Published by Associazione Teriologica Italiana
Hystrix, the Italian Journal of Mammalogy
Volume 24 (1): 43–58, 2013

Available online at:
`http://www.italian-journal-of-mammalogy.it/article/view/6367/pdf`
doi:10.4404/hystrix-24.1-6367

Research Article

Cranial integration and modularity: insights into evolution and development from morphometric data

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Keywords:
allometry
comparative methods
geometric morphometrics
modularity
morphological integration

Article history:
Received: 9 July 2012
Accepted: 17 January 2013

Acknowledgements
I thank Andrea Cardini for inviting me to write this review for the special issue of Hystrix, and him, David Polly and Anna Loy for helpful comments on a previous version of the manuscript.

Abstract

Morphological integration and modularity have become central concepts in evolutionary biology and geometric morphometrics. This review summarizes the most frequently used methods for characterizing and quantifying integration and modularity in morphometric data: principal component analysis and related issues such as the variance of eigenvalues, partial least squares, comparison of covariation among alternative partitions of landmarks, matrix correlation and ordinations of covariance matrices. Allometry is often acting as an integrating factor. Integration and modularity can be studied at different levels: developmental integration is accessible through analyses of covariation of fluctuating asymmetry, genetic integration can be investigated in different experimental protocols that either focus on effects of individual genes or consider the aggregate effect of the whole genome, and several phylogenetic comparative methods are available for studying evolutionary integration. Morphological integration and modularity have been investigated in many species of mammals. The review gives a survey of geometric morphometric studies in some of the groups for which many studies have been published: mice and other rodents, carnivorans, shrews, humans and other primates. This review demonstrates that geometric morphometrics offers an established methodology for studying a wide range of questions concerning integration and modularity, but also points out opportunities for further innovation.

Introduction

The parts of skulls are integrated with each other because they develop, function and evolve jointly. Integration of cranial parts is inevitable because parts share developmental precursors, are packed together tightly in the head, because different functions place demands on different pats of the skull, and because all parts of the head share an evolutionary history (Moore, 1981; Depew et al., 2002; Lieberman, 2011). Yet this integration is not total, but is structured as modules that are relatively independent within the overall integration of the head as a whole (Klingenberg, 2008, 2010). The concepts of integration and modularity are therefore closely linked to each other and need to be discussed jointly.

The methods of geometric morphometrics are suitable for investigating morphological integration and modularity for several reasons. Geometric morphometrics offers a range of flexible and powerful statistical tools for addressing a range of specific biological questions concerning modularity and integration. The combination of geometry with multivariate statistics, which is at the core of geometric morphometrics, ensures that the shape of a structure is characterized completely and without redundancy. These analyses automatically take into account all the spatial relations among morphological landmarks or other geometric features included in the analysis, without the need for the investigator to define, a priori, a set of "traits" to include in the analysis. Which specific aspects of shape are important in answering a particular question is therefore a part of the results of a morphometric analysis. Finally, the various possibilities for visualization of results (Klingenberg, this issue) enable researchers to interpret findings directly in the context of cranial anatomy.

Analyses of morphological integration and modularity have been conducted with geometric morphometric approaches for somewhat more than a decade now (e.g. Klingenberg and Zaklan 2000; Klingenberg et al. 2001a; Bookstein et al. 2003; Klingenberg et al. 2003; Badyaev and Foresman 2004; Bastir and Rosas 2005; Monteiro et al. 2005; Goswami 2006b; Young 2006; Young and Badyaev 2006; Cardini and Elton 2008a; Zelditch et al. 2008; Klingenberg 2009; Ivanović and Kalezić 2010; Jamniczky and Hallgrímsson 2011; Jojić et al. 2012; Martínez-Abadías et al. 2012a). Several different approaches have been used, and new methods continue to be developed by various investigators. It is therefore timely to take stock of the existing work and survey the challenges that remain or new opportunities for innovative research that have emerged recently. This paper briefly summarizes the concepts of morphological integration and modularity, surveys the morphometric methods for studying them, and provides an overview of empirical results from such studies. Because this special issue of Hystrix is devoted to geometric morphometrics, this review will pay special attention to studies of integration and modularity that use geometric morphometric methods. There is a large literature on morphological integration and modularity that is based on traditional morphometric methods. This literature will only be covered as far as it provides unique information and insight on the topic. Likewise, because Hystrix is a journal of mammalogy, this review will primarily concentrate on studies in mammals (including humans) and will only mention a few of the many studies that have been conducted with other organisms.

Morphological integration

Different parts of organisms are coordinated in their sizes and shapes to make up a functional whole. This idea of integration goes back to the beginnings of the study of morphology, where it played a central role in the thinking of pioneers such as Georges Cuvier (Mayr, 1982). The current concepts of integration were conceived by researchers in the 20th century, such as the seminal book by Olson and Miller (1958), and further transformed by linking them width ideas from other fields, such as evolutionary quantitative genetics and developmental biology (Cheverud, 1982a, 1996a; Wagner and Altenberg, 1996; Hallgrímsson et al., 2002; Klingenberg, 2008). Through its developmental and quantitative genetic aspects, morphological integration also closely relates to

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Hystrix, the Italian Journal of Mammalogy ISSN 1825-5272

doi:10.4404/hystrix-24.1-6367
1st July 2013

developmental and evolutionary constraints (Cheverud, 1984, 1996a; Raff, 1996; Wagner, 1996; Wagner and Altenberg, 1996; Arthur, 2001; Klingenberg, 2008; Futuyma, 2010).

Morphological integration can be observed at a variety of different levels, depending on the origin of variation under consideration and the observations under study (Cheverud, 1982a; Klingenberg, 2008). For instance, integration within a species at a particular ontogenetic stage can be investigated by analyzing a sample of individuals of that species, ontogenetic integration can be studied from data derived from different growth stages of one species, or evolutionary integration can be investigated by examining how evolutionary changes in multiple parts are coordinated across a set of related species. Other levels focus more on processes, for example functional integration and developmental integration (Breuker et al., 2006a). The levels of integration mentioned here are not meant as a full enumeration of all possible types, or even of the levels of integration that exist in the literature. At every level, integration is concerned in some way with covariation among the parts or traits, but the origin of variation as well as the conceptual context and focus of investigation differ from level to level.

At each level, morphological integration arises from some sort of interaction among parts. The kind of interaction differs according to the type of integration – it can be developmental interactions for developmental integration, functional interactions for functional integration, shared inheritance by pleiotropy or linkage for genetic integration, concerted evolution among parts for evolutionary integration, and so on.

The interactions that constitute the mechanisms responsible for morphological integration at the different levels are mostly not directly observable. Morphological integration is manifest, however, in the covariation among morphological traits. With appropriate study designs, underlying mechanisms can be inferred from the covariation of morphometric measurements and hypotheses about their effects can be tested. Depending on the level of integration that is of interest, study designs differ, so that covariation of different origins can be analysed and the respective patterns of integration can be compared. For instance, genetic integration requires quantitative genetic designs, such as breeding experiments or measurements from individual for which pedigrees are available (Klingenberg and Leamy, 2001; Myers et al., 2006; Klingenberg et al., 2010; Adams, 2011; Martínez-Abadías et al., 2012a). Developmental integration can be inferred from covariation of fluctuating asymmetry (Klingenberg, 2003), which has been widely used for this purpose (Klingenberg and Zaklan, 2000; Klingenberg et al., 2001a, 2003; Laffont et al., 2009; Ivanović and Kalezić, 2010; Jojić et al., 2011, 2012). For studies of evolutionary integration, data about interspecific variation are used and phylogenetic comparative analyses are used (Monteiro et al., 2005; Drake and Klingenberg, 2010; Monteiro and Nogueira, 2010). Multiple levels of integration can be included in single studies and compared to each other (Klingenberg and McIntyre, 1998; Debat et al., 2000; Klingenberg et al., 2003; Monteiro et al., 2005; Willmore et al., 2005; Young and Badyaev, 2006; Drake and Klingenberg, 2010; Ivanović and Kalezić, 2010; Monteiro and Nogueira, 2010; Gonzalez et al., 2011c; Jamniczky and Hallgrímsson, 2011; Jojić et al., 2012; Klingenberg et al., 2012).

Modularity

Morphological integration is not uniform throughout entire organisms, but tends to be concentrated in certain complexes of parts that are tightly integrated internally, but are relatively independent of other such complexes. Such complexes are called modules. Modularity, therefore, is a concept that is closely connected to integration (e.g. Klingenberg 2008).

In particular, modularity has been prominent in the area of evolutionary developmental biology, where several authors have argued that it provides the flexibility for different traits to evolve more easily under differing or even conflicting functional demands (Raff, 1996; Wagner and Altenberg, 1996; Gerhart and Kirschner, 1997; Kirschner and Gerhart, 1998). Modularity, because of the weak integration among modules, can therefore mitigate the effects of constraints that would apply if variation were fully integrated. Some authors have hypothesized that genetic and developmental modularity should evolve to match functional modularity (Cheverud, 1984, 1996a; Wagner, 1996; Wagner and Altenberg, 1996). Such tests will be especially fruitful where developmental and functional considerations result in different hypotheses of modularity (Breuker et al., 2006a). So far, there is only limited evidence available, and testing this hypothesis with morphometric data has only begun recently (e.g., Klingenberg et al. 2010).

Modularity has become a very popular research topic in recent years and the concept applies to a wide range of fields in biology and beyond (Schlosser and Wagner, 2004; Callebaut and Rasskin-Gutman, 2005). In many of those applications, the starting information is about the actual interactions of interest, for instance, in biochemical or gene regulatory networks, or networks of social or ecological interactions and so on. The analyses therefore dissect those known networks into modules based on the patterns of connectivity among the parts. The context of morphometric studies of modularity differs from these network analyses in that the network of interactions is not directly observable, but that modularity needs to be inferred from patterns of covariation among parts. Depending on the biological question that is to be addressed, the study design can be chosen so that variation and covariation from which modules and interactions are inferred are at the appropriate level. Most often, this is intraspecific variation among individuals of a population, but it is possible to combine this level with the analysis of fluctuating asymmetry for inferring developmental modularity, with quantitative genetic analyses, or with phylogenetically informed comparative analyses across species to study evolutionary modularity (Hallgrímsson et al., 2009; Klingenberg, 2009; Drake and Klingenberg, 2010; Jojić et al., 2011, 2012; Martínez-Abadías et al., 2012a; Sanger et al., 2012).

