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Developmental integration in a complex morphological structure: how distinct are the modules in the mouse mandible?

Christian Peter Klingenberg,[a,*] Katharina Mebus,[b] and Jean-Christophe Auffray[c]

[a]School of Biological Sciences, University of Manchester, 3.614 Stopford Building, Oxford Road, Manchester M13 9PT, UK
[b]Department of Biology, University of Konstanz, 78457 Konstanz, Germany
[c]Institut des Sciences de l'Evolution, CC 064, Université de Montpellier II, 34095 Montpellier Cedex 05, France
*Author for correspondence (e-mail: cpk@man.ac.uk)

SUMMARY The mouse mandible has long served as a model system for studying the development and evolution of complex morphological structures. We used the methods of geometric morphometrics to reassess the hypothesis that the mandible consists of two separate modules: an anterior part bearing the teeth and a posterior part with muscle attachment surfaces and articulating with the skull. The analyses particularly focused on covariation of fluctuating asymmetry, because such covariation is due exclusively to direct interactions between the developmental processes that produce the traits of interest, whereas variation of traits among individuals also reflects other factors. The patterns of fluctuating asymmetry and individual variation were only partly consistent, indicating that developmental processes contribute differentially to variation at different levels. The results were in agreement with the hypothesis that the anterior and posterior parts of the mandible are separate developmental modules. Comparison of all alternative partitions of the landmarks into two contiguous subsets confirmed the hypothesis for the location of the boundary between modules but also underscored that the separation between them is not complete. Modularity is therefore manifest as the relative independence of parts within the framework of overall integration of the mandible as a whole—it is a matter of degrees, not all or nothing.

INTRODUCTION

Modularity appears to be a general characteristic of biological organization, which is observed at levels ranging from the molecular components of cells to the organs of whole organisms (Cheverud 1996; Wagner 1996; Hartwell et al. 1999; von Dassow and Munro 1999; Bolker 2000; Winther 2001). Modules are made internally coherent by manifold and strong interactions among their component parts, but they are relatively independent from other modules and have relatively few or weak connections with other parts of the system. In the context of organismal morphology, modules arise from the spatial pattern of developmental interactions that take place within embryonic fields (Davidson 1993, 2001, ch. 4; Wilkins 2002, p. 255–258). Delimiting modules and examining their correspondence with anatomical features is essential for understanding the development and evolution of morphological structures.

The mouse mandible has long served as a model system for complex morphological structures, that is, structures that are composed of multiple parts with different embryological origins and timing of differentiation (Atchley and Hall 1991; Atchley 1993; Kuratani et al. 1997; Miyake et al. 1997; Depew et al. 2002). The mandible consists of two primary functional units: the alveolar region, which is the anterior part bearing the teeth, and the ascending ramus, which articulates with the rest of the skull and provides surfaces for muscle attachment (Fig. 1). Each of these in turn is composed of several units that are anatomically recognizable and arise from distinct cell populations that differentiate at different times (Atchley and Hall 1991; Atchley 1993; Miyake et al. 1997). Several studies have examined whether the fundamental units of morphological variation in the mandible correspond to these functional units or to the finer subdivision according to embryonic origins (Atchley et al. 1985; Cheverud et al. 1991, 1997; Leamy 1993; Mezey et al. 2000; Klingenberg and Leamy 2001; Klingenberg et al. 2001b).

Here we reassess the question of modular structure in the mouse mandible with a recently developed approach that explicitly considers the developmental origin of covariation among traits (Klingenberg 2003a,c). Covariation among traits can be generated in two principal ways. It can arise from variation that is transmitted from a common source to different traits by direct developmental interactions, such as partitioning of a precursor tissue or inductive signaling from one tissue to an adjacent one. Alternatively, covariation can

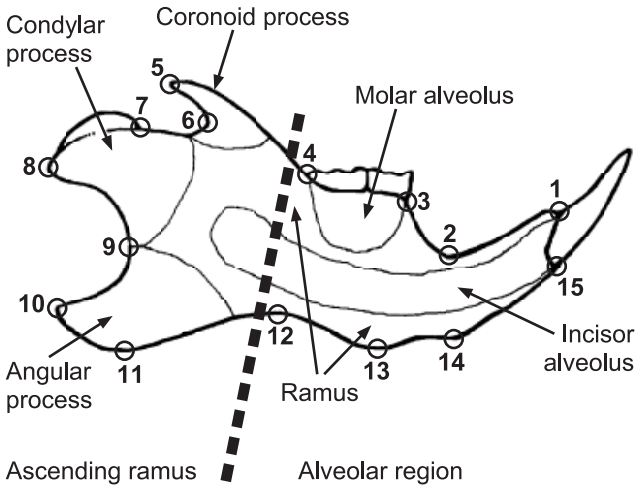


Fig. 1. Anatomical parts of the mouse mandible and landmarks used in this study. The mandible can be subdivided into two functional subunits: the ascending ramus, which serves for the attachment of musculature and for articulation with the skull, and the alveolar region, which bears the teeth. A different subdivision distinguishes the ramus, the coronoid, condylar and angular processes, and the tooth alveoli, which are derived from separate cell populations. Numbered circles denote the 15 landmarks used in this study.

