16·84	18·00	16·06	17·07
SWR	5·55	7·13	—	19·17	16·56	20·13	19·80	19·03	18·47	16·60	18·44	19·66
NZB	6·17	9·35	6·66	—	22·59	24·41	23·10	20·03	23·23	20·74	18·78	19·97
CBAJ	4·91	2·53	6·57	8·17	—	19·41	19·99	15·87	18·95	19·28	16·61	17·47
MOLD	9·02	8·88	10·08	12·69	8·85	—	20·31	20·09	17·39	17·41	18·98	19·14
AEJ	3·51	8·48	6·89	5·68	7·22	10·29	—	13·77	17·58	15·44	15·92	16·53
B6RK	3·68	7·08	6·63	6·87	6·43	12·17	4·72	—	16·27	16·64	11·02	9·66
SJL	6·23	4·77	5·04	7·63	5·36	9·77	8·13	7·23	—	14·17	18·23	17·59
BALB	6·17	6·92	6·30	6·65	6·43	12·52	6·52	5·80	5·68	—	17·72	19·30
BKS	3·40	7·92	6·36	7·23	7·26	9·47	4·44	5·01	8·13	8·55	—	10·02
B10	2·29	6·33	6·18	6·79	5·53	8·43	3·16	4·27	6·62	6·70	3·95	—

Table 4(*a*). *Correlations between the genetic distances (Nei's distance and the % dissimilarity), and the morphometric variables either untransformed, or transformed to* $\log_{10}(X)$. *Spearman's rho was calculated from the ranked data*

	Nei	% Dissim.
Untransformed data		
44 Vars.	0·473	0·597
24 Vars.	0·374	0·477
20 Vars.	0·599	0·632
11 Vars.	0·671	0·675
Log transformation		
44 Vars.	0·516	0·655
24 Vars.	0·449	0·573
20 Vars.	0·497	0·668
11 Vars.	0·676	0·721
Spearman's rho		
44 Vars.	0·343	0·343
24 Vars.	0·280	0·280
20 Vars.	0·401	0·401
11 Vars.	0·565	0·565

Notes: All correlations are significantly different from zero ($P < 0·01$), except for $r = 0·280$ which is significant at $P < 0·05$.

Table 4(*b*). *Correlations among the four sets of morphological variables. (part-whole correlations in parenthesis)*

	44 Vars.	24 Vars.	20 Vars.
24 Vars.	(0·953)	—	—
20 Vars.	(0·840)	0·779	—
11 Vars.	(0·727)	0·685	(0·891)

Note: All correlations significantly different from zero ($P < 0·01$).

conforms well with what is known about the history of these strains. The four substrains of strain C57BL form a single cluster, as do the two substrains of CBA, though as noted above, there may be some inaccuracies in the data for these substrains. Strains SWR and SJL form a loose cluster as might be expected from their origin at different times (1926 and 1955, respectively) from outbred Swiss mice. Strain AEJ, however, does not cluster with strain C57BL even though according to its historical origin (Staats, 1985) it was developed following two crosses to C57BL substrains. Finally, the *M. m. molussinus* strain MOLD is clearly shown to be quite distinct from all other strains of mice, as would be expected.

Cluster analysis of the strains based on the morphometric analysis of the mandible (Fig. 3) gives rather similar results except that the C57BL cluster is looser, and now includes strain AEJ. Similarly, strains CBA/Ca and CBA/J are somewhat more loosely

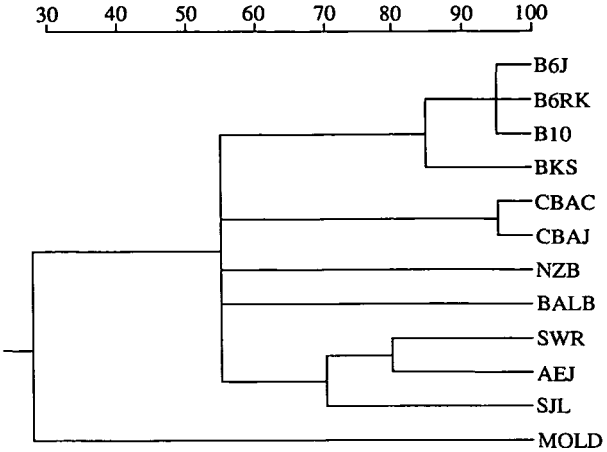

Fig. 2. Average-linkage cluster analysis based on single loci.