A wide variety of morphometric methods have been used to study modularity in mammalian skulls. This diversity partly reflects differences in the concepts of modularity that different investigators use. The details of the definitions that are used can make a substantial difference for the choice of methods and the interpretations of results.

Morphometric methods

Morphometric studies of integration and modularity use a variety of methods that address specific questions, for instance, concerning the patterns of integration within a single structure or the strength of integration between different parts. Usually, a single study includes several different analyses, so that their results can be combined to provide a comprehensive understanding of integration in the structure under study. Some of these methods are the standard tools of geometric morphometrics, such as principal component analysis, whereas others are specialized methods that were developed specifically for this purpose. This overview will focus exclusively on landmark methods (for other overviews of morphometric methods to study integration and modularity, see Goswami and Polly 2010b; Zelditch et al. 2012).

Overall integration in a structure

Integration in a morphological structure means that different parts covary with each other. At the extreme, all components are perfectly correlated, so that variation of the relative positions of landmarks in any small region is sufficient to predict perfectly the variation of the relative positions of the remaining landmarks. If relations are linear, this also means that all variation is contained in a single dimension of shape space. From this reasoning, it follows that principal component analysis, with its natural focus on dimensionality of variation, is an important tool for investigating morphological integration.

Principal component analysis is a traditional method in multivariate statistics (e.g. Jolliffe 2002). In geometric morphometrics, it has sometimes been called relative warp analysis if the analysis uses the shape descriptors from the thin-plate spline approach (Rohlf, 1993), but, in the vast majority of analyses, this is identical to a principal component analysis of landmark coordinates after a Procrustes superimposition. Principal component analysis provides two main results that are particularly relevant for the study of morphological integration: the eigenvalues, which indicate the variance for which each principal component accounts, and the eigenvectors, which indicate the shape features associated with the principal components.

The eigenvalues are the variances of the principal component scores. Because principal components are defined to maximize, successively, the variance for which they account, the first eigenvalue is the largest variance for any linear combination of the variables included in the analysis (subject to a scaling constraint). Similarly, the last eigenvalue is the smallest variance for any linear combination. As a consequence, the eigenvalues offer a convenient way to assess the degree to which variation is concentrated in just one or a few dimensions or spread across many dimensions. In many geometric morphometric studies, a large proportion of the total variation is concentrated in just a few dimensions, so that only the first few eigenvalues are relatively large and drop fairly quickly, before tapering off toward zero (e.g. Klingenberg and McIntyre 1998; Young 2004; Polly 2007; Cooper et al. 2010; Gómez-Robles et al. 2011a; Klingenberg et al. 2012). There are exceptions where variation is spread across very many dimensions and no principal component accounts for a very large share of the total shape variation (e.g. Martínez-Abadías et al. 2012a).

Shape changes associated with principal components, which can be obtained from the eigenvectors, can also be informative about integration and modularity. They indicate whether the dominant aspects of shape variation affect the entire structure or are mostly contained in particular parts or regions. This type of interpretation provides an intuitive idea of the patterns of variation, integration and modularity, but interpreting the shape changes associated with principal components can be quite difficult and clear inference about integration and modularity is often impossible. In particular, the eigenvectors are well defined only if the eigenvalues are clearly distinct. If two or more eigenvalues are identical, the corresponding eigenvectors can be rotated freely in the subspace that they span, so that these principal components are associated with varying combinations of shape features. Therefore, this type of interpretation should be used with considerable caution.

Principal component analysis is also important for the understanding of morphological integration for another reason: an important class of indices of integration are derived from the variance of eigenvalues of a correlation matrix of measurements (Wagner, 1984; Pavlicev et al., 2009; Haber, 2011). If there is complete integration, all variation in the data is in a single dimension of the phenotypic space and the variance of eigenvalues of the correlation matrix is maximal, whereas at the opposite extreme of the spectrum, with not integration whatsoever, all eigenvalues are equal and their variance therefore is zero. Although these indices have primarily been applied with traditional morphometric measurements (lengths, etc.), the index was modified for the context of geometric morphometrics (Young, 2006). As usual in geometric morphometrics, the covariance matrix and not the correlation matrix is used for this purpose (this is, among other reasons, because the eigenvalues of the correlation matrix of Procrustes coordinates are not invariant under rotation of the landmark configurations relative to the coordinate system). To obtain an index of integration that does not depend on the total amount of shape variation in the sample, the eigenvalues of the covariance matrix can be standardized by the total variance in the sample (Young, 2006). This index of integration, sometimes in variants that differ in the way eigenvalues were scaled, has been used in a growing number of studies (Willmore et al., 2006a; Hallgrímsson et al., 2009; Jamniczky and Hallgrímsson, 2009; Ivanović and Kalezić, 2010; Gonzalez et al., 2011b; Jojić et al., 2011; Gómez-Robles and Polly, 2012).

Integration between structures or their parts: partial least squares

Whereas overall integration within a structure is an important topic, many morphometric studies address more specific questions of the integration between specific parts within an overall structure (e.g. the face and neurocranium in the skull) or between entirely separate structures (e.g. the mandible and cranium). Again, both the strength of association between parts and the patterns of covariation are of interest in studies of morphological integration.

The most widely used morphometric method in studies of the patterns of covariation between sets of landmarks is partial least squares (e.g. Tabachnick and Bookstein 1990; Klingenberg and Zaklan 2000; Rohlf and Corti 2000; Klingenberg et al. 2001a; Bookstein et al. 2003; Klingenberg et al. 2003; Mitteroecker and Bookstein 2007; Hautier et al. 2012). This is a method that has a number of common features with principal component analysis, but instead of decomposing the overall variation in a configuration of landmarks into components according to the amounts of variation with which they are associated, partial least squares decomposes a matrix of covariances between two landmark configurations into pairs of axes (one axis for each configuration) that are shape features showing successively maximal covariance with each other. Both the covariance and the shape features are of interest in the context of morphometric integration.

Just as for principal components, partial least squares analysis provides information about the shape change associated with each axis. Shape changes can be visualized and interpreted anatomically in the same way as principal components and other results from morphometric analyses (Klingenberg et al., 2012). The difference is that partial least squares analysis yields the shape features that account for the most covariation between parts rather than overall variation throughout the entire structure under study.

If the analysis examines covariation between two sets of landmarks within a single configuration, such as face and neurocranium, there is a choice whether the analysis should use separate Procrustes superimpositions for the parts (Bastir and Rosas, 2005; Marugán-Lobón and Buscalioni, 2006; Laffont et al., 2009; Bastir et al., 2010; Gkantidis and Halazonetis, 2011; Jamniczky and Hallgrímsson, 2011; Martínez-Abadías et al., 2011; Parsons et al., 2011; Singh et al., 2012) or the data from a single Procrustes fit for the entire structure (Klingenberg and Zaklan, 2000; Bookstein et al., 2003; Klingenberg et al., 2003; Monteiro et al., 2005; Mitteroecker and Bookstein, 2008; Makedonska et al., 2012; Klingenberg and Marugán-Lobón, 2013). Both options are valid, but they may produce markedly different results because they differ in which aspects of covariation they consider (Klingenberg, 2009; Kulemeyer et al., 2009; McCane and Kean, 2011). The approach with separate Procrustes superimpositions focuses on the covariation between the shapes of the parts, each taken separately, and does not consider the covariation that is due to coordinated variation in the relative sizes or arrangement of the parts. By contrast, the method that uses a joint Procrustes fit for both parts examines covariation between parts in the context of the structure as a whole and therefore considers all aspects of covariation, including the relative sizes and arrangement of the parts. Because this additional component of covariation can amount to a significant proportion of the total covariation between parts of a configuration, the difference between the two types of analyses can be fairly substantial (Klingenberg, 2009; Kulemeyer et al., 2009; McCane and Kean, 2011). The effect of a joint Procrustes fit also needs to be taken into account for statistical testing of covariation, for instance by including a new Procrustes fit in each iteration of a permutation test (Klingenberg et al., 2003; Klingenberg, 2009).

Most partial least squares analyses consider two blocks of variables (Rohlf and Corti, 2000). A few studies, however, have used three blocks simultaneously (Bookstein et al., 2003; Monteiro et al., 2005; Gunz and Harvati, 2007). The properties of these multi-block analyses are similar to the two-block analyses, but differ in some details. The shape features extracted for each block of variables are those that most strongly covary across all blocks simultaneously.

To quantify the strength of covariation between parts, it is possible to compute indices such as the RV coefficient (Escoufier, 1973; Klingenberg, 2009). Because the RV coefficient quantifies the total covariation as the sum of all squared covariances between blocks, it is compatible with the framework of partial least squares, where this sum of squared covariances (identical to the sum of squared singular values) also plays an important role (Rohlf and Corti, 2000). The RV coefficient is a multivariate generalization of the squared correlation coefficient but, rather than indicating the degree of association between two variables, it quantifies the strength of association between two sets of variables. It can be used to assess how strong the integration between two sets of landmarks is, either within a single structure where both sets have undergone a joint Procrustes fit or for two configurations of landmarks with separate Procrustes fits. Although the RV coefficient can be computed in both these situations, it is important to note that there can be substantial differences between them, because a simultaneous Procrustes superimposition includes covariance due to variation in the relative sizes and arrangement of the two sub-configurations (Klingenberg, 2009). The RV coefficient is compatible with partial least squares analysis because it is based on related algebra, with the sum of all squared covariances between the two sets of variables as the measure of covariation. As a scalar measure of association between sets of variables, the RV coefficient can be used as an intuitive tool to assess the strength of integration between structures, but it is also useful as a test statistic for permutation tests of association in the context of partial least squares (e.g. as implemented in the MorphoJ software, Klingenberg 2011) or for analyses of modularity (Klingenberg, 2009). All these usages of the RV coefficient are increasingly widespread in various contexts (e.g. Laffont et al. 2009; Drake and Klingenberg 2010; Ivanović and Kalezić 2010; Gómez-Robles and Polly 2012; Jojić et al. 2012; Renaud et al. 2012; Sanger et al. 2012; Klingenberg and Marugán-Lobón 2013).

