

Statistical Analysis of Mandible Form in the Mouse

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Abstract

One of the major problems in modern biology is to understand the development and evolution of the morphological form. Lack of knowledge of the underlying causes of variability of morphological structures inhibits exploration of the morphogenetic integration of development and evolution into a comprehensive theory. We use multivariate statistical analyses on 14 morphological traits and body weight over 9 successive weeks for 6 inbred strains of 221 mice to describe multidimensional morphological variation in the mandible and to test several hypotheses. A genetic distance based on 144 gene loci in inbred mice from Atchley and Fitch (1993) was used to evaluate the relationships between morphological and genetic divergence. Multivariate statistical analyses of these 14 morphological traits reflect four factor patterns of covariation within sex-adjusted mouse strains. Discriminant analysis was used to examine the patterns of divergence among these six strains.

Keywords: morphological form, mandible, inbred mouse, multivariate statistics

Introduction

Morphological form includes size and shape. Since the mandible has been extensively studied experimentally, the mammalian mandible is particularly well-suited for the study of development and evolution of the morphological form (Atchley, et al. 1988). The mammalian mandible derives from cranial neural crest cells and includes four progenitor cell populations: chondroblasts, fibroblasts, osteoblasts and myoblasts. Further cell division and migration differentiate the stem cells into various morphogenetic component including Alveolar, Ramal and 3 processes, i.e., Coronoid, Condylar and Angular process. Interaction among these cells and other surrounding tissues will determine the form of the mandible.

One of the plausible approaches to answer the questions about the evolution of complex morphological structures is to study them from a genetic perspective. However, how the complex morphological divergence and genetic divergence are mutually related is not fully understood.

Previous notable methods include: using conventional quantitative genetic analysis to decompose the phenotypic variability into direct additive and dominance genetic effects, indirect maternal additive effects, indirect maternal dominance genetic effect, genetic covariance between direct additive and indirect maternal additive genetic effects, common environmental effect and residual (Atchley, et al. 1985a); carrying out UPGMA (Unweighted Pair Group Method with Arithmetic Mean) cluster analysis to reveal the developmental history of the mandible by using ICR random-bred mice with two generations (Atchley, et al. 1985a); genetic distance for the 24 strains on 144 loci was

computed to access the genetic affinities of inbred mouse strains of uncertain origin (Atchley and Fitch 1993).

Practically speaking, it will be more efficient and economical to obtain inbred one generation data instead of data from two generations. Herein, we will study how to extract a small set of factor scores accounting for the common variation of morphological traits in one generation data set, explore the relationship between genetic divergence and morphological divergence, between genetic divergence and adult body weight based on one generation's morphological data for 6 inbred strains combined with genetic distance (Atchley and Fitch 1993).

We propose to answer the following questions: (i) Are there sex and strain effects with respect to the 14 traits? (ii) What is the dimensionality of the morphological traits accounting for the common phenotypic variation adjusted by sex and strain effects if they are significant? (iii) How to describe the morphological divergence between strains? Does the genetic divergence in the fixed major gene loci correspond to the morphological divergence in the form of mandible? (iv) Which morphological traits are responsible for the difference between the pairwise strains? (v) Which model is the best one characterizing the body weight's growth curve? Can we incorporate the body weight trajectory into the analysis of the phenotypic variation of the mandible traits? How does the genetic divergence influence the adult body weight?

Materials and Methods

This study included a total of 221 mice from six inbred strains, 14 mandible traits and weekly body weight measurements from week 2 to week 10 (Atchley, et al.1985a). Six

inbred strains were analyzed including A/J, BALB/cByJ, C57BL/6ByJ, SEA/GnJ, SEC1/ReJ, SWR/J (Atchley and Fitch 1993). The mice were sacrificed at the age of ten weeks, enviscerated, skinned and defleshed. Then 19 landmarks were digitized from the skeletal surface of each mandible. Fourteen morphological traits obtained from the 19 landmarks are: Posterior mandible length (POSTMANLEN), Anterior mandible length (ANTMANLEN), Height at mandibular notch (NOTCHHIGH), Height at incisor region (INCISHIGH), Concavity (CONCAVITY), Height of the ascending ramus (RAMUSHIGH), Condyloid width (CONDYLWID), Condyloid length (CONDYLLEN), Coronoid height (CORONHIGH), Coronoid area (CORONSIZE), Angular process length (ANGULARLEN), Tooth bearing area (TOOTHAREA), Superior incisive process curve (SUPERINCIS), Inferior incisive process curve (INFERINCIS). These traits describe the size, shape and functional aspects of the mandible. The data were transformed using natural logarithms. Table 1 gives the summary of strain notation and Table 2 gives the definition of the traits shown as abbreviations. The locations of the landmarks are depicted in Figure 1.

MANOVA to check the sex and strain effect. We used multivariate analysis of variance (Johnson and Wichern 1985) to determine if a statistical significant strain and sex effect exists. The corresponding statistical model is $Y=XB+E$, here Y is a $n*r$ matrix of $r=14$ traits (response variables) measured on $n=221$ subjects; X is a $n*p$ matrix of explanatory variables associated with strain and sex effects; B is a $p*r$ matrix of regression coefficients where $p= 8$; E is an $n*r$ error matrix whose rows are independent identical distributed normal with mean 0 and covariance matrix Σ .

