

Notes and Comments

Cranial Modularity Shifts during Mammalian Evolution

Anjali Goswami*

Committee on Evolutionary Biology, University of Chicago, Chicago, Illinois 60637; and Department of Geology, Field Museum, Chicago, Illinois 60605

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ABSTRACT: The mammalian skull has been studied as several separate functional components for decades, but the study of modularity is a more recent, integrative approach toward quantitative examination of independent subsets of highly correlated traits, or modules. Although most studies of modularity focus on developmental and genetic systems, phenotypic modules have been noted in many diverse morphological structures. However, few studies have provided empirical data for comparing modules across higher taxonomic levels, limiting the ability to assess the broader evolutionary significance of modularity. This study uses 18–32 three-dimensional cranial landmarks to analyze phenotypic modularity in 106 mammalian species and demonstrates that cranial modularity is generally conserved in the evolution of therian mammals (marsupials and placentals) but differs between therians and monotremes, the two extant subclasses of Mammalia. Within therians, cluster analyses identify six distinct modules, but only three modules display significant integration in all species. Monotremes display only two highly integrated modules. Specific hypotheses of functional and developmental influences on cranial bones were tested. Theoretical correlation matrices for bones were constructed on the basis of shared function, tissue origin, or mode of ossification, and all three of these models are significantly correlated with observed correlation matrices for the mammalian cranium.

Keywords: modularity, Mammalia, evolution, development, functional integration.

The correlated evolution of traits is a principal factor in

morphological evolution (Olson and Miller 1958; Vermeij 1973; Emerson and Hastings 1998; Bolker 2000; Pigliucci and Preston 2004). Individual trait variation may be constrained by trait associations, while, conversely, trait associations may promote modifications of functionally related traits. The study of morphological integration allows for examination of broad patterns of trait associations and modularity. The concept of morphological integration was developed by Olson and Miller (1958) based on the idea that traits related by ontogeny or function have greater influence on each other than on traits without shared function or developmental history and that these characters may form discrete groups of highly correlated traits, termed modules. As largely independent units, modules can provide a markedly different perspective of morphological evolution than study of individual traits alone. Furthermore, phenotypic modules may reflect underlying genetic modules, bridging these disparate scales of evolutionary study, and recent studies have empirically or theoretically tied morphological integration to quantitative genetics, molecular pathways, novelty, life-history strategies, and macroevolutionary trends (Cheverud 1982, 1996a, 1996b; Cheverud et al. 1983, 1991, 1997, 2004; Wagner 1995, 1996; Klingenberg and Leamy 2001; Klingenberg et al. 2001a, 2004; Ehrich et al. 2003; Pigliucci and Preston 2004; Schlosser and Wagner 2004).

Trait covariation or correlation measures the coordinated variation of characters. Morphological integration can be partitioned into genetic and evolutionary integration, which differ in their biological origins (Cheverud 1996a). Evolutionary integration is broader, encompassing correlated morphological evolution that is driven by genetics or selection, while genetic integration refers to morphological trait correlations due solely to coinheritance. A variety of microevolutionary studies have examined the relationships among integration and its genetic, developmental, and functional roots in diverse systems, including insect wings (Klingenberg and Zaklan 2000; Klingenberg et al. 2001b), piranhas (Fink and Zelditch 1996; Zelditch et al. 2001), mammalian mandibles and dentition (Atchley et al. 1982; Atchley and Hall 1991; Cheverud et al. 1991,

* Present address: Department of Palaeontology, Natural History Museum, Cromwell Road, London SW7 5BD, United Kingdom; e-mail: a.goswami@nhm.ac.uk.