be due to parallel variation in separate developmental pathways, for instance, from allelic variation in a gene that has a function in both pathways or from simultaneous responses of both pathways to an environmental factor. Because developmental modularity pertains to direct developmental interaction, only the first of these sources of morphological covariation is informative in this context. This component of covariation can be studied separately by analyzing the covariation of fluctuating asymmetry among traits (Klingenberg 2003a,c). Because fluctuating asymmetry stems from random perturbations arising in developmental processes (Palmer 1996; Hallgrímsson et al. 2002; Klingenberg 2003b), covariation of asymmetry of two traits can arise only if the effects of perturbations are transmitted between them through direct interactions of developmental pathways. Therefore, covariation of fluctuating asymmetry is due exclusively to direct developmental interactions of developmental pathways, whereas the covariation of variation among individuals can be due both to direct interactions and to parallel variation of completely separate pathways involved in the development of the traits. Morphometric studies of covariation of asymmetries and of variation among individuals can thus provide an estimate of the role of the two sources of covariation (Klingenberg and Zaklan 2000; Klingenberg et al. 2001a).

Here we apply this approach to test whether the two primary functional units of the mouse mandible are indeed developmental modules. We use the methods of geometric morphometrics to quantify the shape of the mandible, and we present some new extensions of these methods for delimiting modules as partitions of a configuration of landmarks. Analyses of patterns of integration for fluctuating asymmetry and individual variation have also been carried out in mouse mandibles (Leamy 1993) and skulls (Debat et al. 2000). These studies serve as a basis for comparison with our current results.

MATERIALS AND METHODS

Samples and measurements

The mice included in this study were previously used to investigate fluctuating asymmetry in relation to the Robertsonian fusion of chromosomes 4 and 12 (Auffray et al. 2001). Mice from different locations in Belgium were crossed to obtain offspring with different karyotypes, which were reared in the same animal room with food and water provided ad libitum (for further details, see Auffray et al. 2001). The mandibles were cleaned, and the left and right hemimandibles were separated.

Images of the mandibles in lateral (outside) view were obtained with a flatbed scanner, and 15 landmarks were digitized around the outline of the mandible (Fig. 1). To assess measurement error, each mandible was scanned twice, with repositioning of the specimen between scans, and the landmark coordinates were digitized from both replicate images (Palmer 1994; Klingenberg and McIntyre 1998). We included only those specimens for which left and right mandibles were both complete, which produced a final sample size of 90 individuals.

Procrustes superimposition and data correction

The analyses used the methods of geometric morphometrics (Bookstein 1996; Dryden and Mardia 1998), as they have been adapted specifically for the study of left–right asymmetry (Klingenberg and McIntyre 1998; Auffray et al. 1999; Klingenberg et al. 2002). First, the landmark configurations of all left mandibles were reflected to their mirror images by changing the sign of all x coordinates. Then, all configurations were superimposed by a generalized least-squares Procrustes fit and projected onto the shape tangent space at the mean shape (Dryden and Mardia 1998; Rohlf 1999). This procedure extracts shape information by eliminating the variation of landmark configurations that is due to scaling (size differences), position, and orientation. The variation remaining after the Procrustes fit is therefore exclusively the variation in the shape of landmark configurations. After the Procrustes fit, the superimposed configurations were rotated so that the line connecting landmark 1 and the midpoint between landmarks 5 and 10 for the overall mean configuration was horizontal (thus defining the anterior–posterior direction; this rotation has no effect on the statistical analyses but facilitates graphical presentation of results). The landmark coordinates of the configurations superimposed in this way were used for further multivariate analyses. Because there were two-dimensional coordinates from 15 landmarks, there is a total of 30 dimensions in the data, but four degrees of freedom are lost in the Procrustes

procedure when variation of size, position, and orientation is removed, resulting in 26 dimensions in the shape data used in the further analyses.

The data were corrected for the effects of cross (karyotype) and sex by subtracting the differences between the means of the respective groups and the grand mean. The analyses are therefore based on the pooled within-groups variation. Preliminary tests did not indicate a significant heterogeneity of within-group variation among samples, as is consistent with the results from univariate analyses of fluctuating asymmetry (Auffray et al. 2001). Allometric corrections were done by multivariate regression of shape on centroid size (Monteiro 1999). These regressions were done for the mandibles of each side separately and therefore also account for the allometric component of the left–right asymmetry of shape.

Shape variation in the mandible

The relative contributions of variation among individuals, directional asymmetry, fluctuating asymmetry, and measurement error to total variation were assessed by Procrustes analysis of variance (ANOVA) (Klingenberg and McIntyre 1998; Klingenberg et al. 2002). This is an extension for geometric morphometrics of the two-factor ANOVA customary in univariate asymmetry studies (Leamy 1984; Palmer and Strobeck 1986; Palmer 1994), which quantifies the amount of shape variation for the different effects using Procrustes distance.