Factor analysis to reduce the dimensionality of the traits. Several data reduction techniques exist for in multivariate analysis (Johnson and Wichern 1992). Principal components analysis is primarily a dimension reduction approach that is used to construct linear combinations of the original variables that will provide an optimal reduction in the total variation of the data. Although principal components analysis achieves the data reduction to some degree through its factorization of the covariance matrix, it's only a transformation rather than the result of a particular model for analyzing covariance structure. Factor analysis, on the other hand, provides a new set of latent variables, fewer in number than the original variables that account for the common or shared variation. Herein, we are interested in the common covariance structure. Hence, using factor analysis to explore the underlying factors which determine the traits' correlation is more appropriate for our questions.

The general factor analysis model is expressed as

$$X = \Lambda f + e \quad (2.1)$$

where X = p-dimensional vector of observed responses, $X'=(x_1,x_2,\dots,x_p)$

f =q-dimensional vector of unobserved variables called common factors,

$f' = (f_1, f_2, \dots, f_q)$, e = p-dimensional vector of unobserved variables called unique factors,

$e' = (e_1, e_2, \dots, e_p)$ and $\Lambda = p \times q$ matrix of unknown constants called factor coefficients.

Assumptions:

$$E(ee') = \Psi$$

$$\text{cov}(e, f') = 0,$$

Where Ψ is a diagonal matrix, which means the unique parts of each variable are uncorrelated with each other as well as their common parts.

Summary of the statistical framework:

(i) covariance matrix of the response vector X , denoted by Σ_{xx} ,

$$\Sigma_{xx} = \Lambda\Phi\Lambda' + \Psi, \quad (2.2)$$

where Φ is a $q \times q$ symmetric matrix given the correlation between the common factors, and Λ' represents transpose of Λ . Without loss of generality, assume common factors are uncorrelated, then $\Phi = I$, (2.2) becomes

$$\Sigma_{xx} = \Lambda\Lambda' + \Psi, \quad (2.3)$$

(ii) There is no correlation between the original variables under the condition of the common factors, i.e.

$$\rho(X_i, X_j | f_1, f_2, \dots, f_q) = 0 \text{ for all pairs } i \neq j \quad (2.4)$$

where $\rho(X_i, X_j | f_1, f_2, \dots, f_q)$ is the correlation between X_i and X_j conditioned on the factors.

(iii) Equation (2.4) is equivalent to the linear factor model:

$$X_i = c_i + e_i \quad (2.5)$$

where $c_i = \lambda_{i1}f_1 + \lambda_{i2}f_2 + \dots + \lambda_{iq}f_q$, $\text{cov}(c_i, e_i) = 0$, λ_{ik} is the factor coefficient, which is a regression coefficient quantifying the value of trait i coming from common factor k . So $\text{var}(X_i) = \text{var}(c_i) + \text{var}(e_i)$, where $\text{var}(c_i)$ and $\text{var}(e_i)$ are the common variance and unique variance of X_i respectively. The communality of a variable is defined as the portion of a variable's total variance that is accounted for by the common factors.

To improve the interpretation of the factor analysis results, one generally rotates the eigenvectors to "simple structure." There are two rotation methods--orthogonal and oblique depending upon whether there are potentially higher order correlations present.

Under the assumption of having uncorrelated variables, we can use orthogonal rotation such as Varimax algorithm. More generally, in oblique rotation, it is not necessary for factors to be uncorrelated and one can assume a higher order level of correlation among the factors themselves. One might regard orthogonal rotation as a subset of oblique rotations.

Herein, we use the oblique rotation method called the PROMAX algorithm to make the rotation reflect the correlated phenomena in the loadings of the factors and their correlations (Johnson and Wichern 1992). The purpose of the rotation to simple structure is to make a pattern of loadings so that every variable has a high value of loading on a single factor and has small loadings on the remaining factors without changing the covariance matrix.

Determining the number of common factors is an important part of factor analysis and a number of procedures have been proposed to determine the number of factors to be included. Herein, we will choose the number of common factors based on the proportion of sample variance explained and on the ease of interpretation. Factor loadings are estimated by the principal factor method and factor scores are generated by using an ordinary least squares procedure. Also we will adjust the data if there are significantly strain and sex effects before doing factor analysis.

Factor scores. The estimated values of the common factors, called factor scores, are obtained using ordinary least squares procedures (Johnson and Wichern 1992, page 431) producing a 221×4 matrix. Factor score which is the q dimensional vector of j th observation is as following:

$$\hat{f}_j = \begin{bmatrix} \frac{1}{\sqrt{\hat{\lambda}_1}} \hat{e}'_1(x_j - \bar{x}) \\ \frac{1}{\sqrt{\hat{\lambda}_2}} \hat{e}'_2(x_j - \bar{x}) \\ \vdots \\ \frac{1}{\sqrt{\hat{\lambda}_q}} \hat{e}'_q(x_j - \bar{x}) \end{bmatrix}, \text{ where } (\lambda_i, e_i), i=1..q \text{ are eigenvalue-eigenvector pairs of the}$$

sample variance-covariance matrix, x_j is jth observation of p dimensional vector, \bar{x} is the p dimensional overall mean vector.