The RV coefficient is not the only index of association between sets of variables. The simplest way of quantifying covariation is to compute the sum of squared covariances between the two blocks of variables, which is the same as the sum of squared singular values that is computed as part of a partial least squares analysis (Bookstein et al., 1990; Rohlf and Corti, 2000). It is related to the RV coefficient, which is a scaled variant of this sum (scaled by the total within-block covariation). Other measures of covariation between sets of variables, such as the trace correlation (Hooper, 1959; Mardia et al., 1979), which has been used as a measure of integration in geometric morphometrics (Klingenberg et al., 2003, 2004), can show undesirable statistical behaviour. It is therefore recommended to avoid such measures and to use the RV coefficient instead.

Associations between distance matrices

Studying patterns and strength of integration with partial least squares analysis is the most widespread, but not the only approach. An alternative method is based on computing a matrix of all pairwise Procrustes distances between the study units (species average shapes, individuals, etc.) for each part and then comparing the distance matrices for different parts using matrix correlation and Mantel tests (Monteiro et al., 2005). The result of this type of analysis is an overall measure of association between distance matrices of the parts, which can be further used, for example, in cluster analyses to explore the structure of covariation among parts (Monteiro et al., 2005). This method does not provide a direct visualization of the patterns of covariation in the shapes of the parts, which is why these analyses are sometimes are supplemented by a partial least squares analysis that provides those patterns (Monteiro et al., 2005).

The analyses with this approach can be conducted at various levels, for instance among individual within species or among taxa in a phylogenetic comparison (Monteiro and Nogueira, 2010) or variation among individuals and fluctuating asymmetry (Zelditch et al., 2008). This approach has been applied in a range of studies (Monteiro et al., 2005; Zelditch et al., 2008, 2009; Monteiro and Nogueira, 2010; Muñoz-Muñoz et al., 2011; Webster and Zelditch, 2011a,b; Renaud et al., 2012).

Comparing covariance matrices

Patterns of integration can be compared by quantifying the resemblance between covariance matrices of shape variables. A widely used index is matrix correlation, the product moment correlation between corresponding elements in two covariance matrices. Matrix correlations can be tested with a matrix permutation procedure against the null hypothesis that the two covariance matrices are totally unrelated. Both the computation of matrix correlations and the matrix permutation procedure have been specially adapted for the context of geometric morphometrics (Klingenberg and McIntyre, 1998). These adaptations concern the choice whether the diagonal blocks of the matrices, containing the variances and covariances of the landmark coordinates within landmarks, should be included or not. The difference between the two versions sometimes can provide insight into the nature of the resemblance between two covariance matrices, but often both versions provide largely similar results (e.g. Breuker et al. 2006b). Moreover, the matrix permutation test needs to permute landmarks rather than individual coordinates in order to simulate the null hypothesis in a realistic manner (Klingenberg and McIntyre, 1998).

Whereas the matrix correlation is an intuitive measure of resemblance between covariance matrices, it is not the only such measure. Several measures of distances between covariance matrices have been described, which could be used in a similar way (Dryden et al., 2009; Mitteroecker and Bookstein, 2009). For statistical inference based on these measures, however, tests other than the matrix permutation tests will be needed.

For comparison among multiple covariance matrices, it is possible to obtain an ordination of covariance matrices by principal coordinate analysis based on a distance measurement derived from matrix correlation or on other distance measurements among covariance matrices (Debat et al., 2006, 2008, 2009; Jamniczky and Hallgrímsson, 2009; Mitteroecker and Bookstein, 2009; Breno et al., 2011; Gonzalez et al., 2011a). This type of analysis provides scatter plots where the relationships among covariance matrices can be interpreted graphically. It should be noted, however, that the distance measures are not well understood and several others have been described (Dryden et al., 2009). These distance measures differ in which aspects of the covariance matrices they consider. For instance, the distance measure based on matrix correlation is invariant to changes of scale and focuses exclusively on the patterns of covariation, whereas the Riemannian metric (Dryden et al., 2009; Mitteroecker and Bookstein, 2009) considers both the pattern and scale of variation. Two studies compared ordinations derived from different distance measures with the same set of covariance matrices and found some agreement, but also considerable differences (Breno et al., 2011; Gonzalez et al., 2011a). More theoretical work for understanding the nature of the different distance measures and more empirical comparisons of distances are needed.

For all these types of comparisons, a possible complication arises if the structure under study is symmetric in itself, such as the mammalian skull. This type of symmetry is known as object symmetry, and differs from matching symmetry, where there are separate structures on the left and right body sides, such as human hands (Mardia et al., 2000; Klingenberg et al., 2002). For structures with object symmetry, there are two types of landmarks: single landmarks on the midline or midplane and paired landmarks on either side of it. Also, the total shape variation can be divided into separate components of symmetric variation and asymmetry, which occupy orthogonal subspaces of the shape tangent space (Klingenberg et al., 2002). Special care is needed if covariance matrices derived from the two components need to be compared to each other, for instance to compare the patterns of symmetric variation among individual and of fluctuating asymmetry. Because the symmetry and asymmetry components occupy orthogonal subspaces, the whole covariance matrices are uncorrelated, even if there are clear relations in the patterns of shape changes (in whatever dimension there is variation in one component, there is none in the opposite component). A possible solution is to concentrate exclusively on the paired landmarks from one body side (i.e. on one landmark from each pair, after computing Procrustes tangent coordinates for the symmertry or asymmetry components from the entire landmark configurations) and to ignore the landmarks on the midline (Klingenberg et al., 2002). This approach leaves out those aspects of variation that cannot agree between the two components (e.g. for unpaired landmarks, symmetric variation is in the midline or midplane and asymmetry is perpendicular to it) and focuses on those features of shape variation that may or may not match. This limitation and the solution applies to matrix correlation as well as to the computation of the various distance measures for principal coordinate analyses. A growing number of studies have used this approach for computing matrix correlations in animals and plants (Klingenberg et al., 2002; Drake and Klingenberg, 2010; Ivanović and Kalezić, 2010; Breno et al., 2011; Jojić et al., 2011; Klingenberg et al., 2012). It is implemented in the MorphoJ software package (Klingenberg, 2011) and is automatically used by the program in appropriate situations; in other software, users need to make the necessary adjustments manually.

Assessing modularity

Hypotheses of modularity can originate from many types of reasoning, grounded in anatomical, developmental, functional or genetic arguments (Breuker et al., 2006a; Willmore et al., 2006a; Klingenberg, 2008). In the context of geometric morphometrics, hypotheses of modularity are stated in terms of the landmarks that belong to the putative modules. Under such a hypothesis, the relative positions of landmarks belonging to the same module should be integrated relatively tightly, whereas integration between modules should be weaker. By contrast, if the landmarks are partitioned into subsets differently, so that their divisions do not coincide with the boundaries of true modules, the strong within-module covariation contributes to the covariation among subsets, and the overall covariation among subsets of landmarks is therefore expected to be stronger. This prediction can be assessed empirically by quantifying covariation among the sets of landmarks belonging to the different hypothesized modules and comparing this with the strength of covariation among subsets of landmarks that have been partitioned in different ways (Klingenberg, 2009).

To quantify the strength of covariation, the RV coefficient between the Procrustes coordinates of the sets of landmarks can be used if there are just two hypothesized modules or, for more than two modules, a multi-set RV coefficient can be computed by averaging the RV coefficients for all pairwise combinations of the subsets of landmarks (Klingenberg, 2009). The RV coefficient or multi-set RV coefficient is first computed for the partition of landmarks into subsets that coincide with the hypothesis of modularity, and then for a number of alternative partitions of the landmarks. Depending on the number of hypothesized modules and landmarks, this may be a full enumeration of all possible partitions or a large number of random partitions.

Also, depending on the biological context, investigators may choose to restrict the alternative partitions to those that are spatially contiguous. If disjoint sets of landmarks are not considered to be plausible candidates for modules, for instance for developmental modules where the interactions responsible for the integration within modules are tissue-bound processes such as the diffusion of signalling factors, it may be undesirable to include partitions of landmarks with disjoint sets as part of the comparison. A criterion for the spatial contiguity of subsets can be defined by requiring subsets to be connected by the edges of an graph that specifies which landmarks are considered to be adjacent to each other (Klingenberg, 2009). Whether disjoint subsets of landmarks are plausible as potential modules or whether each module needs to be spatially contiguous depends entirely on the biological context of each specific analysis.

A growing number of studies have used this method for testing hypotheses of modularity (Hallgrímsson et al., 2009; Klingenberg, 2009; Bruner et al., 2010; Drake and Klingenberg, 2010; Ivanović and Kalezić, 2010; Klingenberg et al., 2010; Jojić et al., 2011; Burgio et al., 2012a; Jojić et al., 2012; Kimmel et al., 2012; Lewton, 2012; Martínez-Abadías et al., 2012a; Sanger et al., 2012; Sydney et al., 2012; Klingenberg and Marugán-Lobón, 2013). Beyond biological structures, the method has even been used to evaluate modularity in archaeological artefacts (González-José and Charlin, 2012).

Several other methods also exist that assess models of modularity in shape data within a variety of different statistical frameworks (e.g. Mitteroecker and Bookstein 2007; Márquez 2008. Jojić et al. (2012) conducted a direct comparison, using the same data, between the method described above and traditional morphometric approaches (Cheverud, 1995, 1996b) and found that both approaches produced compatible results and supported the same conclusions concerning modularity. This comparison is encouraging and suggests that comparisons of results across studies are possible.