As a global test for comparisons of the covariance matrices for individual variation and fluctuating asymmetry, we used a matrix permutation approach (Mantel 1967; Cheverud et al. 1989), as modified specifically for geometric morphometrics (Klingenberg and McIntyre 1998). The test statistic is the matrix correlation, that is, the product moment correlation of corresponding elements of the two matrices being compared. The test simulates the null hypothesis of no relationship among two covariance matrices by randomly reshuffling the landmarks in one of the matrices (i.e., permutation of rows and columns of the matrix, keeping the x and y coordinates of each landmark together as a pair) (Klingenberg and McIntyre 1998). For each test, 10,000 such randomization runs were performed, and the matrix correlation of each run was compared with the one for the original comparison.

The patterns of shape variation in the mandible were displayed with principal component analysis (Jolliffe 1986). This technique extracts new variables, the principal components, which successively account for the maximum amount of variation in multivariate data, subject to the condition that each principal component is uncorrelated with all preceding ones. Geometrically, they can be interpreted as those directions of the multidimensional shape space that account for the most scatter among data points. The first few principal components can therefore be used as a summary of the main features of shape variation. The shape changes associated with the principal components can be visualized and interpreted as patterns of variation, but they should not be expected to correspond to particular biological processes.

Because the principal components correspond to directions in the multivariate space of the data, it is straightforward to compare them with each other by the angles between them. These angles can be computed as the arccosine of the vector correlation between the principal components (the inner product of the coefficients; for details, see Klingenberg 1996). To assess the statistical significance of these angles, we compared them with the distribution of angles between pairs of random vectors in 26-dimensional space.

Covariation between parts of the mandible

Previous studies reported that the division of the mouse mandible into the alveolar region and ascending ramus is a major feature of its variation (Leamy 1993; Cheverud et al. 1997; Mezey et al. 2000). For our study, we therefore divided the landmarks into subsets corresponding to these two regions before the analyses (Fig. 1). We included both landmarks 4 and 12 in the alveolar region. For landmark 4, this is straightforward because the landmark is defined in part by the position of the molar teeth. For landmark 12, we chose this allocation because the lower contour of the mandible is directly associated with the incisor alveolus that extends far posteriorly inside the ramus, whereas the relation of this landmark to the ventral flange of the masseteric muscle attachment area, which extends forward from the angular process, is not consistent among specimens.

To quantify the covariation between parts of the mandible, we computed the trace correlation between the coordinates of the respective subsets of landmarks (Hooper 1959; Mardia et al. 1979, pp. 170–171). The trace correlation r_T is a measure of the association between pairs of multivariate observations that is analogous in many ways to the familiar product moment correlation used in standard univariate statistics. The trace correlation ranges from zero if the two sets of variables are completely independent to one if the two sets are perfectly redundant. Moreover, as for the univariate product moment correlation, the squared trace correlation can be interpreted as the proportion of the total variance in one set of variables that is explained by a multivariate regression on the other set of variables (for details, see Hooper 1959).

As a statistical test of the covariation between the alveolar region and ascending ramus of the mandible, we used a permutation approach (Klingenberg and Zaklan 2000). To simulate the null hypothesis of no covariation, the trace correlation was computed repeatedly from modified data where the two parts of the mandible were reassigned to each other randomly. The possible effects of Procrustes fitting on the covariation of landmark positions were taken into account by including a Procrustes fit in the permutation step. For the analysis of individual variation, the test used the landmark coordinates after Procrustes superimposition of the left–right means of original configurations (corrected for sex, cross, and size). For fluctuating asymmetry, the overall mean shape was added to the individual left–right differences (also corrected for sex, cross, and size), because the mean configuration is important for the Procrustes fit. For each permutation step, the landmarks of the ascending ramus and of the alveolar region were separated into two sets, randomly exchanged among individuals, and combined again to generate a new data set under the null hypothesis of complete independence between the two modules. A new Procrustes fit was carried out with this data set, and the trace correlation was computed and compared with the one for the original data. This procedure was repeated 10,000 times for each test.

To display the patterns of covariation between the two parts of the mandible, we used the partial least-squares method (Tucker 1958; Bookstein et al. 1990, 2003; Rohlf and Corti 2000). This approach extracts pairs of new variables that account for the maximal amount of covariation between two sets of variables, in our case, the coordinates of the two sets of landmarks. To visualize these features of joint variation graphically, we rescaled the partial least-squares coefficients to account for the number of landmarks (for details, see Klingenberg and Zaklan 2000) and displayed them as a change from the average shape.

Exploring alternative partitions of the mandible

If the mandible consists of two modules that are distinct units of morphological variation (Cheverud et al. 1997; Mezey et al. 2000), then the landmarks in each module should be relatively independent of the landmarks in the other module but strongly interdependent within modules. Therefore, the correlation between modules is expected to be relatively low. If the landmarks of the mandible were partitioned in a way that is inconsistent with the modular boundary, one would expect an increase of the correlation between subsets, because some of the strong intramodular correlations would link the two subsets. Accordingly, the correlation between the landmarks of the alveolar region and those of the ascending ramus (Fig. 1) should be lower than the correlation between subsets of landmarks partitioned in any other way. To test this hypothesis, we computed the trace correlations across subsets for all possible partitions of the 15 landmarks into two sets of seven and eight landmarks that were contiguous along the mandible outline, both for individual variation and for fluctuating asymmetry.