We use factor scores as inputs to the following analysis of the covariance patterns among morphological traits and body weight curve.

Measurement of the morphological divergence and its relationship to genetic

divergence. The Mahalanobis statistical distance (Mahalanobis 1936) between two points $x=(x_1, \dots, x_p)^T$ and $y=(y_1, \dots, y_p)^T$ in the p-dimensional space R^p is defined as

$$\sqrt{D^2} = d_s(x, y) = \sqrt{(x - y)^T S^{-1} (x - y)}, \text{ where } S \text{ is the covariance matrix. From the}$$

definition, the Mahalanobis distance is a Euclidean distance weighted by the sample variance-covariance matrix. The Mahalanobis distance takes the intercorrelations of morphological traits into account and also gives a measurement of morphological divergence of different strains. The data used will be adjusted by sex effect if there is significant sex effect.

As we know phenotypic variation can be partitioned into genotypic variation and environmental variation. An interesting question is whether the pair-wise genetic divergence in the fixed major gene loci corresponds to the pair-wise morphological divergence in mandible form. Our hypothesis is to test the linear relationship between

genetic divergence and morphological divergence. The source of genetic distance matrix for the 144 loci for the 6 inbred strains of mice came from Atchley and Fitch's paper (1993)—“Genetic affinities of inbred mouse strains of uncertain origin”, where genetic distance is defined as the percentage of the different fixed alleles between a pair of strains.

Discriminant analysis. Factor analysis can tell the patterns of covariance of morphological traits and Mahalanobis distance can give a measurement to measure the morphological divergence. But which morphological traits are responsible to differentiate the mouse strains have not been explored. A multigroup discriminant analysis (the data after adjusting the sex effect if there is a significant sex effect) of the 6 inbred mice strains will produce the optimal subset of traits that maximize the separation of the strains and will answer the question “which morphological traits are responsible for discriminating these six strains of inbred mice?”. Fisher's method for discriminating among several populations was motivated to obtain a reasonable representation of the population that contains only a few linear combinations of the observations. The Fisher's Sample Linear discriminant function was computed by the following formular:

Let $\hat{\lambda}_1, \hat{\lambda}_2, \dots, \hat{\lambda}_s > 0$ denote the $s \leq \min(q-1, p)$ nonzero eigenvalues of $W^{-1}\hat{B}_0$ and $\hat{e}_1, \hat{e}_2, \dots, \hat{e}_s$ be the corresponding eigenvectors (scaled so that $\hat{e}' S_{pooled} \hat{e} = 1$). Then the vector of coefficients \hat{l} that maximizes the ratio (which measures the variability between the groups relative to the common variability within groups):

$$\frac{\hat{l}' \hat{B}_0 \hat{l}}{\hat{l}' \hat{W} \hat{l}} = \frac{\hat{l}' \left(\sum_{i=1}^q (\bar{x}_i - \bar{x})(\bar{x}_i - \bar{x})' \right) \hat{l}}{\hat{l}' \left(\sum_{i=1}^q \sum_{j=1}^{n_i} (\bar{x}_{ij} - \bar{x}_i)(\bar{x}_{ij} - \bar{x}_i)' \right) \hat{l}}$$

is given by $\hat{l}_1 = \hat{e}_1$. The linear combination $\hat{l}_m' x$ is called the sample m^{th} discriminant, $m \leq s$ and $\hat{B}_0 = \sum_{i=1}^q (\bar{x}_i - \bar{x})(\bar{x}_i - \bar{x})'$ is the sample

between group matrix, $W = \sum_{i=1}^q (n_i - 1) S_i = \sum_{i=1}^q \sum_{j=1}^{n_i} (\bar{x}_{ij} - \bar{x}_i)(\bar{x}_{ij} - \bar{x}_i)'$ is the sample within group matrix.

growth curve models for body weight. We propose three models to fit the measured body weight's growth curve.

The first model is a longitudinal model with variance and covariance matrix of AR(1) type which captured the property of variance and covariance of the error term: $y_{ij} = \beta_0 + \beta_1 d_j + e_{ij}$. Here, y_{ij} represents the j th measurement on the i th object ($i = 1, \dots, 221$; $j = 1, \dots, 9$), d_j is the corresponding day, β_0 and β_1 are regression coefficients and $\text{var}(e_i) = \text{AR}(1)$, e_i 's are independent identical distributed vectors.

The second model is a nonlinear logistic model with fixed effect:

$y_{ij} = b_1 / (1 + \exp[-(d_j - b_2) / b_3]) + e_{ij}$. Here y_{ij} represents the j th measurement on the i th object ($i = 1, \dots, 221$; $j = 1, \dots, 9$), d_j is the corresponding day, b_1, b_2, b_3 are the fixed-effects parameters, e_{ij} are the residual errors assumed to be independent identical distributed with mean 0 and variance σ_e^2 . The advantage of this model is that we can easily get the physically important values from the estimates, such as the inflection point is b_2 , and the maximum slope is $b_1 / (4 * b_3)$.