Whereas testing a-priori hypotheses of modularity is an important aspect of the study of modularity, investigators often want to find modules with exploratory analyses when no such hypotheses are available. This means that the task is to search for a partition of landmarks into subsets so that the covariation between subsets is minimal and strong covariation of relative landmark positions is mainly confined within subsets. It is tempting to use the combinatorial approach that is part of the approach outlined above, and simply to search for that partition of landmarks that results in the weakest covariation between subsets (lowest RV coefficient or multi-set RV coefficient). However, the problem with this idea is that there always is at least one partition that provides the weakest covariation, even if there is no modularity at all in the structure under study. Accordingly, this approach is not suitable for an exploratory search for modules, but should be restricted to the test of a-priori hypotheses (Klingenberg, 2009).

A different strategy is to use some clustering technique as an exploratory approach to find modules based on a measure of covariation among landmarks or regions of the structure under study (e.g. Monteiro et al. 2005; Goswami 2006a,b; Willmore et al. 2006a; Goswami 2007; Zelditch et al. 2008, 2009; Makedonska et al. 2012). Goswami (2006a,b, 2007) used a scalar measure of covariance among landmarks, which combined information about both the degree of association and the angles between the relative shifts of landmarks, in a hierarchical cluster analysis to identify modules in mammalian skulls. Other studies used cluster analysis to examine the larger-scale structure of variation among parts of a structure (Monteiro et al., 2005; Zelditch et al., 2008, 2009). Because clustering algorithms always produce clusters, even when the data do not contain such a hierarchical structure (Sneath and Sokal, 1973), some considerable caution is required when interpreting the results from such exploratory analyses. It is tempting to use the approach outlined above for testing the resulting modules, but there is a danger of circular reasoning if the clustering itself was based on information about covariation among landmarks. Much further work is required to develop methods for testing for modular structure in situations where no a-priori hypotheses are available.

Allometry as an integrating factor

Allometry, the association between size and shape, is a factor that contributes to morphological integration (Klingenberg et al., 2001a; Mitteroecker and Bookstein, 2007; Klingenberg, 2009). Because the relationship between size and shape is often nearly linear, allometry generates shape variation that is primarily in one direction in shape tangent space. If there is a relatively large amount of size variation and allometry is sufficiently strong, this size-related component of shape variation can make up a substantial proportion of shape variation. As a result, a considerable proportion of the total shape variation may be concentrated in the direction of the allometric effects. If allometric variation is sufficiently abundant relative to variation from other sources, it manifests itself as increased integration of shape variation. Also, allometry often affects all parts of a structure or organism jointly, leading to integrated change of the entire configuration of landmarks under study. Integration from allometry can vary in its effects, and it may or may not interfere with the analysis of modularity (Klingenberg, 2009; Jojić et al., 2012).

In geometric morphometrics, the most straightforward and most frequently used approach for analysing allometry is multivariate regression of shape on size (e.g. Loy et al. 1998; Monteiro 1999; Cardini 2003; Drake and Klingenberg 2008; Rodríguez-Mendoza et al. 2011; Sidlauskas et al. 2011; Weisensee and Jantz 2011; Klingenberg et al. 2012; Martínez-Abadías et al. 2012b; Ponssa and Candioti 2012). The most widely used shape measures are centroid size and log-transformed centroid size (log-transformed centroid size is particularly useful when the range of sizes is very large and much of the size-related shape change occurs among smaller specimens). The direction of allometry can be characterized by the vector of regression coefficients, which is the shape change that is expected for an increase of size by one unit (usually centroid size or log-transformed centroid size is used; note that the magnitude of this vector depends on the units of measurement or the basis used for the log transformation). To correct for the effects of allometry in a dataset, investigators can compute the residuals from the regression of shape on size and use those residuals in further analyses of integration or modularity (e.g. Klingenberg 2009; Jojić et al. 2012; Klingenberg and Marugán-Lobón 2013).

Depending on the context of a study, different levels of allometry may be relevant: ontogenetic allometry from size variation due to growth, evolutionary allometry due to evolutionary size changes among species, and static allometry due to individual size variation at a given ontogenetic stage within a single population (e.g. Cock 1966; Gould 1966; Cheverud 1982b; Klingenberg and Zimmermann 1992; Klingenberg 1996, 1998). When data for multiple levels are available within a single study, comparing them explicitly and relating them to patterns of integration may provide insight into the origins of observed patterns of variation. Examples of such studies include comparisons of static and ontogenetic allometry (Drake and Klingenberg, 2008; Weisensee and Jantz, 2011), ontogenetic and evolutionary allometry (Gonzalez et al., 2011c) and of static allometry within populations and evolutionary allometry among taxa (Klingenberg et al., 2012). All of these types of allometry can contribute to integration at the respective levels of variation.

Martínez-Abadías et al. (2012b) used multivariate regression to relate skull shape not only to the centroid size of the skull, but also to chondrocranial length and to estimated brain volume in mice and humans. Like allometry, these relations to additional quantities, which are linked with important developmental factors and processes, contribute to variation across the skull and can contribute to overall integration. The interplay of the different processes is likely to be complex and difficult to untangle with morphometric methods (e.g. Hallgrímsson et al. 2009).

Sample sizes

Integration and modularity concern the patterns of variation and covariation about the mean of shape or other morphological traits. Because morphometric data usually have a high dimensionality, estimating the patterns of covariation is inherently a quite ambitious statistical undertaking and requires a substantial sample size. Theoretical considerations and simulations indicate that estimates of mean shape using Procrustes superimposition are well behaved, provided shape variation is sufficiently concentrated around the mean shape (Dryden and Mardia, 1998; Rohlf, 2003), which applies for most biological data even on large taxonomic scales (Marcus et al., 2000). This is much less clear, however, for measures of variation around the mean, which are of central importance for studies of integration and modularity.

Some studies have used rarefaction methods, subsampling from the original data, to examine the statistical behaviour of estimates of integration and modularity (Polly, 2005; Goswami, 2006a; Cardini and Elton, 2007; Goswami and Polly, 2010b). There is a possibility, however, that rarefaction procedures provide an overoptimistic view of the required sample sizes because they ignore the fact that even the total dataset is a sample drawn from some population. An alternative to rarefaction is the bootstrap, which is a resampling method that explicitly considers that the total dataset itself has been obtained by sampling (Efron and Tibshirani, 1993). This method has also been used to assess the reliability of summary statistics such as centroid size and the total variance of shape and suggested that relatively modest sample sizes can give reasonable estimates of those statistics (Cardini and Elton, 2007).

There are also some simple theoretical considerations that can guide the choice of sample sizes. Because most analyses of integration use covariance matrices in one way or another, sample sizes need to provide sufficiently reliable estimates of covariance matrices. The usual recommendation for multivariate analyses is that sample size should be substantially larger than the dimensionality of the data, which is often very large in geometric morphometrics because of the high dimensionality of the shape tangent space (nearly two or three times the number of landmarks, depending on whether the data are in 2D or 3D – but only about half if the data have object symmetry and only the symmetric or asymmetric component of variation are used; Klingenberg et al. 2002). If integration is strong, so that the vast majority of variation is contained within a lower-dimensional subspace of the shape tangent space, a considerably lower sample size may be sufficient. This reasoning suggests an informal procedure for determining whether sample size is sufficient: a preliminary principal component analysis is used to check the dimensionality of the data, and if the sample size is much larger than the number of principal components that account for most of the total shape variance (e.g. 95%), the sample size should be sufficient for analyses of integration and modularity.

Levels of integration and study designs

Morphological integration applies at a range at different levels, which can provide information about different biological processes concerning variation in a structure (Klingenberg, 2008). This is similar to allometry, where levels such as static, ontogenetic and evolutionary allometry have long been known and discussed extensively (Cock, 1966; Gould, 1966; Cheverud, 1982b; Klingenberg and Zimmermann, 1992; Klingenberg, 1996, 1998). For allometry, the levels are defined by the process that produces the size variation involved in the allometric effects (individual variation within populations at a given ontogenetic stage for static allometry, growth for ontogenetic allometry, evolutionary change of size for evolutionary allometry). Similarly, the levels of morphological integration are defined by the processes responsible for the morphological variation.

The different levels apply to the same structures, but examine integration and modularity in different contexts (Klingenberg, 2008). It is therefore possible to study integration at multiple levels jointly with the same set of landmarks or other morphometric data. Depending on which level is of interest, the data have to be collected according to different study designs—data from multiple related species are needed for studies of evolutionary integration, genetic information is needed to infer genetic integration, an understanding of biomechanical interactions provides information about functional integration, and so on. It is therefore important to tailor the study design to the specific questions being asked. Often, however, a single study design can provide information on integration at multiple levels. This possibility is easily overlooked because the vast majority of studies of integration have focussed entirely on the level of intraspecific or intra-population variation.

Some authors have hypothesized that genetic and developmental modularity evolve adaptively to match functional modularity—traits that are involved jointly in particular functions should evolve to share common developmental pathways and common genetic control of morphological variation (Cheverud, 1984, 1996a; Wagner, 1996; Wagner and Altenberg, 1996). This “matching hypothesis” can be tested by comparing modularity across levels: functional versus developmental and genetic modularity (Breuker et al., 2006a). Such tests are expected to be particularly fruitful if there are reasons to expect differences between levels in how traits are grouped together as modules, so that it will become apparent if modularity at one level “wins” over another level (Breuker et al., 2006a). Only few analyses have conducted such tests of the matching hypothesis so far (Breuker et al., 2006a; Klingenberg et al., 2010), but there are many opportunities for future studies. Further topics for comparison across levels comes from the idea that the dominant direction of genetic variation in shape space, the first principal component of the genetic covariance matrix, within populations is a “line of least resistance” for evolutionary change (Schluter, 1996; Renaud and Auffray, this issue) or, if multiple principal components account for substantial variation, that there may a plane or subspace of least resistance (Martínez-Abadías et al., 2012a). Overall, therefore, studies comparing patterns of integration and modularity across different levels are a promising area for future studies.

Integration within populations and species is important because it constitutes a basis for comparison and figures prominently in evolutionary theory (e.g., Steppan et al. 2002). Accordingly, it is justified that the vast majority of studies of integration and modularity have focussed on this level of variation (often, such studies do not specify the level of variation, but authors simply write about "modularity" or "morphological integration" without any additional qualifier). Even studies of modularity at large taxonomic scale mostly have concentrated on comparisons of intraspecific modularity (e.g. Steppan 1997a,b; Goswami 2006a,b, 2007; Sanger et al. 2012). Whereas this interest in intraspecific integration and modularity is clearly justified, other levels of variation are providing interesting information as well, and this information is often available from the same samples.