1997, 2004; Atchley 1993; Badyaev and Foresman 2000, 2004; Klingenberg and Leamy 2001; Klingenberg et al. 2001a, 2003; Cheverud 2004; Klingenberg 2004; Polly 2005), and the mammalian skull (Cheverud 1982, 1988, 1989, 1995, 1996a, 1996b; Cheverud et al. 1983; Zelditch 1988; Zelditch and Carmichael 1989a, 1989b; Steppan 1997a, 1997b; Ackermann and Cheverud 2000, 2004a, 2004b; Marroig and Cheverud 2001, 2005; Marroig et al. 2004; Zelditch and Moscarella 2004). Across these systems, there has been a focus on the relationships between genetic and phenotypic integration (Cheverud 1982, 1996b, 2004; Cheverud et al. 1983, 1997, 2004; Atchley 1993; Klingenberg and Leamy 2001; Klingenberg et al. 2001a, 2004) and between development and phenotypic integration (Cheverud 1984, 1996a; Zelditch 1988; Zelditch and Carmichael 1989a, 1989b; Atchley and Hall 1991; Atchley 1993; Klingenberg and Nijhout 1999; Klingenberg and Zaklan 2000; Klingenberg et al. 2001b, 2003; Klingenberg 2002, 2004; Zelditch and Moscarella 2004; Badyaev et al. 2005).

Although many workers have stressed the potential of hypotheses of modularity to unite the genetic, developmental, and morphological scales of evolutionary study (Cheverud 1996a; Bolker 2000; Zelditch et al. 2001), few comparative studies above the genus level have ever been conducted (but see Cheverud 1989; Marroig and Cheverud 2001; Ackermann and Cheverud 2004b). Previous small-scale studies (within species or genera) provide a strong foundation for examining modularity, but few studies have provided empirical data for comparing modules across higher taxonomic levels, limiting the ability to assess the broader evolutionary significance of modularity. This study assesses cranial modularity across the class Mammalia and addresses the following questions: Do discrete modules exist? Are modules conserved across large clades? and Does cranial modularity reflect specific functional or developmental relationships among skull bones? This study provides the phylogenetic breadth and perspective necessary to isolate and test competing hypotheses of modularity by including taxa that vary in potentially influential factors, such as diet, encephalization, and heterochrony.

The mammalian skull offers many advantages for analysis of modularity. First, it is well studied developmentally, anatomically, and functionally. Second, the fossil record of mammals is sufficiently complete for testing hypotheses of morphological evolution and for incorporating morphologies not represented in extant taxa (Carroll 1988). Third, many previous studies of morphological integration have used the mammalian skull as a model system (Cheverud 1982, 1988, 1989, 1995, 1996a, 1996b; Zelditch and Carmichael 1989a, 1989b; Ackermann and Cheverud 2000, 2004b; Badyaev and Foresman 2000, 2004; Zelditch and Moscarella 2004; Badyaev et al. 2005), providing an es-

sential starting point for assessing modules and their distribution across larger clades.

Methods

Landmarks

In this study, three-dimensional data were gathered with an Immersion Microscribe G2X digitizer, which has a reported accuracy of 0.23 mm and a measured error of 0.03 mm. Within Theria, 57 cranial landmarks (table A1 in the appendix in the online edition of the *American Naturalist*; fig. 1) of definite homology across all therians (e.g., tripartite sutures) were digitized. In most fossil taxa, the zygomatic arch is broken, and thus, the zygomatic landmarks (ZPR in table A1) are excluded from the analyses for six of the nine fossil taxa. The three Pleistocene carnivorans (*Smilodon fatalis*, *Panthera atrox*, and *Canis dirus*) are represented by numerous complete specimens, allowing for inclusion of the zygomatic region in analyses. Cranial vault landmarks (VLT in table A1) were difficult to detect in four carnivoran species (*Melursus*, *Ailuropoda*, *Ailurus*, and *Herpestes*), probably because of early suturing of the vault, and were excluded from analyses. Orbital landmarks (ORB in table A1) were undetectable and excluded in two carnivoran species (*Melursus* and *Taxidea*).