The third model is a nonlinear logistic model with random effect. Pinheiro and Bates (1995) propose the following logistic nonlinear mixed model for a growth curve model: $y_{ij} = (b_1 + u_i) / (1 + \exp[-(d_j - b_2) / b_3]) + e_{ij}$. Here, y_{ij} represents the j th measurement on the i th object ($i = 1, \dots, 221$; $j = 1, \dots, 9$), d_j is the corresponding day, b_1, b_2, b_3 are the fixed-effects parameters, u_i ($i = 1, \dots, 221$) are the random-effect parameters assumed to be independent identical distributed with mean 0 and variance σ_μ^2 and e_{ij} are the residual errors assumed to be independent identical distributed with mean 0 and variance σ_ϵ^2 and independent of the u_i .

By using information criteria, AIC (Akaike Information Criterion, $AIC = -2(\text{maximum log likelihood}) + 2 * \text{number of free parameters}$) and BIC (Schwarz Bayesian Information Criterion, $BIC = -2(\text{maximum log likelihood}) + \log(\text{number of observations}) * \text{number of free parameters}$) (for a review of the use of information criterion in model selection, see, e.g., Burnham and Anderson, 2002), we can select the best model which has the smallest AIC and BIC values.

We used factor analysis to study the covariance patterns among growth function and factor scores of morphological traits obtained by factor analysis and use linear regression analysis to explore the linear relationship between genetic distance and adult body weight.

Results

This project examines the following questions: (i) does significant sex and strain effects occur with respect to the 14 morphological traits; (ii) is there a significant correspondence between divergence between these mouse strains and genetic divergence

in large numbers of fixed major gene loci; (iii) which morphological traits are responsible for the difference between the pairwise strains; (iv) which model best characterizes the body weight's growth curve; the relationship between the body weight and morphological trait factors; and (v) does genetic divergence in major gene loci correspond to differences the adult body weight.

Strain and sex effects: MANOVA shows there are significant overall strain and sex effects. Univariate analysis of variance (See Table 3) indicates significant strain effects for all traits, and significant sexual dimorphism for all mandible traits except there is no significant sex effect for traits ANTMANLEN, CONDYLLLEN, CORONHIGH, SUPERINCIS, INFRINCIS.

Dimensionality of morphological traits: Using the sex and strain adjusted data, we found that the first four factors explained 9% of the common variance in the form of mandible, while the fifth factor explained only 3% variation of the common variance and was not included in subsequent analysis.

In factor 1, all the factor coefficients are positive, suggesting that all the traits covary in the same direction. The variables with the largest positive factor coefficients are Tootharea, Ramushigh, Angularlen, Postmanlen and Concavity. Thus, these traits reflect measurements of the tooth area, Ramus process and Concavity. For simplicity, we denoted factor 1 as height and length of the mandible.

In factor 2, Coronsize, Coronhigh, Notchhigh have larger positive factor coefficients, with an inverse relationship between Coronsize and Coronhigh versus Concavity, Condyllen, Angularlen and Ramushigh. These traits reflect the measurement of Coronoid process. So we designated factor 2 as Coronoid process.

In factor 3, Superincis have larger negative factor coefficients while Inferincis have larger positive factor coefficients, which means if we increase one unit of factor 3, we will decrease Superior incisive process curve and increase Inferior incisive process curve. These traits describe the height of the incisor Alveolar. We named the factor3 as curvature of the incisive process.

In factor 4, Antmanlen and Superincis have the larger positive factor coefficients. These traits characterize the length of Anterior. We defined factor 4 as Anterior length.

From exploratory factor analysis, we have extracted the 4 factors from 14 traits named as: Factor1-- height and length of the mandible; Factor 2-- Coronoid process; Factor 3-- curvature of the incisive process; Factor 4-- Anterior length. These four factors explain 97% of the common variance. Coronsize has the largest communality as 0.96 and Tootharea have the third largest communality as 0.71 of the 14 traits. These two traits are the only two traits reflecting the area of the traits. As we know, communality describes the portion of total variance accounted by common variance. The smallest two communalities are 0.14 and 0.18 which relate to Condylwid and Condyllen respectively, which means this factor model can not well explain Condylar process by the four common factors, the unique factors account most of the total variation of the Condylar process. Moreover, because Condylar region is a major region of postnatal growth in the mandible(Atchley, et al. 1985a), the unique factors are probably related to the postnatal growth.

Although the mandible seems to be a single bone, the patterns of covariance of mandible demonstrate the ontogenetic history of these morphological traits and reflect several developmental and functional skeletal units.

Morphological divergence between strains. We use Mahalanobis distance to quantify the morphological divergence between strains. Table 5 gives the Mahalanobis distance and the genetic distance between the six strains. As we mentioned before, genetic distance represents the percentage of the different fixed alleles between a pair of strains with respect to 144 loci for 6 inbred strains of mice (Atchley and Fitch 1993). The Mahalanobis distance between strain SEC1/ReJ and C57BL/6ByJ is the largest, the smallest distance occurs between strain SEA/GnJ and BALB/cByJ, while the genetic distance between strain SEA/GnJ and SWR/J is the greatest, the smallest distance occurs between strain SEC1/ReJ and BALB/cByJ, consist with the fact that SEC1/ReJ founders which have BALB/cByJ as their ancestor included possibly 50%~75% BALB/cByJ genes (Atchley and Fitch 1993).

All pairwise distances are statistically significant ($p < 0.0001$).