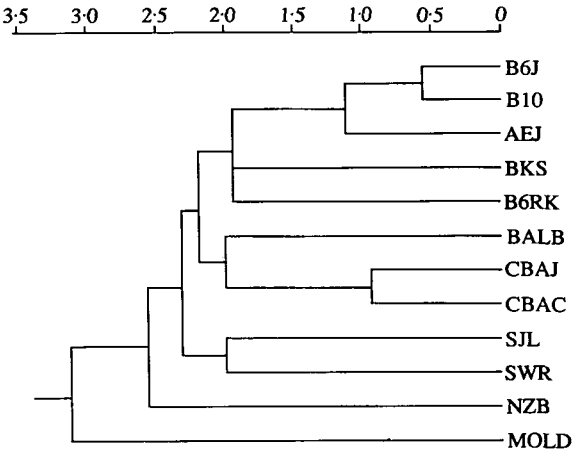

Fig. 3. Average-linkage cluster analysis based on 11 mandible measurements.

clustered. Strain MOLD is shown to be distinct, but not quite as clearly as with the single locus analysis.

Finally, cluster analysis of all 44 variables on 7 bones (Fig. 4) re-establishes the tight C57BL cluster, eliminates AEJ from that cluster, and shows NZB to be more distinct than MOLD from the other strains. Inspection of the morphometric data (Table 2) shows that strain NZB mice are extremely large, and have an unusual shape of pelvis.

The correlations among the genetic and morphometric distances across all strains, before and after suitable linearising (ie. logarithmic) transformations of the morphometric data are shown in Table 4. Among the morphological variables, distances estimated from the two sets of bones based on 24 and 20 measurements had a correlation of $r = 0·78$, and the mandible variables correlated reasonably well ($r = 0·72$) with the set of 24 variables.

In all cases, Nei's genetic distance had a lower correlation with the morphometric distances than did the percent dissimilarity, though as expected, the Spearman's rank correlations were identical. Dis-

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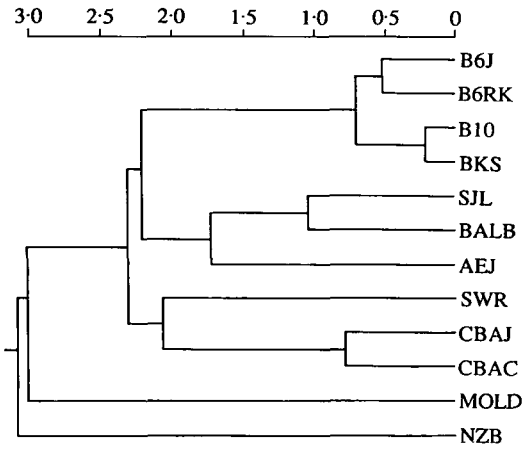

Fig. 4. Average-linkage cluster analysis based on 44 variables from seven bones.

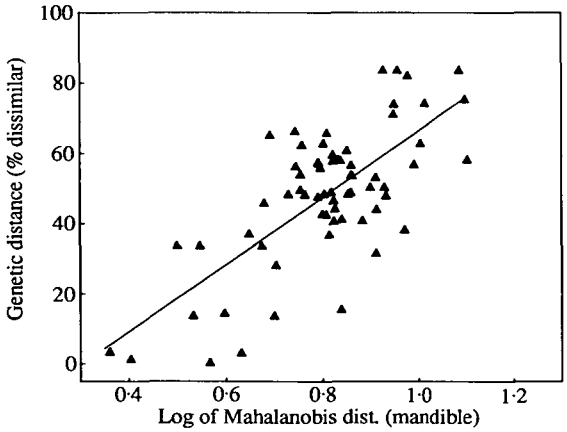

Fig. 5. Morphometric ($\log_{10}$ Mahalanobis distance) distances based on the 11 mandible measurements versus genetic (% dissimilarity) distances. ($r = 0{\cdot}72$, $P < 0{\cdot}01$). The line ($Y = -29{\cdot}1 + 95{\cdot}4X$) shows the regression of percentage dissimilarity on the $\log_{10}$ Mahalanobis distance.