Although landmarks are homologous across all therians, landmarks between therian and monotreme crania are less consistent. This issue is caused by differences in the presence of bones in therians and monotremes and between the two extant monotreme families. Several landmarks used for therians are not found in one or both monotremes. For this reason, in comparison with the 57 landmarks digitized in therians, 39 landmarks were digitized for *Ornithorhynchus* (duck-billed platypus), of which nine do not have homologues in therians (table A1; fig. 2). Only 30 landmarks were consistently identified and digitized for *Tachyglossus* (echidna), of which 15 do not have therian homologues and nine do not have homologues in *Ornithorhynchus* (table A1). In the two monotremes, the nonhomologous landmarks were chosen to represent similar physical locations as landmarks in the therian taxa, in cases where bones are absent in all therians (e.g., septomaxilla, ectopterygoid) or in one or both monotremes (e.g., jugal). Although use of nonhomologous landmarks is a potential source of error, it is unavoidable in comparisons across Mammalia because of the marked differences in cranial morphology between therians and monotremes.

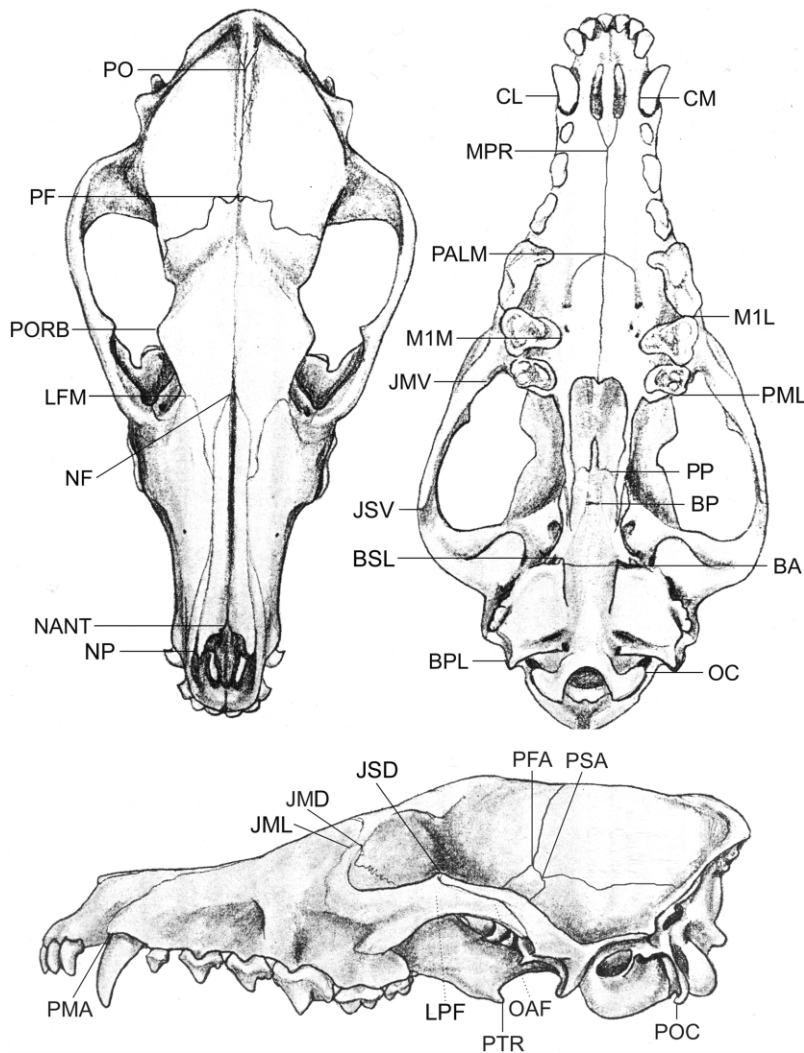


Figure 1: Cranial landmarks for *Vulpes vulpes* (red fox). Symmetrical landmarks are shown on one side only. Labels as in table A1 in the appendix in the online edition of the *American Naturalist*. Modified from Gilbert (1980).