Relationship between genetic divergence and morphological divergence. Our hypothesis is that the observed morphological divergence obtained by patterns of covariance of mandible traits can be linearly explained by the genetic divergence. Linear regression is used to test this hypothesis (Figure 2). There is no significant linear relationship between these two distances ($p_value = 0.14$). The possible reasons why morphological divergence and genetic divergence do not covary include the followings:

(i) It may be possible that use of these 144 major gene loci, which were selected as being associated with many developmental systems, not only for mandible traits, decreases the chances of detecting a significant linear relationship between genetic divergence and morphological divergence. Our morphological divergence was measured by Mahalanobis distance of the mandible traits which were controlled by a small subset of 144 major

genes. (ii) Morphological divergence were not a unique function of the genetic distance, but also depended on their environmental effects (Atchley, et al.1985a).

It is possible in some situations that genetic distance may be determined by relatively few major genes related to mandible traits and that may increase the chances of detecting significant linear relationship between genetic divergence and morphological divergence.

Discriminant analysis. An interesting question is which traits are responsible for the differences in morphological form between these various inbred mouse strains. Table6 gives the four discriminant function vectors that account for 96% of the total morphological variance. Vector 1, which discriminates primarily strains C57BL/6ByJ and SEC1/ReJ, has the largest coefficients on Incishigh and Antmanlen related to Alveolar part, with an inverse relationship between Incishigh versus Postmanlen. Vector 2, which marks the difference of strain SWR/J from strain BALB/cByJ and C57BL/6Byj, has large coefficients on Ramushigh and Angularlen related to secondary Chondroblasts process. Vector3, which distinguishes strain A/J from strain SEC1/ReJ and SWR/J, has large coefficients on Notchhigh, with an inverse relationship between Notchhigh versus Concavity and Coronhigh, associated with Ramus component and Coronoid process. Vector 4, differentiating strain A/J and strain SEA/GnJ, has large coefficients on Angularlen, with an inverse relationship between Angularlen versus Coronsize and Inferincis, associated with Angular process, Coronoid process and Alveolar component. Vectors1,2,3 and 4 account for 49.62%, 23.66%, 12.78% and 10.13% of the total variance respectively, which implies that the form of the Alveolar process explains the most of the total variance by distinguishing between the strain C57BL/6Byj and

SEC1/ReJ. Likewise in Table 5, the Mahalanobis distance between strain C57BL/6Byj and SEC1/ReJ is the largest of all the pairwise strains.

Model selection. We use three models to fit the body weight's growth curve. They are Longitudinal with AR (1) model, Nonlinear model with fixed effect and Nonlinear model with random effect. From the model selection statistics in Table 7, we can choose the best model by using information criteria, AIC and BIC, such that the model with the smallest AIC and BIC values is the best model. In this study, the best model is the nonlinear model with a random effect to fit the measured body weight's growth curve. Also we get the estimates of all the parameters $\hat{b}_1 = 3.18, \hat{b}_2 = 6.56, \hat{b}_3 = 11.63, \hat{\sigma}_u^2 = 0.017, \hat{\sigma}_e^2 = 0.009$, estimated inflection point=7, maximum slope=0.068.

Relationship between growth curve and morphological factor scores. In Table 8, we use exploratory factor analysis to study the patterns of covariance between the growth curves and factor scores obtained from the previous factor analysis in Table 4. The columns give the resultant factor patterns for the new set of factors denoted as "hidden variables". The factor pattern shows that curvature of the incisive process has a stronger correlation with the adult body weight starting from week 3; early body weight at week 2 uniquely falls into one category, the other factors are not correlated with the body weight.

Relationship between genetic divergence and the adult body weight. Table 9 gives the Mahalanobis distance of pairwise strains with respect to body weight at week 10 and the genetic distance between the six strains. By doing linear regression (see Figure 3), we can see that there is no significant linear relationship between genetic and morphological divergence in body weight at 10 weeks of age for these 6 strains.

Relationship between genetic divergence and the discriminant function scores.

From discriminant analysis as used in Table 6, we can also calculate the discriminant function scores for each of 221 mice. Figure 4 plots the relationship between genetic divergence and the Mahalanobis distance with respect to discriminant function scores. No linear relationship is apparent (p-value=0.13).

Discussion

Factor analysis. Through exploratory factor analysis, we define four factors based on patterns of covariance of morphological traits, but we do not know whether these four factors are related to the underlying causal factors forced by progenitor cell populations (chondroblasts, fibroblasts, osteoblasts and myoblasts).

We assume here that the mechanism how the underlying causal factors control the development and evolution of the morphological form of the mandible is not clear. What we can get from factor analysis are the reduction of dimensionality of morphological traits and easy computation using factor scores instead of the original traits, moreover, the patterns of covariation among the traits and the extent of intercorrelations between individual traits and regions of the mandible.

If the data are normally distributed, we can use confirmatory factor analysis to check the model fit obtained from exploratory factor analysis. Here, although the log transformation improved the normality to some extent, the data can not be regarded as normal data. That is the reason why confirmatory factor analysis is not applicable here. Exploration of other statistics tool such as nonparametric Bayesian in this context will be a promising approach.