The following sections will focus on three of the other levels of integration that have been relatively well studied: developmental integration (accessible via analyses of integration in fluctuating asymmetry), genetic integration and evolutionary integration.

Fluctuating asymmetry and developmental integration

The developmental basis of morphological integration is critically important for understanding evolutionary processes, but it cannot be observed directly from morphological data and needs to be inferred. Morphological integration can be the result of developmental interactions between precursors of the traits, or it can result from environmental variation or genetic differences that affect multiple traits simultaneously, even if those traits are separate and do not interact in their development. A tool that can be used for inferring direct interactions between the developmental pathways that produce different morphological traits is fluctuating asymmetry (Klingenberg, 2003, 2005). Because fluctuating asymmetry originates from random perturbations in developmental processes, it can only produce covariation of asymmetry between two traits if the effects of the perturbations are transmitted between precursors of the traits by developmental interactions. If there are no such interactions, asymmetries of the traits are uncorrelated. The integration of fluctuating asymmetry can therefore be used as a tool to assess the developmental origin of morphological integration.

Methods for the study of fluctuating asymmetry have been firmly established in geometric morphometrics, both for the situation where there are two separate structures on the left and right body sides (matching symmetry) and for object symmetry, where the entire structure is symmetric in itself because the axis or plane of symmetry passes through it (Klingenberg and McIntyre, 1998; Mardia et al., 2000; Klingenberg et al., 2002). For matching symmetry, there are separate landmark configurations for the left and right sides that are subjected jointly to a Procrustes superimposition after reflection of all configurations from one side (e.g. all configurations from the left side). Asymmetry can be computed from the differences between the shapes and sizes of the left and right sides. For object symmetry, a copy of each landmark configuration is reflected to its mirror image and paired landmarks are relabelled so that the reflected copy can be fit together with the untransformed copy, and all copies are then used together in a Procrustes superimposition. Asymmetry can be computed from the differences between the original and reflected and relabelled configurations after the Procrustes fit (for more details, see Klingenberg et al. 2002). This type of analysis has also been generalized for complex types of symmetry (Savriama and Klingenberg, 2011; Savriama et al., 2012).

Fluctuating asymmetry is used in an increasing number of analyses to investigate the developmental basis of integration (Klingenberg and Zaklan, 2000; Klingenberg et al., 2001a, 2003; Hallgrímsson et al., 2004; Breuker et al., 2006b; Young and Badyaev, 2006; Zelditch et al., 2008; Klingenberg, 2009; Laffont et al., 2009; Zelditch et al., 2009; Drake and Klingenberg, 2010; Ivanović and Kalezić, 2010; Klingenberg et al., 2010; Jamniczky and Hallgrímsson, 2011; Jojić et al., 2011; Webster and Zelditch, 2011a; Jojić et al., 2012; Klingenberg et al., 2012). Other studies have collected the same type of data for different purposes, such as comparing the patterns of developmental instability and canalization (Debat et al., 2000; Santos et al., 2005; Willmore et al., 2005, 2006a,b; Debat et al., 2008, 2009; Breno et al., 2011). Together, these studies constitute a growing database that can be used to assess the importance of direct developmental interactions for integration in morphological structures. Unfortunately, the results of these studies are quite heterogeneous at the moment and do not allow any generalizations. This heterogeneity is at least partly due to true biological differences, but it is possible that heterogeneity of experimental designs, rearing procedures and morphometric analyses also contributes to the variation. At this time, it seems advisable to investigate the developmental origin of integration on a case-by-case basis—it is possible, however, that general trends will emerge if more case studies are done.

Genetic integration

Because patterns of genetic variation and covariation are critical determinants of evolutionary change (Lande, 1979; Roff, 1997), patterns of genetic integration have long received considerable attention in evolutionary biology. In particular, many studies have focused on model systems such as the mouse mandible (Atchley et al., 1985; Atchley and Hall, 1991; Atchley, 1993; Cheverud, 1996a; Mezey et al., 2000; Klingenberg et al., 2001b, 2004; Burgio et al., 2012a) and the *Drosophila* wing (Fernández Iriarte et al., 2003; Houle et al., 2003; Mezey and Houle, 2005; Santos et al., 2005). More and more studies are specifically addressing questions concerning genetic integration of complex structures in these models, but also in a growing number of non-model organisms including humans (Martínez-Abadías et al., 2009, 2012a). Whereas most genetic studies have used traditional morphometric approaches, the methods of geometric morphometrics are becoming more widespread in genetics.

Several distinct approaches exist for genetic studies that relate to morphological integration. Perhaps the most straightforward approach is to examine the effects of specific mutations on the strength and patterns of integration. Studies of this kind have been conducted in mice (Hallgrímsson et al., 2006; Willmore et al., 2006b; Hallgrímsson et al., 2009; Martínez-Abadías et al., 2011; Parsons et al., 2011) and *Drosophila* wings (Debat et al., 2006, 2009, 2011). These experiments have the potential to reveal possible mechanisms that contribute to integration, but most studies so far have yielded complex results, so that it is difficult or impossible to draw clear-cut conclusions.

A second approach is to use gene mapping techniques to find quantitative trait loci (QTLs) that affect the shape of a structure and to examine integration and modularity of their effects. QTL studies require information on genetic markers and shape data for a suitably structured study population (e.g. the F2 or a later generation from a cross between two inbred lines). Such studies have been done in mice with traditional morphometric methods (Cheverud et al., 1997; Leamy et al., 1999; Mezey et al., 2000; Ehrich et al., 2003) and with geometric morphometric approaches (Klingenberg et al., 2001b; Workman et al., 2002; Klingenberg et al., 2004). Although the results of these studies depend considerably on the specific methods and data used, they have tended to show that the genetic architecture of shape is quite complex, with many QTLs affecting shape, and that nonadditive effects are important. Other studies used different genetic designs where phenotypic changes can be related to substitutions of chromosomal regions between strains, have broadly supported these results (Burgio et al., 2009; Boell et al., 2011; Burgio et al., 2012a,b).

Yet another strategy is to investigate genetic variation in a population without separating the effects of individual loci at all, but to focus on the aggregate effect of the whole genome on a set of morphological traits. The patterns and amount of genetic variation can be obtained from genetic covariance matrices, which can be estimated from shape data in a breeding experiment or in a study population of individuals for which mutual genealogical relationships are known. These requirements can be met relatively easily for many types of organisms and, accordingly, quantitative genetic studies of shape have been conducted in a wide range of plants and animals, including humans (Klingenberg and Leamy, 2001; Mezey and Houle, 2005; Willmore et al., 2005; Myers et al., 2006; Gómez et al., 2009; Martínez-Abadías et al., 2009; Klingenberg et al., 2010; Adams, 2011; Martínez-Abadías et al., 2012a). Several studies have specifically considered questions about genetic integration or modularity. Because the genetic covariance matrix can be used to predict the response to selection, it is possible to simulate localized selection focused on a particular part and examine whether the response is also local or global throughout the entire structure under study (Klingenberg and Leamy, 2001; Martínez-Abadías et al., 2009; Klingenberg et al., 2010; Martínez-Abadías et al., 2012a). Also, it is possible to conduct tests of modularity using genetic covariance matrices (Klingenberg et al., 2010; Martínez-Abadías et al., 2012a).

If integration of the total genetic variation in a population is strong, so that the first principal component of the genetic covariance matrix accounts for a disproportionate share of the total genetic variation, then evolution by drift or selection can occur more easily in the direction of that first principal component than in other directions. In other words, this first principal component acts as a "genetic line of least resistance" (Schluter, 1996). If there is not a single principal component that accounts for an unusually large share of the genetic variation, but two or a few principal components together account for much of the total genetic variation, they can instead form a plane or subspace of least resistance (Martínez-Abadías et al., 2012a). In this way, genetic integration may have a substantial potential influence on long-term evolutionary outcomes.

Even though genetic studies of integration and modularity have become easier with advances in statistical methods and genotyping technology, investigating the genetic basis of morphological integration and modularity remains challenging. Most studies are limited to some extent by statistical power to detect genetic effects or by the uncertainty about estimates of genetic parameters due to limited sample sizes. Further work, using large experimental designs or extensive pedigree data, will be necessary to characterize the exact nature of genetic integration and modularity in complex structures such as the mammalian skull.

Evolutionary integration: comparative methods

Integration and modularity not only apply to the patterns of covariation within populations and species, but also to the patterns of covariation among evolutionary changes of shape in a clade of related taxa. At this macroevolutionary scale, studies of integration and modularity need to use comparative methods to take into account the phylogenetic structure of variation. With this proviso, all the morphometric methods for investigating patterns of covariation and modularity can also be used at this level (Klingenberg and Marugán-Lobón, 2013).

To assess whether there is phylogenetic signal in the morphometric data, a permutation test has been described that simulates the null hypothesis of a complete lack of phylogenetic structure by randomly swapping the shape data across the taxa in the phylogeny (Klingenberg and Gidaszewski, 2010). This test is now widely used in comparative studies of shape (e.g. Gidaszewski et al. 2009; Figueirido et al. 2010; Álvarez et al. 2011a; Fortuny et al. 2011; Meloro et al. 2011; Perez et al. 2011; Álvarez and Perez 2013; Brusatte et al. 2012; Klingenberg et al. 2012; Meloro and Jones 2012; Sanger et al. 2012; Klingenberg and Marugán-Lobón 2013). In the majority of studies, there is a significant phylogenetic signal, so that phylogenetic comparative methods should normally be used for analyses of evolutionary integration and modularity (Klingenberg and Gidaszewski, 2010; Klingenberg and Marugán-Lobón, 2013).

Phylogenetic comparative methods are now well known and widely available, particularly independent contrasts and phylogenetic generalized least squares (Felsenstein, 1985; Rohlf, 2001; Felsenstein, 2004). Both these methods have been shown to provide equivalent results (Rohlf, 2001; Blomberg et al., 2012), both can easily accommodate multivariate data, and both can therefore be used in the context of geometric morphometrics.