Specimens

Data were gathered from 1,644 specimens, representing 106 species (97 extant and nine fossil; table A2 in the appendix in the online edition of the *American Naturalist*). Monotremes are represented by the two extant families (Tachyglossidae and Ornithorhynchidae), marsupials are represented by three orders (Dasyuromorphia, Diprotodontia, and Peramelia), and placental mammals are represented by two orders (Carnivora and Primates). These taxa were chosen to provide phylogenetic, morphological, ecological, and developmental breadth and to allow comparisons at multiple taxonomic levels, thereby providing the potential for bridging micro- and macroevolutionary scales of analysis. Most species are represented by 15–22

specimens, although a few rare or extinct species are represented by slightly fewer (11–14) specimens (table A2). Male and female specimens are equally or nearly equally represented in all species. Rarefaction analyses showed that landmark correlation matrices for this data set are stable at these sample sizes (Goswami 2006).

Data Analysis

Original three-dimensional data were subjected to a series of operations before analysis. Data were recorded in Microsoft Excel (Redmond, WA) format directly from the digitizer into a laptop computer. Larger specimens were digitized in two orientations and unified in Mathematica

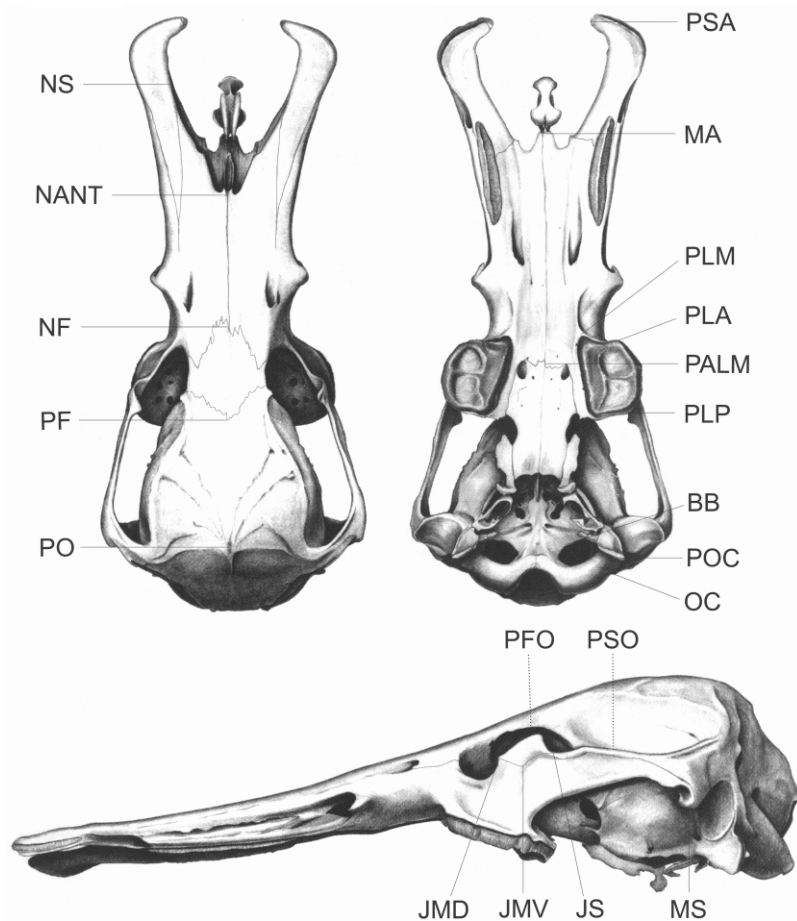


Figure 2: Cranial landmarks for *Ornithorhynchus anatinus* (duck-billed platypus). Symmetrical landmarks are shown on one side only. Labels as in table A1 in the appendix in the online edition of the *American Naturalist*. Modified from Musser and Archer (1998).