tances based on the percent dissimilarity correlated highly with the distances based on morphometry. Before the logarithmic transformation they ranged from 0·48 to 0·68, but they were increased to from 0·57 to 0·72 after transformation. A plot of genetic distances based on the single loci and the mandible morphometric distance is shown in Fig. 5. This graph is equivalent to the one published by Wayne & O'Brien (1986) in which they found a non-significant correlation between the variables except that in this study there was better representation of closely related strains, and the logarithm of the Mahalanobis distance has been plotted against percent dissimilarity, whereas Wayne & O'Brien plotted Nei's genetic distance against the Mahalanobis distance.

The simulations of sampling variance of the genetic distances resulted in correlations of $0{\cdot}69 \pm 0{\cdot}08$, $0{\cdot}76 \pm 0{\cdot}04$ and $0{\cdot}86 \pm 0{\cdot}01$ for samples sizes of 10, 20 and 30 loci, respectively. This suggests that samples of about 10 cladistically informative loci would, on average, give about as good an estimate of the percent genetic similarity between mouse strains as would estimates based on the morphometric characters. This was further confirmed by regression analysis. Linear regression of the percent dissimilarity between all strains on the logarithm of the Mahalanobis distances between strains based on mandible data (data in Table 3) gave a regression equation of:

$$Y = -29{\cdot}1 + 95{\cdot}4X,$$

where Y is the estimated percent dissimilarity, and X is the log of the Mahalanobis distance. Comparison of the observed percent dissimilarity with the estimated dissimilarity based on the above equation gave a mean absolute deviation of $11{\cdot}01 \pm 8{\cdot}04$. i.e. on average, an estimate of the percentage genetic dissimilarity from a knowledge of the Mahalanobis distance would be in error by about 11%. A similar calculation based on all 44 variables gave a similar estimate of 11·4%. In comparison, a simulated sample based on 10 loci, assuming that the observed dissimilarities are true dissimilarities gave a mean absolute deviation of observed minus estimated dissimilarities of 9·9%.

4. Discussion

The cluster analysis of the 12 mouse strains based on single-gene markers corresponds very closely with known similarities amongst these strains. Closely related substrains such as the four C57BL and two CBA substrains formed tight clusters (possibly too tight), whereas strain MOLD was clearly quite distinct from all the laboratory strains. Thus, this particular collection of inbred strains seems to be well suited to studies of genetic and morphometric distances, and the compiled single-locus data are probably as true a reflection of the real genetic similarities between the strains as can be obtained.

This study shows that, based on a these inbred mouse strains, there is a strong and highly significant correlation of $r = 0{\cdot}58$ to $0{\cdot}72$ between genetic distances as estimated from single loci and the morphometric variables, transformed to a favourable scale. The proportion of the total variation in the morphometric characters that may be 'explained' by variation in the single-gene characters is given by R^2, which ranged from 34–52% depending on the choice of bones measured. The only major discrepancy between the two sets of data concerned strain NZB mice. These mice are not particularly distinct according to their single-gene markers, but they are morphologically unusual in being extremely large, and having an unusual shaped pelvis. Whether these characteristics are a result of the accumulation of many polygenes or just a few major genes is not clear. If there had been deliberate selection for increased body weight in the base population from which this strain was derived (as is entirely possible), then it would probably have changed both the size and shape

of the mice without having much influence on the frequency of the single-gene markers. In such circumstance it is a matter of interpretation as to whether the morphometric or the single-locus markers offer a better estimate of true genetic differences between the strains. Minor disagreements between the two measures may also be noted. Strain AEJ only appears to be similar to the C57BL substrains for mandible shape though the historical record notes a cross and backcross to C57BL substrains. Likewise, there is some disagreement over the tightness of the clusters of closely related substrains, possibly due to under-estimation of genetic divergence for the single-gene markers.