Statistical significance and biological significance. The result of statistical data analysis should be used with caution. Statistical insignificance should not be automatically assumed to represent biological insignificance (Tacha et al. 1982). Further investigation will be necessary to give good biological interpretation. For example, the possible reasons why morphological and genetic divergence does not covary are due to:

(i) small sample size of inbred strains which may cause bias; (ii) it may be possible that use of these 144 major gene loci, which were selected as being associated with many developmental systems, not only for mandible traits, decreases the chances of detecting a significant linear relationship between genetic divergence and morphological divergence; (iii) Morphological divergence were not a unique function of the genetic distance, but also depended on their environmental effects (Atchley, et al.1985a). In this project, we have only six inbred strains of mice. Although we have 221 observations, genetically speaking, we really have only 6 observations because inbred strains are isogenic. Collecting morphological data with more strains and studying the genetic divergence based on a smaller subset of 144 major gene loci associated with the mandible traits will make more accurate and meaningful results. Also discriminant function scores and genetic distance do not covary even though they are both measures between groups variation.

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Appendix

When we scan the data, we find that trait4—Incishigh violates the normality although the factor analysis does not require normality assumption, and we tried to use Box-Cox transformation to correct for non-normality, but no further improvement occurs. One could do a Box-Cox transformation on the log data as well. We also tried to detect the outliers in a statistical sense, but still we can not find any biological support to exclude the outliers. In this project, we include all the observations and all the traits, and do log transformation on trait and body weight variables. Figure 5 shows the QQ-plot of the log transformation of the traits.

Tables

Table 1: summary of strain notation

Strain code	strain	Total Number of observations	Number of males	Number of females
A	A/J	15	10	5
B	BALB/cByJ	36	20	16
F	C57BL/6Byj	38	22	16
L	SEA/GnJ	51	23	28
M	SEC1/ReJ	41	23	18
O	SWR/J	40	18	22
Total		221	116	105

**Table 2: definition of the 14 traits and their short descriptive code
(Posterior traits are in italics)**

measurement\position	Anterior	Region	<i>Posterior</i>	<i>region</i>
Length	t2:ANTMANLEN=d(4,6)	Body	t1:POSTMANLEN=d(1,4) t8:CONDYLLEN=m(16-17,14-19) t11:ANGULARLEN=m(1-2,3-19)	<i>body</i> <i>condylar Process</i> <i>angular Process</i>
Height	t4:INCISHIGH=d(5,8) t13:SUPERINCIS=d(8,4-6) t14:INFERINCIS=d(5,4-6)	Body Body Body	t3:NOTCHHIGH=d(3,14) t6:RAMUSHIGH=d(16,2-4) t5:CONCAVITY=d(3,2-4) t9:CORONHIGH=d(12,14)	<i>angular+ramus</i> <i>condylar+body+angular</i> <i>body+angular</i> <i>coronoid Process</i>
Width			t7:CONDYLWID=d(15,18)	<i>condylar Process</i>
Area	t12:TOOTHAREA=s(3,4,5,6,7,8,9,11)	body+Alveolar	t10:CORONSIZE=s(11,12,13,14)	<i>coronoid Process</i>

Where t2 = trait2, ANTMANLEN is the descriptive code of trait2 defined as d(4,6). d(4,6) is the distance between landmarks 4 and 6, m(16-17,14-19) is the distance from the midpoint of a line from 16-17 to the midpoint of a line from 14-19, d(16,2-4) is the vertical distance from 16 to the line 2-4, s(11,12,13,14) is the area enclosed by landmarks 11,12,13,14.

Table 3: Univariate analysis of strain and sex effect with respect to traits

test/trait	1	2	3	4	5	6	7	8	9	10	11	12	13	14
strain effect	*	*	*	*	*	*	*	*	*	*	*	*	*	*
sex effect	*	0	*	*	*	*	*	0	0	*	*	*	0	0

Note: * represents trait has significant effect ($p < 0.05$), 0 represents trait has no significant effect.

Table 4: The PROMAX rotated factor pattern matrix for the 14 traits analysis (data used after adjusting the sex and strain effects)

Traits		Factor1	Factor2	Factor3	Factor4	Communality
trait1	POSTMANLEN	0.652	-0.071	-0.293	-0.217	0.563
trait2	ANTMANLEN	0.397	0.196	0.303	0.482	0.520
trait3	NOTCHHIGH	0.401	0.337	0.145	0.139	0.315
trait4	INCISHIGH	0.478	0.175	-0.070	0.250	0.327
trait5	CONCAVITY	0.596	-0.337	-0.027	-0.212	0.514
trait6	RAMUSHIGH	0.703	-0.236	0.203	-0.228	0.644
trait7	CONDYLWID	0.369	-0.046	0.040	-0.001	0.140
trait8	CONDYLLEN	0.266	-0.305	0.114	-0.015	0.177
trait9	CORONHIGH	0.248	0.528	-0.118	-0.248	0.416
trait10	CORONSIZE	0.229	0.894	-0.089	-0.317	0.960
trait11	ANGULARLEN	0.656	-0.250	0.106	-0.252	0.568
trait12	TOOTHAREA	0.771	0.044	-0.002	0.331	0.706
trait13	SUPERINCIS	0.274	-0.044	-0.831	0.333	0.878
trait14	INFERINCIS	0.141	0.154	0.589	0.131	0.408