The analysis of evolutionary integration and modularity can use covariance matrices computed from independent contrasts or the estimated matrix of evolutionary covariances from phylogenetic generalized linear models with the same morphometric tools as they are used for other levels of variation. Overall patterns and the amounts and dimensionality of evolutionary variation can be studied with principal component analysis of the covariance matrix of independent contrasts (Klingenberg et al., 2012; Klingenberg and Marugán-Lobón, 2013). Estimating evolutionary allometry and correcting for allometric effects of evolutionary changes in shape can be achieved by multivariate regression of independent contrasts of shape onto independent contrasts of size (usually centroid size or log-transformed centroid size; Figueirido et al. 2010; Swiderski and Zelditch 2010; Perez et al. 2011; Klingenberg et al. 2012; Klingenberg and Marugán-Lobón 2013). Evolutionary integration between different structures or parts can be studied with partial least squares analysis of independent contrasts (Bastir et al., 2010; Klingenberg and Marugán-Lobón, 2013) or with partial least squares analysis of covariance matrices from phylogenetic generalized least squares (Dornburg et al., 2011; Meloro et al., 2011). Modularity tests also can be conducted with covariance matrices computed from independent contrasts (Drake and Klingenberg, 2010; Klingenberg and Marugán-Lobón, 2013). These analyses use the same computations for evolutionary integration and modularity that are used in analyses at different levels and the results are directly comparable across levels. Phylogenetic comparative methods are only beginning to be used in geometric morphometrics, but it is likely that they will become much more widespread in the near future.

These methods are related to the method of mapping morphometric data onto phylogenies using squared change parsimony, which has been used increasingly in recent years (Klingenberg and Ekau, 1996; Rohlf, 2002; Nicola et al., 2003; Linde et al., 2004; Macholán, 2006; Sidlauskas, 2008; Astúa, 2009; Gidaszewski et al., 2009; Figueirido et al., 2010; Klingenberg and Gidaszewski, 2010; De Esteban-Trivigno, 2011a,b; Dornburg et al., 2011; Fortuny et al., 2011; Monteiro and Nogueira, 2011; Brusatte et al., 2012; Klingenberg et al., 2012; Meloro and Jones, 2012; Klingenberg and Marugán-Lobón, 2013). This approach provides a direct visualization of evolutionary changes in shape space, and therefore is a useful tool for understanding the evolutionary history of morphological structures. Whereas analyses using comparative methods such as independent contrasts provide summary information about patterns of shape changes on all branches of the phylogeny, this graphical approach is complementary because it provides information about changes on particular branches and the occupation of the shape space by different subclades within the group under study. The two approaches are therefore complementary and can be used in combination with each other (Klingenberg and Marugán-Lobón, 2013).

As an alternative to phylogenetic comparative methods, other studies have used an approach based on taxonomic hierarchy, using distances between average shapes for different taxonomic levels (within species, among species within genera, among genera, etc.) to construct distance matrices for different parts of a structure and then to characterize integration using the relations among distance matrices (Monteiro et al., 2005; Monteiro and Nogueira, 2010).

Integration and modularity of cranial shape in mammals

So far, this review has focused on the methodology for analysing morphological integration and modularity. For the remaining part of this paper, however, I will organize the discussion around the primary mammalian systems that have been used in morphometric studies and what their results imply for our understanding of mammalian evolution. The summary that follows is an overview of work on morphological integration that has been done in the main mammalian study systems (both model and "non-model" systems), mostly with geometric morphometric methods. Because the literature relating to integration and modularity in mammalian skulls has become so large, it is impossible to present a complete survey. I apologise to the authors whose work I had to omit.

High-level comparisons

Morphometric analyses at large phylogenetic scales can be challenging because of difficulties in identifying homologous landmarks and the sheer scale of variation, which can pose challenges to morphometric procedures such as the tangent space approximation to Kendall's shape space. Nevertheless, empirical analyses have demonstrated that analyses of skull shape can be feasible, even for analyses across all mammalian orders (Marcus et al., 2000).

Some studies compared modularity and integration across mammals with different methods and found conserved features (Goswami, 2006a; Porto et al., 2009) and identified possible consequences for macroevolutionary processes (Marroig et al., 2009; Goswami and Polly, 2010a). Other studies focused on comparing between specific, phylogenetically remote groups, such as the comparison between didelphid marsupials and New-World monkeys (Shirai and Marroig, 2010) or carnivorans and primates (Goswami and Polly, 2010a). Some studies have compared aspects of integration and allometry between mice and humans or other primates to identify commonalities in the developmental origin of variation (Hallgrímsson et al., 2004; Martínez-Abadías et al., 2012b). Other studies compared major clades in terms of evolutionary integration to examine whether variation in diet brings about similar responses in different clades (Wroe and Milne, 2007; De Esteban-Trivigno, 2011a; Goswami et al., 2011). A general interpretation of the results is difficult, because these studies used very different methods and sampling strategies.

These studies demonstrate that analyses of integration at very large phylogenetic scale are feasible. Much more work is required to establish patterns of evolutionary integration across the mammalian phylogeny and within-taxon patterns of integration.

Mouse and other rodents

The mouse mandible has long been a model for development and evolution of complex morphological structures (Atchley and Hall, 1991; Klingenberg and Navarro, 2012). It is composed of several units with distinct developmental origins, functional roles and, to some extent, separate inheritance and evolutionary history. Yet, the mandible is still relatively simple, by comparison to structures such as the cranium, so that it can be studied relatively easily. Accordingly, many studies have investigated the mandible in the context of development, genetics and evolution.

A particular focus of the research on morphological integration and modularity in the mouse mandible was the hypothesis that the alveolar region and ascending ramus are two distinct modules. Sometimes, these modules were further subdivided into smaller elements (Atchley and Hall, 1991; Atchley, 1993). Evidence for modularity first came from studies using traditional morphometrics in combination with different quantitative genetic approaches in laboratory mice (Atchley et al., 1985; Leamy, 1993; Cheverud, 1996a; Cheverud et al., 1997; Mezey et al., 2000; Ehrich et al., 2003) and later also from studies using geometric morphometrics (Klingenberg and Leamy, 2001; Klingenberg et al., 2001b, 2003, 2004; Klingenberg, 2009; Burgio et al., 2012a; Renaud et al., 2012). Studies in hybrids between different subspecies of house mice indicate that hybridization is affecting different parts of the mandible differently, so that the overall effect on the shape of the entire mandible is complex (Renaud et al., 2012). Experimental studies suggest that bone remodelling under mechanical loading is important for integration and modularity in the mandible (Lightfoot and German, 1998; Tagliaro et al., 2009; Renaud et al., 2010; Vecchione et al., 2010). Histological studies further support the subdivision of the mouse mandible into two modules, as the difference between alveolar region and ascending ramus is also apparent in the processes of bone growth and remodelling in postnatal development (Martinez-Maza et al., 2012).

For wild populations of the house mouse, both plasticity and integration have been shown to be important factors for the evolution of mandible shape (Renaud and Auffray, 2009; Boell and Tautz, 2011; Siahsarvie et al., 2012). Allometry has been reported to be an integrating factor in mandibular shape variation in a Robertsonian chromosome polymorphism in the house mouse, but differential response of the alveolar region and ascending ramus was also found (Sans-Fuentes et el., 2009; Muñoz-Muñoz et al., 2011). Similar modularity was found in the mandible of yellow-necked field mice (Jojić et al., 2007, 2012). Renaud et al. (2007) reported allometry in the evolution of mandible outline shape in murids, indicating that size is an integrating factor, but also found a strong effect of dietary specialization. A detailed study of integration and modularity in a species of deer mouse found complex patterns for individual variation and fluctuating asymmetry (Zelditch et al., 2008).

Studies of integration in the mandible have also been conducted in a wide range of other rodents. In marmots and squirrels (Sciuridae), integration in the mandible has been investigated with a range of different morphometric approaches and both a modular structure of covariation and allometry have been reported (Velhagen and Roth, 1997; Cardini, 2003; Cardini and Tongiorgi, 2003; Cardini and Thorington, 2006; Zelditch et al., 2009; Swiderski and Zelditch, 2010). A series of papers examined morphological variation and integration in the mandible of spiny rats (Echimyidae) using a range of geometric morphometric methods and in relation to phylogeny, geography and ecological variables (Monteiro et al., 2003a, 2005; Monteiro and dos Reis, 2005; Perez et al., 2009). Similar analyses were also conducted for the whole group of caviomorph rodents (Álvarez et al., 2011b,a; Álvarez and Perez, 2013). Hautier et al. (2012) studied variation in the mandible and cranium of hystricognathous rodents and the integration between mandible and cranium.

The whole skull also has been used in many studies of morphological integration, particularly in laboratory mouse, where various experimental approaches have been used to investigate the mechanisms involved in cranial integration. Because the mouse is one of the classical "model organisms", its craniofacial development is known in great detail and many genetic and other experimental resources are available for it (Depew et al., 2002; Chai and Maxson, 2006). In particular, for laboratory mice, it is possible to investigate development directly by conducting morphometric studies including a range of ontogenetic stages (Willmore et al., 2006a; Zelditch et al., 2006). This is now possible even for embryos (Young et al., 2007; Boughner et al., 2008; Parsons et al., 2008, 2011), although careful attention to experimental procedures is required because of the potential for artefacts (Schmidt et al., 2010). These direct analyses of development complement genetic approaches, where mutations are used that disrupt specific developmental processes (Hallgrímsson et al., 2006; Willmore et al., 2006b; Hallgrímsson et al., 2009; Martínez-Abadías et al., 2011; Parsons et al., 2011) or where QTLs for different skull regions are sought for understanding the genetic origin of modularity (Leamy et al., 1999). In addition, experimental approaches can be used for investigating specific processes, for example to demonstrate the importance of bone remodelling for cranial morphology (Lightfoot and German, 1998; Vecchione et al., 2010). Note that integration does not only concern the skull itself, but that there is also extensive integration between the skull and surrounding soft tissue (Jamniczky and Hallgrímsson, 2011).