The results do not support Wayne & O'Brien's (1986) claim that 'morphological change and biochemical change are poorly coupled'. The data used in this study were deliberately collected so as to be as far as possible comparable with that presented by Wayne & O'Brien (1986), but without the deficiencies present in their data. Clearly, very closely related strains such as substrains of the same inbred strain should be similar according to both genetic and morphometric criteria, if morphometric and genetic distances are correlated. This was investigated in this study by including several pairs of such strains, whereas Wayne & O'Brien only included a single pair (C57BL/6 and C57BL/10) in their study. Their assertion that strains DBA/1 and DBA/2, SJL and SWR, and CBA and C3H were substrains separated after more than 40 generations of brother × sister mating appears to be in error as it is not in agreement with the historical record according to Rice & O'Brien (1980) and Staats (1985). Similarly, a very distantly related group such as a sub-species should appear very dissimilar both genetically and morphometrically, and this was verified using the MOLD strain in this study. No such strain was used by Wayne & O'Brien. It is not surprising that their study failed to detect such a correlation; restricting the range of variation usually decreases a correlation. The present study also included data on seven bones, rather than the single bone used by Wayne & O'Brien.

There were other similarities between the studies. The morphometric data in both studies were actually collected by the same person (MFWF), and analysed in an identical fashion using identical methods for correcting for size, and the same computer programs for the analysis, and in both cases the genetic distances came from the open literature. Thus, the two studies should be very comparable.

An important difference between the two studies, however, was that Wayne & O'Brien presented genealogical data on some of their strains from which they estimated the 'divergence time' between strains. They noted that 'the genetic distance estimates should increase proportionately with the amount of time the populations have been reproductively isolated'. Their 'divergence time' was correlated with the genetic distance ($r = 0{\cdot}73$, $P < 0{\cdot}05$), but not with the (untransformed) morphological ($r = 0{\cdot}24$, $P > 0{\cdot}05$) distance between strains, and they concluded that 'the present results would imply that molecular evolution is often constant and time-dependent; the precise prediction of the molecular clock theorum'.

Unfortunately, some of their data is inaccurate and misleading. For example, their genealogies show BALB/c and DBA/2 originating from the same base population in about 1920, whereas the historical record shows that these strains have no known common ancestry. Similarly, strain A is shown as coming from the same base population when the historical record shows it to be the result of a cross between the Cold Spring Harbor albino and the Bagg albino (Staats, 1985). However, even if these data were accepted, their suggestion that genetic divergence in these strains is time-dependent is untenable. Fig 6 shows their Fig 4 re-drawn to include the origin, with an added regression line showing genetic distance as a function of divergence time. If genetic distance is time-dependent, then it should be possible to predict the genetic distance between pairs of strains from a knowledge of the divergence time, using regression. Such a regression line should pass through the origin; at time zero there should be no distance, and distance should increase from then on at an almost constant rate (the molecular clock). However, with these data the line meets the X-axis at about 40 years. This implies that all the strains were genetically identical for the first 40 years of their existence (up until about 1945) and then they began to diverge rapidly. At first sight this might seem to confirm the suggestion of Fitch & Atchley (1985*a*) that 'Evolution in inbred strains appears rapid'. However, Fitch & Atchley (1985*b*) later accepted that the data supported the more reasonable suggestion that the ancestral heterozygosity was 0·2 or more, 'and that inbreeding accompanied by selection for heterozygosity can account for the remainder of the documented divergence'. There is no evidence for such rapid evolution in inbred mice.

Obviously, the strains studied by Wayne & O'Brien were not identical for the first 40 years. A much more satisfactory explanation for the data in Fig. 6 is that the genetic distance is a reflexion of inbreeding at the time the strains were separated. The data fall into three groups. A single point after 45 years represents divergence between C57BL/6 and C57BL/10, a pair of sublines separated after about 14 years of brother × sister mating. As there would be little residual heterozygosity in such a strain, these substrains would not be expected to be very divergent. The middle group, separated for 50–70 years represents differences among pairs of strains such as C57L and C57BL/6 which have one parent in common, DBA/1 and DBA/2 which are sublines of a strain which was not fully inbred, and CBA and C3H which were developed from the same cross. These were