RESULTS

Sources of variation

The Procrustes ANOVA yielded significant effects of individuals, sides, and their interaction on mandible shape (Table 1). The main effect of sides indicates directional asymmetry in the shape of the mandibles. The significant individual × side interaction and the relative magnitudes of the respective mean squares show that fluctuating asymmetry exceeds measurement error and therefore can be used for the subsequent analyses.

Table 1. Procrustes analysis of variance of the amounts of shape variation attributable to different sources

Source	Sums of Squares	Degrees of Freedom	Mean Squares × 10^6
Individuals	0.2908	2314	0.1256***
Sides	0.0071	26	0.2746***
Individuals × sides	0.0858	2314	0.0371***
Measurement error	0.0565	4680	0.0121

Sums of squares and mean squares are in units of squared Procrustes distance (Klingenberg and McIntyre 1998).
***$P < 0.001$

Global comparison of individual variation and fluctuating asymmetry

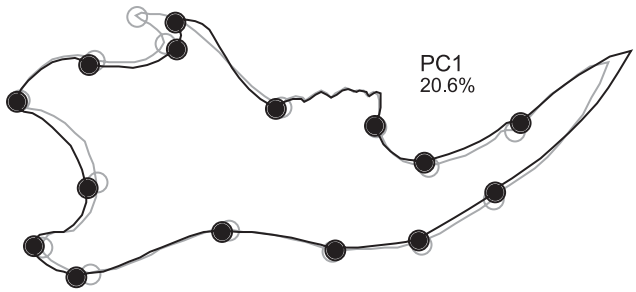
The comparisons of the covariance matrices for the variation among individuals and for fluctuating asymmetry using a matrix permutation test highlighted some special features of the matrices. The matrix permutation test that compared the whole covariance matrices including the diagonal blocks (the variances of the coordinates and covariances between x and y coordinates at each landmark) yielded a matrix correlation of 0.79, which was not statistically significant in the matrix permutation test ($P = 0.66$, with 10,000 random permutations of landmarks). In contrast, if the diagonal blocks were excluded so that only the covariation among landmarks was considered, but not the variation at each landmark on its own, the matrix correlation is only 0.37, but it is statistically significant ($P = 0.018$, with 10,000 random permutations).

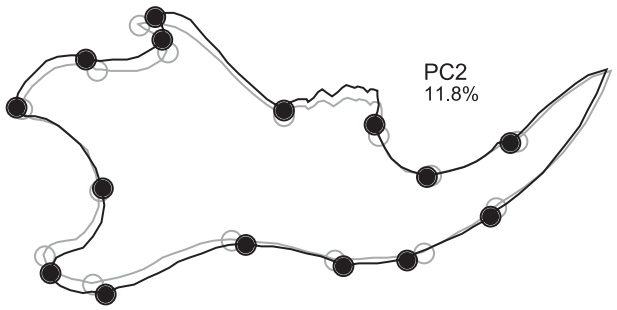
Although these results may appear contradictory at first sight, they reflect the special nature of the covariance matrices under study. The dominant pattern in the covariance matrices for both individual variation and fluctuating asymmetry is a relatively large amount of local variation at each landmark combined with substantially smaller covariances among landmarks. The test including the diagonal blocks reflects this overall similarity (with a contrast between diagonal and off-diagonal entries), but the matrix correlation is not statistically significant because the order of landmarks according to their amounts of within-landmark variation is not the same for the two covariance matrices. The overall agreement was weaker for the test that focused only on the covariances among landmarks, but the correlation was significant because the covariances among landmarks for individual variation and for fluctuating asymmetry were patterned similarly. Overall, therefore, there were clear differences as well as some shared patterns between the covariance matrices for individual variation and fluctuating asymmetry.

Patterns of variation in the mandible

The first three principal components accounted for about 45% of the total variance both for individual variation and for fluctuating asymmetry. Because there is a total of 26 dimensions, this indicates that much of the shape variation is concentrated in only a few of them.

There seemed to be little agreement of specific principal component patterns between the analyses of individual variation and fluctuating asymmetry (Figs. 2 and 3), reflecting the result of the matrix permutation test. The angles between corresponding principal component vectors were all greater than 72 degrees and did not differ from the angles expected





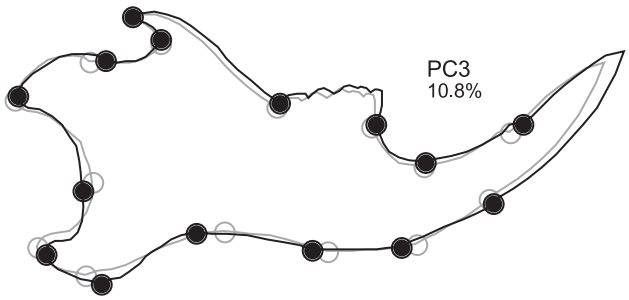
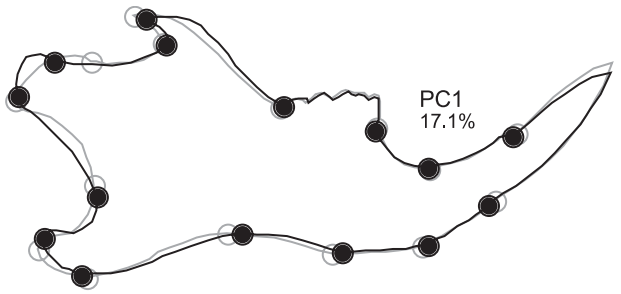
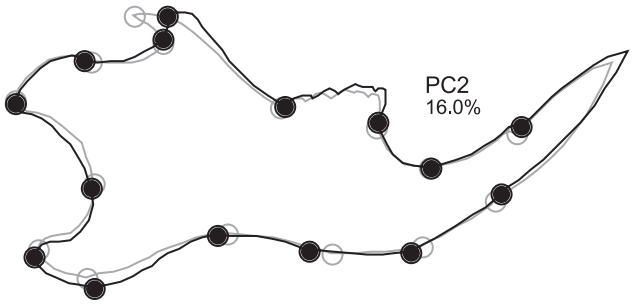