Overall, these studies support the idea that the complexity of cranial integration reflects the complexity of cranial development (Hallgrímsson et al., 2009). In addition, patterns and strengths of integration in the rodent skull change over ontogeny (Zelditch, 2005; Willmore et al., 2006a; Zelditch et al., 2006; Gonzalez et al., 2011a).

Integration in the skull has been studied extensively with a range of methods. Several studies quantified the degree of integration in the rodent skull with a measure derived from the variance of eigenvalues of the covariance matrix of shape variables (Willmore et al., 2006a; Hallgrímsson et al., 2009; Jamniczky and Hallgrímsson, 2009; Gonzalez et al., 2011b; Jojić et al., 2011). Some studies used partial least squares analysis to investigate patterns of integration between different skull regions such as the dorsal and ventral sides (Rohlf and Corti, 2000; Corti et al., 2001; Monteiro et al., 2003a; Macholán et al., 2008; Burgio et al., 2009). Tests of modularity have yielded mixed results, with some supporting and others inconsistent with a-priori hypotheses (Hallgrímsson et al., 2009; Jamniczky and Hallgrímsson, 2011; Jojić et al., 2011). The patterns of integration for individual variation and fluctuating asymmetry appear to be related in some cases and totally dissimilar in others (Debat et al., 2000; Hallgrímsson et al., 2004; Breno et al., 2011; Jojić et al., 2011), indicating that the role of developmental interactions in determining patterns of integration in the skull is unclear.

Allometry appears to be an important contributing factor to integration in rodent skulls and can produce strong integration for large-scale phylogenetic comparison, but not necessarily at smaller scales (Roth, 1996; Monteiro et al., 1999; Cardini and O'Higgins, 2004; Cardini and Thorington, 2006; Hautier et al., 2012; Martínez-Abadías et al., 2012b). Note, however, that cranial allometries themselves can evolve and therefore differ among taxa (Cardini and Thorington, 2006; Wilson and Sánchez-Villagra, 2010).

Integration has not only been studied in the mandible and cranium of rodents, but also in the teeth, both for the entire molar row and for individual molar teeth. Laffont et al. (2009) found significant covariation among mandibular molars of voles for individual variation but not for fluctuating asymmetry, and thus suggested that factors other than direct developmental interactions were responsible for integration. A search for QTLs affecting the shape of the mandibular molar row in laboratory mice found 18 putative QTLs, which had effects involving complex shape changes involving all three molars in combination, rather than changes limited mostly to a single tooth (relative to the remaining landmarks), and thus suggested that the genetic architecture of shape is integrated throughout the molar row (Workman et al., 2002). Patterns of within-population integration of molar tooth shape have been shown to coincide with directions of evolutionary diversification, suggesting that these patterns of integration can function as lines of least resistance (Renaud et al., 2005, 2011; Renaud and Auffray, this issue).

Although a multitude of studies have addressed questions on craniofacial integration and modularity in rodents, many gaps remain in our knowledge. Because so much is known about mouse development and genetics, furthering our understanding of mechanisms and consequences of integration and modularity in rodents must remain a priority for further research.

Carnivorans

Studies of cranial integration and modularity and of the evolution of skull shape in carnivorans are quite abundant. Patterns of cranial integration in carnivorans vary, partly in accordance with phylogenetic relatedness and also with diet (Goswami, 2006b). A considerable degree of evolutionary integration was found in studies that combined samples of carnivorans and carnivorous marsupials, and also appeared to be associated with differences in diet (Wroe and Milne, 2007; Goswami et al., 2011). The patterns of integration uncovered by these studies feature general contrasts between relatively short, broad and elongate, slender skulls that have also been reported in studies of carnivorans alone (Drake and Klingenberg, 2010). That diet can be a crucial factor for evolution of skull shape has been shown by studies examining transitions to herbivory, which are accompanied by marked shifts in morphology and functional aspects such as bite force (Christiansen and Wroe, 2007; Figueirido et al., 2010, 2011, 2012). In the extreme, such as for the evolution of sabre-toothed cats, such morphological and functional changes can bring about major shifts in the patterns of integration by comparison to other, related taxa (Christiansen, 2008, 2012).

For evolutionary change across the Carnivora, a pattern of modularity between the face and braincase has been observed, which also holds within species in the gray wolf and in domestic dogs (Drake and Klingenberg, 2010). This modular pattern of integration is also found for fluctuating asymmetry, indicating that it has arises from direct developmental interactions within the modules of the face and braincase, but that there are few such interactions between face and braincase (Drake and Klingenberg, 2010). The same modular structure of covariation in the skull also appears to have facilitated the explosive evolution of skull shapes in domestic dogs under domestication and selection by breeders (Drake and Klingenberg, 2008, 2010; Drake, 2011). Integrated evolution also has been shown for the ramus and corpus of the mandible (Meloro et al., 2011).

Shrews

Patterns of integration for variation among individuals, fluctuating asymmetry and variation among species in the mandible of shrews were found to correspond clearly, and intraspecific patterns of integration were similar in different species (Badyaev and Foresman, 2000, 2004; Young and Badyaev, 2006). These results indicate that patterns of integration are evolutionarily stable and that developmental interactions are important in determining patterns of integration within and among species. Functional aspects, assessed by the locations of muscle insertions on the mandible, appear to be important determinants of the patterns of integration (Badyaev and Foresman, 2000, 2004; Young and Badyaev, 2006) and bone remodelling in the mandible under muscle loading is related to morphological variation (Young and Badyaev, 2010).

A study combining morphometric analyses of shrew molar teeth in several populations and a numerical model of development of the tooth showed clear similarities of phenotypic variation in tooth shape among populations and correspondence to the patterns of variation produced by the developmental model (Polly, 2005).

Humans and other primates

The literature on morphological integration in the skull of humans and other primates is massive and dates back several decades. Accordingly, it is also very heterogeneous in terms of the morphometric methods and biological concepts that were used in different studies. Many studies use traditional morphometric methods for investigating morphological integration in primates, as approaches for investigating integration in the context of geometric morphometrics have only been developed relatively late. Primates were used as the study system in several key papers that revived the interest in morphological integration and related subjects such as allometry (e.g. Gould 1975; Cheverud 1982a,b; Cheverud et al. 1989; Cheverud 1995; Ackermann and Cheverud 2000; Marroig and Cheverud 2001; Ackermann 2005; Marroig and Cheverud 2005; Shirai and Marroig 2010). After geometric morphometric techniques were established, applications concerning cranial integration and allometry in primates, including humans, have become increasingly widespread (e.g. O'Higgins and Jones 1998; Collard and O'Higgins 2001; Lieberman et al. 2002; Penin et al. 2002; Singleton 2002; Bookstein et al. 2003; Frost et al. 2003; Bastir et al. 2004; Hallgrímsson et al. 2004; Mitteroecker et al. 2004; Bastir and Rosas 2005; Willmore et al. 2005; Mitteroecker and Bookstein 2008; Martínez-Abadías et al. 2009; Makedonska et al. 2012; Martínez-Abadías et al. 2012a; Singh et al. 2012). In accordance with the focus of this special issue of Hystrix, I will concentrate primarily on papers about cranial integration and modularity that use geometric morphometric methods.

Many studies of morphological integration in the primate skull are concerned with the covariation of specific anatomical or developmental components, which has been a been the subject of extensive discussion in physical anthropology and primatology (e.g.Lieberman 2011). A wide variety of different divisions of the skull into parts and study designs have been used, but many studies use partial least squares to extract the patterns of covariation between parts from morphometric data. Examples include parts such as the cranium and mandible (Bastir et al., 2005), face and neurocranium (Mitteroecker and Bookstein, 2008), the face, cranial base and cranial vault (Bookstein et al., 2003; Bastir and Rosas, 2006; Gkantidis and Halazonetis, 2011; McCane and Kean, 2011; Makedonska et al., 2012; Singh et al., 2012), cranial base, cranial fossa and mandibular ramus (Bastir and Rosas, 2005), the parietal, occipital and temporal bones in the posterior braincase (Gunz and Harvati, 2007), oral and zygomatic components of the face (Makedonska et al., 2012), or between parts of the mandible (Harvati et al., 2011). Baab et al. (2010) used partial least squares to study the association of cranial shape robustness, a general feature of cranial morphology that might itself be a form of integration. In general, these analyses tend to show that integration between different cranial parts is fairly strong, tests against the null hypothesis of independence usually yield statistically significant results if sufficiently large sample sizes are available, and the patterns of covariation revealed by the shape changes associated with partial least squares axes often suggest biological explanations for the origin or adaptive value of integration.

Partial least squares analysis has also been used for studying integration between skull and soft tissues, for example between the brain and skull (Bastir et al., 2010). An association between the soft tissues of the face and the underlying skull was also found in this way (McCane and Kean, 2011), as well as with a different method (Halazonetis, 2007).

Tests of hypotheses on modularity, using comparisons of the strength of association between subsets of landmarks (Klingenberg, 2009), have been conducted for two examples in humans. In a study of the shape of the human brain in the midsagittal plane cortical and subcortical regions behaved as separate modules (Bruner et al., 2010). By contrast, the face, cranial base and cranial vault in human skulls did not behave as separate modules, neither for genetic nor phenotypic covariation, but variation appeared to be integrated throughout the entire skull (Martínez-Abadías et al., 2012a). Hypotheses of modularity can also be formulated for postcranial traits—one study used similar methods for studying modularity in the pelvis across a spectrum of primates (Lewton, 2012).