Table 5: Mahalanobis distance (upper triangular region) and genetic distance matrix (lower triangular distance)

From	A/J	BALB/cByJ	C57BL/6Byj	SEA/GnJ	SEC1/ReJ	SWR/J
A/J	0	8.21	8.67	6.61	8.32	7.77
BALB/cByJ	26.9	0	7.92	5.03	5.43	8.48
C57BL/6Byj	48.5	47.1	0	7.98	10.24	6.85
SEA/GnJ	36.4	23.4	42.9	0	5.67	6.15
SEC1/ReJ	24.7	9.6	41.5	26.8	0	8.31
SWR/J	48	47.6	50	57.3	39.1	0

Note: Each distance below the diagonal represents the percentage of the different fixed alleles between a pair of strains with respect to 144 loci for 6 inbred strains of mice (Source: Atchley, Fitch, 1993, "Genetic affinities of inbred mouse strains of uncertain origin"). Each distance above the diagonal represents the Mahalanobis distance of pairwise strains with respect to 14 traits.

Table 6: Discriminant function analysis of 14 traits for the 6 inbred strains of mice(data adjusted by the sex effect)

Trait:	Can1	Can2	Can3	Can4
POSTMANLEN	-0.385	0.416	0.018	0.015
ANTMANLEN	0.452	-0.032	0.160	-0.188
NOTCHHIGH	-0.014	0.436	-0.426	-0.117
INCISHIGH	0.547	0.275	-0.081	-0.196
CONCAVITY	-0.041	0.255	0.324	-0.176
RAMUSHIGH	-0.146	0.501	0.243	-0.142
CONDYLWID	-0.057	0.205	-0.312	-0.219
CONDYLLEN	0.114	0.433	-0.128	0.286
CORONHIGH	0.153	0.419	0.335	0.268
CORONSIZE	0.133	0.179	0.096	0.428
ANGULARLEN	0.024	0.555	0.202	-0.384
TOOTHAREA	0.106	0.143	0.097	0.238
SUPERINCIS	-0.184	0.130	0.179	-0.173
INFERINCIS	0.364	0.276	0.055	0.345
Var %	49.620	23.660	12.780	10.130
Strain means				
A/J	0.442	-1.308	4.522	-3.561
BALB/cByJ	-2.243	3.104	-0.125	0.732
C57BL/6Byj	5.213	2.717	-0.481	-0.555
SEA/GnJ	-1.227	-0.781	1.539	1.880
SEC1/ReJ	-4.397	-0.446	-1.717	-1.497
SWR/J	2.973	-3.431	-1.328	0.341

Table 7: Model selection of body weight growth curve

Model	AIC	BIC
Model1--Longitudinal with AR(1)	-1824	-1817
Model2--Nonlinear with fixed effect	-1836	-1813
Model3--Nonlinear with random effect	-3064	-3047

Table 8: Factor pattern of growth function and factor scores

Symbol	Description	Hidden variable1	Hidden variable2	Hidden variable3	Hidden variable4
1	height and length of the mandible	0.151	-0.470	0.233	0.591
2	Coronoid process	0.416	0.397	0.478	-0.122
3	curvature of the incisive process	0.553	0.147	0.264	0.489
4	Anterior length	0.351	0.472	0.466	0.040
bw1	Body weight at week 2	0.547	0.637	-0.239	0.030
bw2	Body weight at week 3	0.639	0.460	-0.378	0.201
Bw3	Body weight at week 4	0.788	0.014	-0.363	0.213
Bw4	Body weight at week 5	0.873	-0.229	-0.223	0.037
Bw5	Body weight at week 6	0.916	-0.170	-0.051	-0.084
Bw6	Body weight at week 7	0.899	-0.200	0.066	-0.170
Bw7	Body weight at week 8	0.932	-0.188	0.125	-0.187
Bw8	Body weight at week 9	0.925	-0.142	0.109	-0.119
Bw9	Body weight at week 10	0.893	-0.171	0.089	-0.172

Table 9: Mahalanobis distance (upper triangular region) and genetic distance matrix (lower triangular distance)

From	A/J	BALB/cByJ	C57BL/6Byj	SEA/GnJ	SEC1/ReJ	SWR/J
A/J	0	0.042	0.201	0.447	1.505	1.163
BALB/cByJ	26.9	0	0.244	0.49	1.547	1.205
C57BL/6Byj	48.5	47.1	0	0.246	1.304	0.961
SEA/GnJ	36.4	23.4	42.9	0	1.058	0.715
SEC1/ReJ	24.7	9.6	41.5	26.8	0	0.342
SWR/J	48	47.6	50	57.3	39.1	0

Note: Each distance below the diagonal represents the percentage of the different fixed alleles between a pair of strains with respect to 144 loci for 6 inbred strains of mice (Source:Atchley,Fitch,1993,"Genetic affinities of inbred mouse strains of uncertain origin"). Each distance above the diagonal represents the Mahalanobis distance of pairwise strains with respect to body weight at week 10.