Fig. 2. Principal components for individual variation. Each diagram shows the average shape (open circles, gray outline) and the shape corresponding to a score of +0.07 Procrustes units for the respective principal component (solid circles, black outline). The percentages indicate the proportion of the total shape variance among individuals for which each principal component accounts.





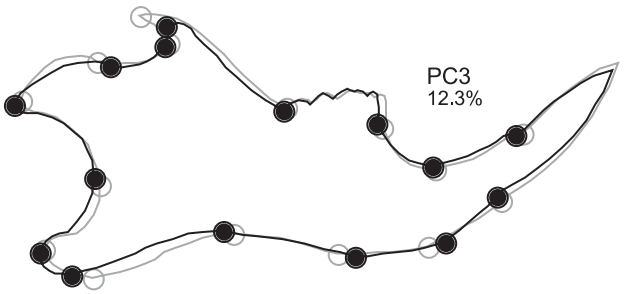


Fig. 3. Principal components for fluctuating asymmetry. Each diagram shows the average shape (open circles, gray outline) and the shape corresponding to a score of +0.07 Procrustes units for the respective principal component (solid circles, black outline). The percentages indicate the proportion of the total shape variance for fluctuating asymmetry for which each principal component accounts.

between random vectors. Even if possible rearrangements in the order of principal components were considered, there was no apparent one-to-one resemblance between the principal components from the two analyses.

As a general trend, the largest landmark shifts tended to be concentrated in the ascending ramus for the first three principal components (Figs. 2 and 3). A few recurrent patterns of local shape changes appeared in several of the principal components. In the ascending ramus, there were various changes in the relative sizes and positions of the coronoid, condylar, and angular processes. In the alveolar region, the landmarks along the ventral contour of the mandible tended to move in anterior or posterior direction, which appeared to relate to changes in the radius of bending of the incisor alveolus, and there was a dorsoventral dilation or compression of the molar region. These local changes contributed to the overall patterns of variation in various combinations. It is not apparent from the principal components to what extent these combinations may be constrained.

Covariation between alveolar region and ascending ramus

There was a moderate degree of covariation between the landmark subsets of the alveolar region and ascending ramus, as indicated by the squared trace correlations computed for individual variation ($r_T^2 = 0.412$) and for fluctuating asymmetry ($r_T^2 = 0.399$). Despite the similarity of these correlations, the permutation test indicated that the anterior–posterior

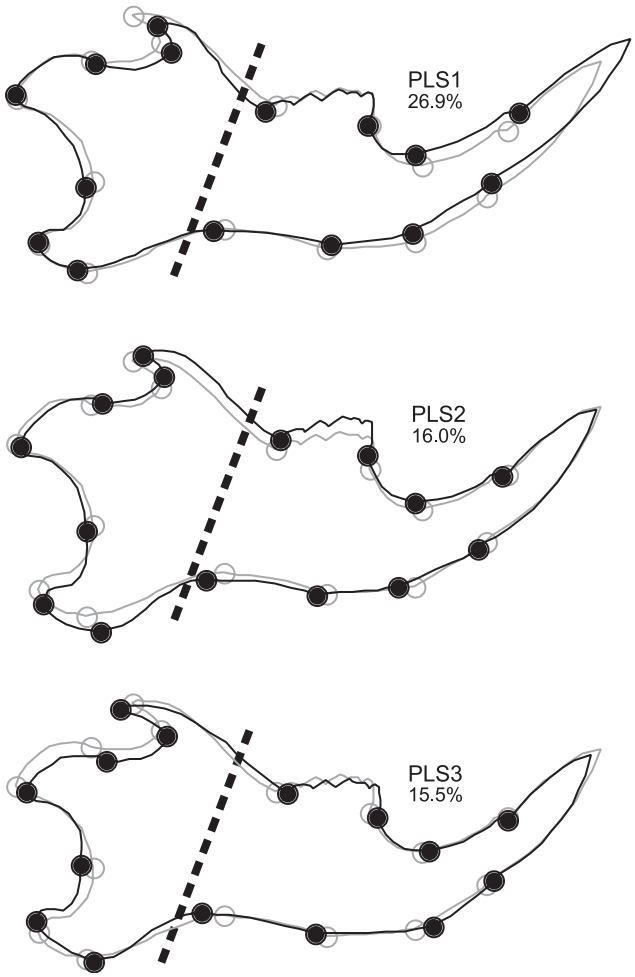


Fig. 4. Patterns of among-individual covariation between alveolar region and ascending ramus. Each diagram shows a pair of shape changes in the two parts (separated by a dashed line) that maximize the covariance between them, as computed by partial least-squares analysis (Rohlf and Corti 2000). The percentages given with each diagram indicate the proportion of the sum of singular values (a measure of the total covariance between sets of landmarks) for which each pair accounts.