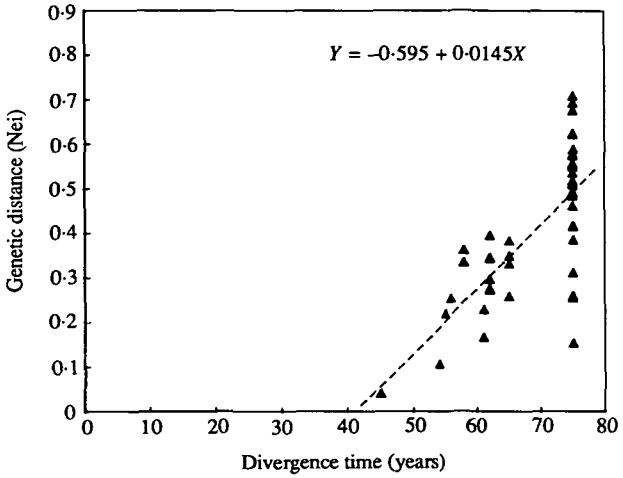

Fig. 6. Data of Wayne & O'Brien (1986) on 'divergence time, and genetic distances among their sample of 15 inbred mouse strains, re-drawn to show the origin and the regression of genetic distance on time. Note that this appears to predict that all strains were identical for the first 40 years, and are now diverging rapidly. A more plausable explanation is that distances are a reflection of inbreeding at the time of separation, coupled with the fact that it takes some years to produce a fully-inbred strain (see text).

separations of related pairs which would be expected to be more divergent than C57BL/6 and C57BL/10. Finally, the group separated for 70–80 years represents differences among strains of independent (e.g. DBA/2 and BALB/c) or nearly independent origin, which could be expected to be highly divergent. The association with time in Fig. 6 is an artifact arising from variation in inbreeding at the time the colonies were separated, and the fact that it takes several years to produce an inbred strain. Obviously, the initial separation had to be among non-inbred strains as there were no inbred strains at that time. Later separations involved partially-inbred strains, which took a few years to produce, and the last separation was within a fully-inbred strain, which again took time to produce. The genetic distances between the strains are not diverging as predicted from the regression line; rather they are staying almost constant, or diverging at the most at the rate predicted by joining the point at 45 years (C57BL/6 vs C57BL/10) with the origin.

Wayne & O'Brien's failure to include any strains separated for less than about 40 years was probably because of their reliance on published data for the estimates of genetic distance. Only in the case of well-established strains was such data available. The data in Fig. 6 therefore provide no evidence of a genetic clock (apart possibly from the separation between C57BL/6 and C57BL/10), and can not be used to validate the estimates of genetic distance as there is no independent estimate of the inbreeding present in the colonies at the time of separation of each pair of strains which would be the single most important factor determining their divergence.

Although data on single-gene markers are often more informative than morphometric data, the simulations of sampling variation for single-locus data, and the regression studies of estimated and observed percent dissimilarity for the morphometric and simulated data should be noted. These suggest that sampling of about 10–20 cladistically-informative loci would give a correlation between genetic distance estimates of 0·69 to 0·76. Assuming that about 20% of loci are polymorphic (Rice & O'Brien 1980), a sample of 50–100 loci would normally need to be examined to give estimates of genetic distance comparable in precision to the morphometric data presented here. Studies of closely related substrains would require the sampling of many more loci in order to find some which were informative. As noted above, in studies of closely related substrains of mice, study of morphometric data has already been shown to be useful (Festing, 1973) whereas studies of single loci are usually uninformative because the substrains do not differ.

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Note Added in Proof

Fitch & Atchley (1987) also concluded that there was no correlation between distances based on single loci and mandible shape. However, like Wayne and O'Brien (1986) their data had few closely related substrains such as C57BL/6 and C57BL/10, and no distantly related strains such as MOLD. Also, in considering relationships such as that between CBA and C3H they took no account of the probable effects of more than 100 generations of brother × sister mating. This would double the additive genetic variance between strains (Fitch & Atchley 1985*b*), and allow ample time for the accumulation of mutations affecting mandible shape (Festing 1973).

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