Because allometry has long been a central theme in primate ontogeny and evolution (e.g. Gould 1975), many morphometric studies have examined allometry through growth and static allometry within populations (e.g. O'Higgins and Jones 1998; Collard and O'Higgins 2001; Ponce de León and Zollikofer 2001; Lieberman et al. 2002; Penin et al. 2002; Strand Viðarsdóttir et al. 2002; Bastir and Rosas 2004; Berge and Penin 2004; Cobb and O'Higgins 2004; Mitteroecker et al. 2004; Zollikofer and Ponce de León 2004; Mitteroecker et al. 2005; Bastir et al. 2006; Bulygina et al. 2006; Bastir et al. 2007; Marroig 2007; Sardi et al. 2007; Morimoto et al. 2008; Baab and McNulty 2009; Gonzalez et al. 2010, 2011c; Weisensee and Jantz 2011; Martínez-Abadías et al. 2012b; Sardi and Ramírez Rozzi 2012). Other studies have considered only adult specimens, but from several species, and therefore concern evolutionary allometry, with a possible contribution from static allometry within species (e.g. Singleton 2002; Frost et al. 2003; Rosas and Bastir 2004; Cardini and Elton 2008b; Bastir et al. 2010; Elton et al. 2010; Bastir et al. 2011; Gilbert 2011; Ito et al. 2011). It is also possible to use these allometric approaches to investigate growth of the human face (Hennessy and Moss, 2001; Velemínská et al., 2012) or allometry of the brain (Bruner et al., 2010) and, related to it, the endocranial cavity (Neubauer et al., 2009; Gunz et al., 2010; Neubauer et al., 2010; Gunz et al., 2012). In most of these studies, pronounced allometry has been found, and allometry may therefore be an important integrating factor in the primate head. Some studies have therefore applied size correction, by using residuals from the multivariate regression of shape on size, before further analyses of integration (e.g. Bastir et al. 2011; Martínez-Abadías et al. 2012a).

Several studies have compared ontogenetic trajectories among populations and species (e.g. Collard and O'Higgins 2001; Ponce de León and Zollikofer 2001; Penin et al. 2002; Strand Viðarsdóttir et al. 2002; Berge and Penin 2004; Cobb and O'Higgins 2004; Mitteroecker et al. 2004, 2005; Mitteroecker and Bookstein 2008; Gunz et al. 2010, 2012; Sardi and Ramírez Rozzi 2012). If data from multiple populations or species are available and ages of specimens are known, it is possible to compare ontogenetic and evolutionary allometry to assess whether evolution occurred by ontogenetic scaling (Gonzalez et al., 2011c). This kind of study is important because it directly relates to explanations of evolutionary change by ontogenetic scaling, possibly through processes such as heterochrony (Klingenberg, 1998; Lieberman, 2011). In turn, these evolutionary processes can be crucial determinants for macroevolutionary patterns, such as evolutionary allometry. Evolutionary phenomena, in turn, depend on the availability of genetic variation on which natural selection and drift can act. So far, only few analyses combine geometric morphometric approaches with quantitative genetic analyses, but two of these studies have been conducted in primates: macaques (Willmore et al., 2005) and humans (Martínez-Abadías et al., 2009, 2012a). Willmore et al. (2005) studied the developmental basis of genetic and phenotypic integration by comparing covariance patterns of genetic and environmental variation as well as fluctuating asymmetry. Correspondences between patterns of variation were not very strong, but statistically significant and therefore suggest that direct developmental interactions are involved in shaping genetic integration, but perhaps make only a relatively small contribution. Martínez-Abadías et al. (2009, 2012a) used hypothetical selection for localized shape changes in the human skull and consistently found that the predicted responses affected the entire skull, indicating that genetic variation for human skull shape is highly integrated. Consistent with this finding, a test rejected the hypothesis that the face, cranial base and cranial vault are distinct modules concerning genetic variation (Martínez-Abadías et al., 2012a). Given the far-reaching implications of genetic integration for the evolution of the human head, further analyses of the genetic basis of cranial shape variation in primates are urgently needed.

The strong genetic integration for cranial shape that is apparent in the results on humans (Martínez-Abadías et al., 2009, 2012a) has implications for the understanding of primate and particularly human evolution. Because simulations of selection for different localized shape features gave similar responses involving global shape changes throughout the skull (Martínez-Abadías et al., 2012a), it is clear that the shape change in response to selection is not a reliable guide for inferring the specific features under selection and, above all, that there is no direct correspondence between the shape change that was selected for and the resulting evolutionary response. This adds to the complexities for inferring past selection, for instance, in human evolution (Lieberman, 2008, 2011). The second implication is that it is doubtful whether the skull can be divided into anatomical or functional subunits that are independent of each other in their evolution and therefore can be used as distinct cladistic characters for inferring phylogeny (Skelton and McHenry, 1992; Strait et al., 1997; Cardini and Elton, 2008a; González-José et al., 2008). If the genetic variation of skull shape observed in this one human population is representative of past populations (for which it is the best evidence that is currently available), the evolution in all parts of the skull is highly interdependent. Different putative modules in the skull are therefore unlikely to provide independent information on phylogeny. Moreover, because cranial integration is associated with strong evolutionary constraints (Martínez-Abadías et al., 2012a), it also makes it plausible that independent evolutionary changes in different evolutionary lineages produce similar shape changes, which would help to account for the homoplasy that has made it difficult to infer phylogenies from craniodental characters (e.g. Skelton and McHenry 1992; Strait et al. 1997; Wood and Lonergan 2008).

Some studies have found that different parts of the human skull, such as the face, temporal bone or cranial base, reflect factors such as population history and adaptation to climates to different degrees (Harvati and Weaver, 2006; Perez and Monteiro, 2009; von Cramon-Taubadel, 2011b; von Cramon-Taubadel and Smith, 2012). This implies some degree of modularity at the evolutionary level. To reconcile this observation with the strong genetic integration in the skull, one can hypothesize that this evolutionary modularity results from differential effects of selection on different parts of the skull, each in its own developmental and functional contexts. Any such hypothesis must necessarily remain rather speculative because of the various difficulties inherent in inferring past selection regimes (or tests of selection versus the null hypothesis of random drift). For instance, tests of such hypotheses almost inevitably make unrealistic assumptions, such as constancy of genetic covariance matrices over time or proportionality of genetic and phenotypic covariance matrices (e.g. Perez and Monteiro 2009; von Cramon-Taubadel 2009; Smith 2011).

There have been numerous morphometric studies of teeth in humans and other primates (Robinson et al., 2001; Martinón-Torres et al., 2006; Kieser et al., 2007; White, 2009; Gómez-Robles et al., 2011b; Grieco et al., 2013). Integration in the dentition has clear functional significance because it relates directly to occlusion during biting and chewing. Therefore, it is not surprising that integration has been shown among the shapes of premolar teeth and throughout the premolars and molars (Gómez-Robles et al., 2011a; Gómez-Robles and Polly, 2012). It appears that evolutionary allometry in the shape of teeth can be quite strong and evolution by allometric scaling has also been demonstrated (Martinón-Torres et al., 2006; Singleton et al., 2011).

Studies in humans offer some special opportunities for investigating factors that contribute to craniofacial shape variation. Some studies have investigated plasticity in the skull by following the consequences of transitions in the mode of subsistence in human populations, for instance from hunter-gatherer to agricultural modes, and have found shape changes in the skull and mandible (Paschetta et al., 2010; von Cramon-Taubadel, 2011a). Because these shape changes are likely to relate to differences in the consistency of food and requirements for mastication, they can be informative about functional effects of mastication and bone remodelling under mechanical loads, and are therefore relevant for this source of integration. A different approach is to take advantage of opportunities like the quasi-experimental modifications of the skull through intentional deformation (Cheverud et al., 1992; Kohn et al., 1993; Perez, 2007; Martínez-Abadías et al., 2009). And finally, the data concerning dysmorphologies from diseases with known aetiologies and localized origins are a very rich, so far greatly underexploited, resource for the study of integration in the skull (e.g. Tobin et al. 2008; Richtsmeier and DeLeon 2009; Heuzé et al. 2012).

Conclusions

This article has reviewed some of the vast and still rapidly growing literature on morphological integration and modularity. Two primary conclusions arise from this survey: first, there is now an established and diverse set of tools for investigating morphological integration and modularity within the framework of geometric morphometrics and, second, a large and increasing number of studies have applied these methods to mammalian systems so that a general picture starts to emerge, but many opportunities remain for filling in big gaps in current knowledge. Also, there are several major challenges for innovations in methodology and development of new experimental protocols to tie the study of integration and modularity to functional, genetic and phylogenetic aspects of craniofacial evolution.

Although numerous studies on integration and modularity have been published, many of them using mammals, it is surprisingly difficult to use this information for making comparisons or developing generalizations across larger taxa. Authors differ in how many "routine" statistics they provide in their papers: statistics such as eigenvalues or matrix correlations between covariance matrices for individual variation and fluctuating asymmetry, which are all relevant for studies of morphological integration, are reported in some papers but not in others. It is fairly simple for authors to include such statistics in their papers; the incentive for them is that the paper may be cited a few times more often as a result (which is also an argument that might convince editors and reviewers anxious to improve the impact factor of their journal). Also, there is a need for carrying out analyses of integration in additional species, even if a similar study already exists in a related species. This will facilitate comparative studies on the evolution of integration and modularity in the mammalian skull, which will benefit from the rapid growth of knowledge in this area and will in turn contribute to it.

Challenges that require new developments remain in several areas. In many of the cases I will mention here, it will also be useful to gain a better understanding and to provide more accessible explanations of how the existing methods work and what assumptions they make. For comparisons of integration across taxa, it will be important to develop methods for comparing many covariance matrices simultaneously without losing too much information. Improving the methodology for exploratory searches for modules in morphometric data also remains a challenge, and it clearly is a daunting one, both from biological and statistical points of view. There are many possibilities for incorporating phylogenetic comparative approaches and quantitative genetics into analyses of integration and modularity to encompass the spectrum from micro- to macroevolutionary perspectives. There are promising new possibilities in the emerging synthesis of morphometric and biomechanical approaches (e.g. O'Higgins et al. 2011), which may lead to new ways of understanding the functional aspects of integration and modularity. Finally, there are new opportunities to relate the study of integration and modularity to insights and experimental protocols from developmental biology (Young et al., 2010; Parsons et al., 2011; Kimmel et al., 2012).

I am optimistic that morphometric studies on integration and modularity in the mammalian skull will contribute substantially to a comprehensive and unified understanding of the developmental, functional and historical aspects of the evolution of complex morphological structures (Breuker et al., 2006a; Klingenberg, 2010). This is a major contribution of geometric morphometrics to evolutionary biology, which will in turn consolidate geometric morphometrics as an important discipline in 21st-century biology.

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Associate Editor: A. Cardini