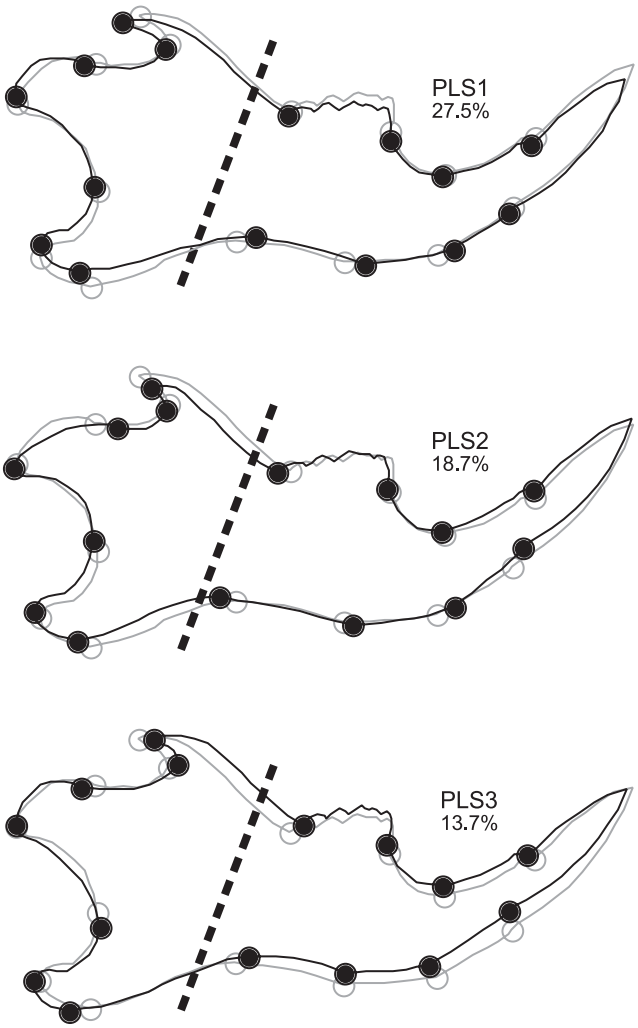


Fig. 5. Patterns of covariation between alveolar process and ascending ramus for fluctuating asymmetry. Each diagram shows a pair of shape changes in the two parts (separated by a dashed line) that maximize the covariance between them, as computed by partial least-squares analysis (Rohlf and Corti 2000). The percentages given with each diagram indicate the proportion of the sum of singular values (a measure of the total covariance between sets of landmarks) for which each pair accounts.

covariation was statistically significant only for individual variation ($P = 0.013$) but not for fluctuating asymmetry ($P = 0.131$). It appears that the Procrustes fit had a substantial influence on the covariation between these subsets of landmarks, because both permutation tests gave highly significant results ($P < 0.0001$) if Procrustes fitting was not included as a part of the permutation procedure.

The observed covariation between the anterior and posterior parts of the mandible was concentrated in just a few features of shape for both individual variation and fluctuating asymmetry, because the partial least-squares analysis showed that 3 dimensions (of a possible 14) accounted for nearly 60% of the covariation. The patterns of covariation between the two parts of the mandible included changes like overall lengthening or broadening of the mandible but also simultaneous localized changes such as changes in the posterior attachment processes combined with shifts of landmarks in the alveolar region (Figs. 4 and 5). There appeared to be no correspondence between the results for individual variation and fluctuating asymmetry. Moreover, the shape features extracted by partial least squares analysis did not match the principal components (angular comparisons did not find a significant departure from the expectation for pairs of random vectors), neither for individual variation nor for fluctuating asymmetry.