5.0 (Wolfram Research, Champaign, IL) using a least squares algorithm. Seven landmarks were used to orient the two positions into a single frame. A new least squares analysis script was written because of the unavailability of an existing program that can treat specimens with missing data. The unification program written for this analysis used seven primary orienting landmarks to compute a least squares fit. If any of these were missing, they were excluded from the calculation. If more than four of the orienting landmarks were missing, unification was aborted. The least squares fit was used to reorient all of the landmarks in the second orientation, and missing data points remained missing. The least squares value (the sum of the squared distances between the seven orienting landmarks between the two positions) was used as a measure of unification error. Specimens with a high unification error (>0.25 mm per landmark, similar to the accuracy of digitization) were removed from the data set.

Following unification of individual specimens, reflection

of symmetrical points was conducted to fill in missing data. Six midline landmarks were used to denote an axis of reflection for 50 symmetrical landmarks (one landmark, anterolingual M1, was captured only on the right side in this study). It should be noted that use of this algorithm masked fluctuating asymmetry, which constitutes a minor, though interesting, component of morphological variation and thus was not considered in this study. As in the unification routine, the fit of the midline landmarks to a plane provided a measure of error. Specimens with high reflection mirror errors (i.e., the midline landmarks deviate more than 0.25 mm per landmark from the defined midline plane, because of either original measurement error or asymmetry in the specimen) were excluded from further analysis. In general, missing data comprised only a small part of the data set, from 2% to 5%. However, these methods are useful for incorporating landmarks from delicate parts of the skull, such as the pterygoids or auditory bullae,

and prove useful for fossil taxa, which typically have many more missing data.

Following reflection, all specimens of a single taxon were oriented to the same position, as any differences in orientation would result in erroneous landmark covariances. A Procrustes algorithm, written in Mathematica 5.0, treats missing data by excluding individual missing data points from computation of the least squares fit. In standard Procrustes algorithms that allow missing data, a missing data point necessitates the removal of entire landmarks or specimens from consideration, which would greatly reduce the final data set. The new Procrustes algorithm rotates and translates each specimen to find an optimal least squares fit among the specimens. Scaling, a common Procrustes procedure, was not applied to specimens, to reduce the effect of inducing covariances through Procrustes fitting. Thus, size is not removed from the data set before analysis.

For analysis, only landmarks from the midline (6–8) and right side of the skull (11–26) were used, as any variation as a result of asymmetry had already been confounded by implementation of the mirror algorithm described above. For each species, landmarks with excessive missing data (more than two missing values) were removed from further analysis. Midline and right-side landmarks were used to calculate vector dot product-moment covariance matrices for individual species in Mathematica 5.0.1, using the following algorithms:

$$C_{ii'} = \frac{1}{N-1} \sum_j (\vec{x}_{ij} - \vec{u}_i) \cdot (\vec{x}_{ij'} - \vec{u}_{i'}),$$

where \vec{x}_{ij} is the vector of coordinates of the three-dimensional landmark i of individual j (after Procrustes superimposition) and \vec{u}_i is the vector of mean coordinates for landmark i . These steps produced a 32×32 (therian), 22×22 (*Ornithorhynchus*), or 18×18 (*Tachyglossus*) landmark covariance-variance matrix for each species, which was then used in cluster analyses to determine patterns of cranial modularity.

Cluster analyses used Ward's method of linkage, in Matlab 6.5 (Mathworks, Natick, MA), to determine whether conserved discrete clusters exist. Ward's method is preferable to other linkage algorithms because it minimizes the variance of a cluster rather than simply minimizing distances within a cluster. Because cluster analysis must produce clusters, linked landmarks may not necessarily have strong covariances among them and thus may not represent biological modules. Therefore, resulting clusters were determined to be modules only if the mean correlation among landmarks within each cluster was significantly greater than 0, as determined by Fisher's z -transformation and Student's t -test. Landmark variance-

covariance matrices were converted to landmark correlation matrices by dividing the covariances of landmarks a and b by the variances of the respective landmarks.