Figures:

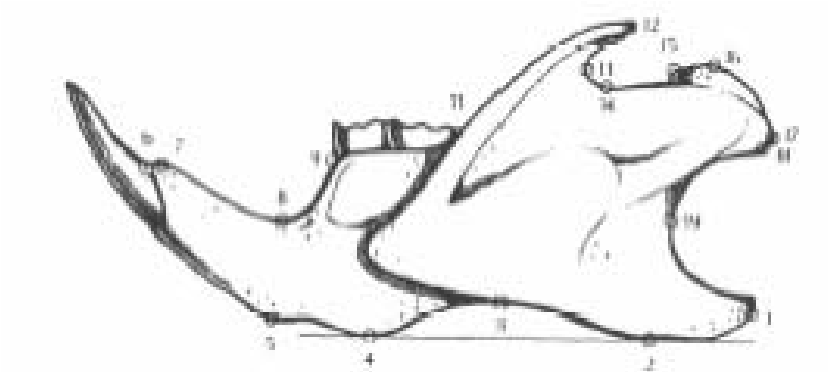


FIGURE 1.—Outline of the mature mouse mandible denoting the position of morphological landmarks used to describe the traits presented in Table 1.

Figure 1: landmarks of the mature mouse mandible.(from p. 581 of Atchley, W. R., et al., 1985. “Genetic analysis of size-scaling patterns in the mouse mandible.” *Genetics* 111:579-595, 1985.)

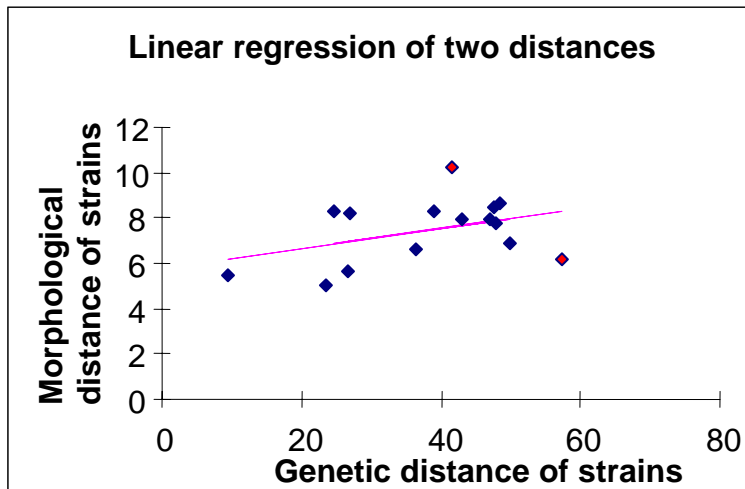


Fig 2: Linear regression of genetic and morphological distance (data adjusted by sex effect)

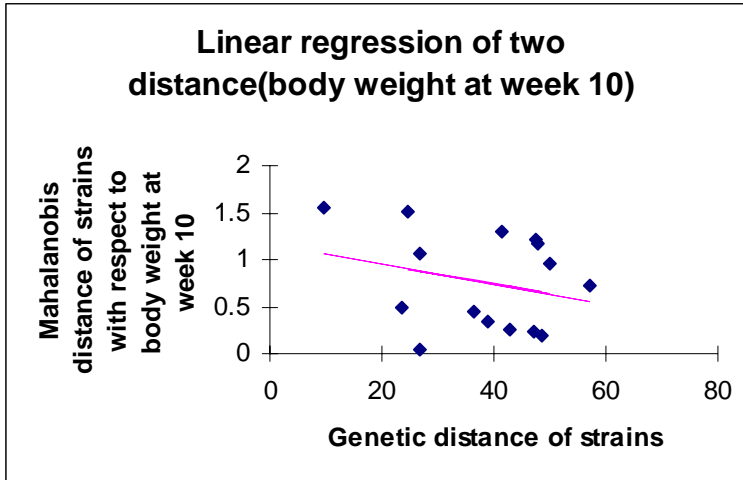


Fig 3: Linear regression of genetic and Mahalanobis distance between strains with respect to body weight at week 10($p_value=0.34$, $R^2 =7\%$)

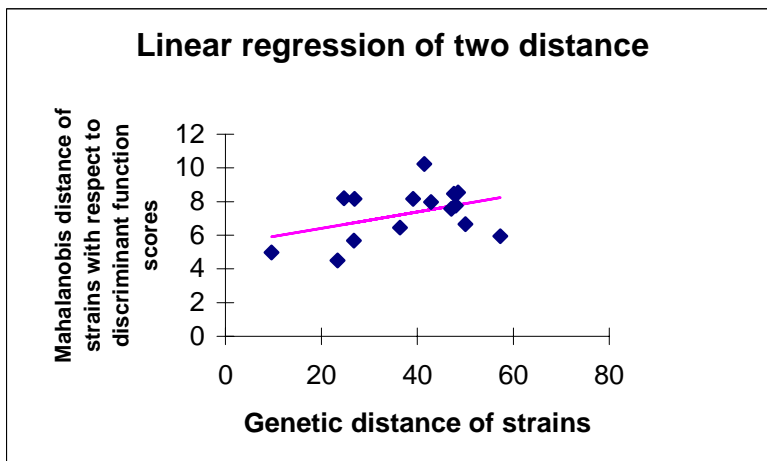


Fig 4: Linear regression of genetic and Mahalanobis distance between strains with respect to discriminant function scores obtained by discriminant analysis($p_value=0.13$, $R^2 =17\%$)

Fig 5: QQ-plot of the log transformation of the traits