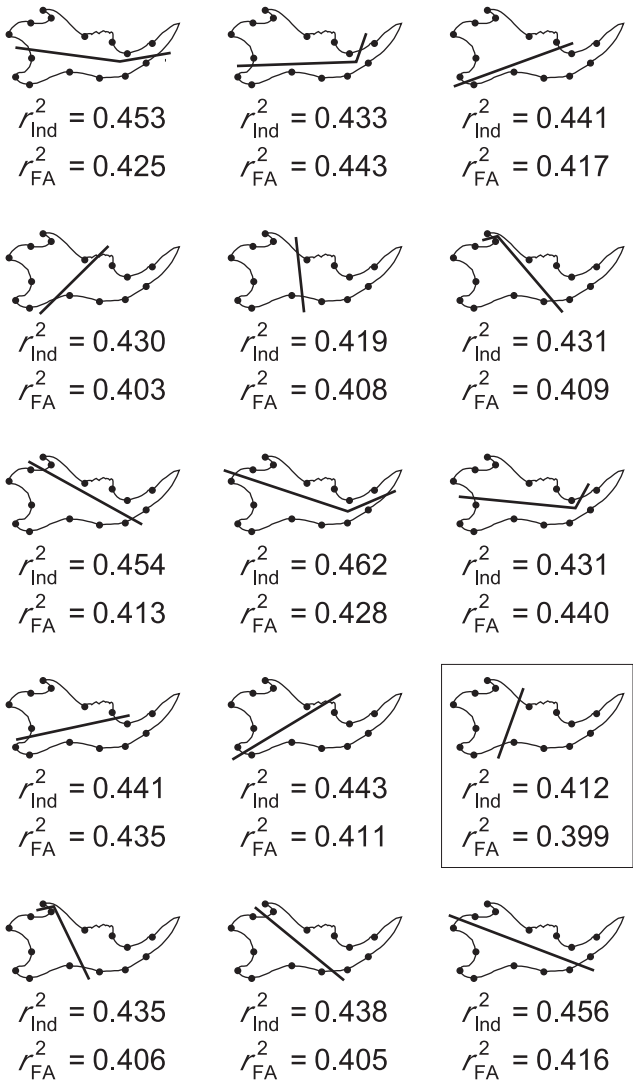


Fig. 6. Alternative partitioning of the mandible into subsets containing seven and eight landmarks. The r^2 values are the multiple correlations between the partitions for individual variation (Ind) and fluctuating asymmetry (FA). The rectangle points out the a priori partition into alveolar region and ascending ramus.

Delimiting the modules: comparison of alternative partitions

The partition of landmarks into two groups corresponding to the alveolar region and ascending ramus (Fig. 1) yielded the lowest trace correlations of landmark positions between subsets for both individual variation and fluctuating asymmetry (Fig. 6). The a priori partition therefore better reflects the modular structure than does any other partition. For individual variation, the squared trace correlations ranged from 0.412 to 0.462, whereas for fluctuating asymmetry, they ranged from 0.399 to 0.443. These ranges indicate that the differences in trace correlations among alternative partitions of landmarks are fairly small. This analysis confirms the distinction of the alveolar region and ascending ramus as separate modules, but the data also indicate that their separation from each other is relatively weak.

DISCUSSION

The mouse mandible is a structure composed of several subunits, which are derived from distinct developmental origins and assemble to form an integrated whole (Atchley and Hall 1991; Depew et al. 2002). This complexity of mandibular structure manifests itself in the results of our analyses through morphometric variation that is richly patterned, with diverse features of variation affecting mandibular shape at a range of different spatial scales.

Comparisons of patterns of variation

Our analyses showed that the patterns of mandibular shape variation among individuals coincided with the patterns of fluctuating asymmetry only partially. This finding suggests that different processes are involved in generating the variation observed at the two levels. This would mean that the genetic and environmental factors causing variation among individuals produce patterns of variation that do not have a direct equivalent in the within-individual processes that generate differences between body sides.

This result broadly agrees with a similar study comparing individual variation and fluctuating asymmetry in morphological landmarks of the dorsal side of the mouse skull (Debat et al. 2000). In that study, as in the present one, the matrix permutation test indicated no congruence between the covariance matrices for individual variation and fluctuating asymmetry, and there was no similarity of principal component patterns. Debat et al. (2000) particularly emphasized the possible difference in the buffering processes against variation from different sources. Because the landmarks were defined by features such as the nasal capsule, zygomatic arch, orbit, and the cranial vault, the structure considered by Debat et al. (2000) was an even more heterogeneous assemblage of parts than the mandible with its different subunits. Accordingly, a broad variety of processes takes part in the spatial patterning of such structures, which may interact in different ways with genetic and environmental factors (Leamy et al. 1999).

Similar studies in other animals have produced widely variable results. For the pharyngeal jaws of a cichlid fish species, there was no correspondence between the patterns of individual variation and fluctuating asymmetry (Klingenberg et al. 2002). Because a trophic polymorphism was one of the components of variation among individuals, which does not have an equivalent process at the within-individual level of fluctuating asymmetry, the discrepancy may be due to this difference in the factors contributing to morphological

variation. Fundamentally different results, however, emerged from studies of the wings of flies (Klingenberg and McIntyre 1998; Klingenberg and Zaklan 2000) and bumblebees (Klingenberg et al. 2001a), for which the covariance matrices of individual variation and fluctuating asymmetry consistently showed clear correspondence. In comparison with vertebrate jaws and skulls, insect wings have a much simpler structure and development, because they originate from a single epithelium folded over to form a pouch whose opposite sides are apposed to each other to produce the double layer of cuticle of the finished wing (Waddington 1940; Held 2002). It is possible that this relatively simple organization of wings and the processes that produce them also impose consistent patterns on morphological variation within and among individuals, whereas morphological variation of more complex composite structures is inherently more flexible. As an alternative explanation, Debat et al. (2000, p. 429) suggested that strong natural selection on the shape symmetry of insect wings might conceal differences in the developmental processes responsible for variation within and among individuals. Because the detailed selection regimes are not known for any of these examples, no firm conclusions can be drawn.

Covariation between anterior and posterior parts

Some previous studies have found a division of the mandible into two distinct modules, the alveolar region and the ascending ramus (Atchley et al. 1985; Leamy 1993; Cheverud et al. 1997; Mezey et al. 2000), whereas others did not confirm this (Klingenberg and Leamy 2001; Klingenberg et al. 2001b). This study used geometric morphometrics to analyze covariation between the two parts and found a moderate degree of multivariate correlation between them for both individual variation and fluctuating asymmetry. However, with a permutation test that accounted for the possible influence of the Procrustes superimposition on the correlation, only the correlation for individual variation was confirmed as statistically significant but not the one for fluctuating asymmetry. This means that the data presented here are consistent with the hypothesis of two separate developmental modules in the mandible. The data would not even contradict the extreme model of complete absence of correlated asymmetry, as it would be expected for total developmental independence. The significant correlation between the alveolar region and ascending ramus for individual variation might derive from parallel variation in the two modules due to genetic or environmental variation.

Our results agree with those reported in an earlier study of integration of fluctuating asymmetry among linear distance measurements in the mouse mandible (Leamy 1993). In that study, the correlations for individual variation within the alveolar region and the ascending ramus and between the two modules were of similar magnitude. For fluctuating asymmetry, however, the correlations within modules tended to be greater than the correlations between modules (but correlations of asymmetry between modules were statistically significant). Therefore, Leamy's (1993) analysis also suggests that variation in the mouse mandible is characterized by developmental integration within modules that are partially independent from each other and that integration across modules is stronger in the variation among individuals.

The comparison of this study of mouse mandibles to similar studies conducted in fly wings reveals some interesting differences. The shape variation of anterior and posterior compartments of fly wings is tightly integrated (Klingenberg and Zaklan 2000), even though they are separate cell lineages (García-Bellido et al. 1973; Dahmann and Basler 1999; Held 2002). Not only is there a highly significant covariation between the positions of landmarks in the two compartments, but the axes of maximal covariance between compartments extracted by partial least-squares analysis closely matched the principal components, indicating that covariation between the two compartments can account for the bulk of shape variation across the entire wing and that the entire wing corresponds to a single integrated module (Klingenberg and Zaklan 2000). In contrast, for the mouse mandibles investigated here, there was only a moderate degree of covariation between modules. Features of shape variation that are specific to each module make up a considerable proportion of the total variation and are reflected by the principal components. By definition, the partial least-squares analysis does not consider these localized patterns of variation, and the abundance of intramodular variation therefore accounts for the difference between the two analyses.

Locating the boundary between modules

To investigate the location of the boundary between the two principal modules, we compared alternative partitions of the mandible. This analysis is based on the expectation that the correlation between landmarks of two separate modules should be lower than for any other partition into two subsets. Any other partition would combine some of the landmarks from both modules in each of the new subsets, and the strong correlations that link landmarks within each module should therefore produce a correlation between the artificial subsets that is higher than the correlation between the true modules. Indeed, the a priori partition of the mandible into alveolar region and ascending ramus produced a lower correlation between subsets of landmarks than any other partitioning for both individual variation and fluctuating asymmetry. This analysis therefore supports the hypothesis that the alveolar region and ascending ramus are natural modules, confirming the results of earlier studies (Atchley et al. 1985; Cheverud et al. 1991, 1997; Leamy 1993; Mezey et al. 2000).

Although the a priori partition produced the lowest correlations between subsets of landmarks, the differences in the correlations among all possible partitions were not very large. This indicates that the alveolar region and ascending ramus are modules that are separate from each other to some degree but that they are not completely independent. This result confirms other studies reporting evidence of integration between the anterior and posterior parts of the mandible. For example, the fluctuating asymmetry of interlandmark distances was shown to be partially integrated across the mandible (Leamy 1993), and selection for shape features belonging entirely to one of the modules was predicted to elicit responses throughout the whole mandible (Klingenberg and Leamy 2001). Modularity in the mandible appears to be a question of degrees, not simply a black-or-white matter.

The analysis of covariation of asymmetry adds new evidence indicating that this modular structure is generated, at least in part, by direct developmental interactions that primarily take place within modules. By identifying the relative contributions of direct developmental interactions and parallel variation of separate pathways to the total morphological variation, this study therefore presents an advance toward a more mechanistic view of modularity and morphological integration. Much remains to be done, however, to understand how the many processes involved in forming the mandible interact to join its component parts together into a coherent functional unit.

A challenge for future studies will be to determine whether there is an additional set of modules at the smaller scale of the angular, condylar, and coronoid processes in the ascending ramus, the molar alveolus, and the base of the incisor (Atchley and Hall 1991; Depew et al. 2002). The recurrent patterns involving these smaller regions found in the principal components and partial least-squares analyses suggest this possibility. The units of integration might be even smaller, for instance, the attachment areas of individual muscles (Badyaev and Foresman 2000). Unfortunately, this question cannot be addressed with the current data because each of these smaller units contains only very few landmarks, which do not suffice to examine whether variation within these regions is integrated and relatively independent from surrounding parts, as it would be expected in a module. For this purpose, it will be necessary to use a data with greater spatial resolution than the set of landmarks considered here.

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