Theoretical Correlation Matrices

To test the relationship of observed patterns of landmark integration to shared functional and developmental relationships among bones, theoretical landmark correlation matrices were constructed, following the methodology used by Marroig et al. (2004). Four landmark integration matrices were used, based on functional groups, tissue origin (neural crest or paraxial mesoderm), mode of ossification (dermal or endochondral), and tissue origin and mode of ossification combined (table A1). For each pair of landmarks, shared functional or developmental relationship was scored as 1, and no relationship was scored as 0. For the combined developmental matrix, each type of developmental relationship was scored as 1 such that the theoretical correlation value for pairs of landmarks could range from 0 to 2. Theoretical correlation matrices were compared to landmark correlation matrices for each therian species and for each order (Carnivora, Primates, Dasyuromorphia, Peramelia, Diprotodontia) with matrix correlation analysis and Mantel's test (Marroig et al. 2004) in Mathematica 5.0. The 11 therian species (six fossil and five Recent) with missing landmarks were excluded from theoretical correlation matrix analyses (table A2).

Results

Therians

Cluster analyses consistently recovered six discrete landmark clusters in most therians (fig. 3). Individual species matrices generally produced the same clusters as their respective orders, with some exceptions. Observed discrete clusters are indicated for each species in table A2. These clusters correspond generally to cranial regions, including the oral, zygomatic, orbital, vault, and basicranial regions (fig. 4). The oral region is further divided into an anterior oral-nasal group and a posterior molar group. Correlations among landmarks within a single module (table A2) are significantly higher than those between modules, with mean correlation within modules of 0.43 and mean correlation between modules of 0.05. However, there are marked differences among modules, in that some modules (anterior oral-nasal, molar, and basicranial) show significantly higher within-module landmark correlations than the other modules (orbit, zygomatic-pteryoid, vault).

The anterior oral-nasal module includes the anterior dentition and facial skeleton (table A1; fig. 3). It forms a discrete cluster in 96 out of 104 therian species and shows

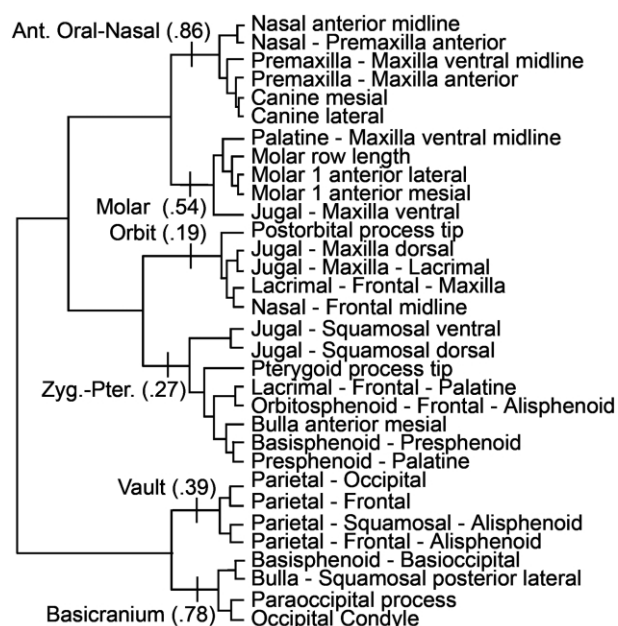


Figure 3: Cluster analysis of cranial traits for a therian (placental), *Canis lupus* (gray wolf), with mean within-group correlations for identified modules.

landmark correlations significantly greater than that between modules in 95 species (table A2; fig. 3). The molar module is composed of molar, palatal, and anterior zygomatic landmarks (table A1; fig. 3). Molar landmarks form a discrete cluster in 79 species and show landmark correlations significantly greater than that between modules in 69 species (table A2). Within Primates in particular, the molar group often does not form a discrete cluster. Instead, in 18 of the 38 primate species sampled here, the molar landmarks are clustered around, but not within, the oral-nasal landmarks and do not show significantly greater correlations than observed between modules.

The orbit module is composed of landmarks within both the facial and the neurocranial skeletons (table A1; fig. 3). Landmark correlations among orbit landmarks are significantly lower than those of the anterior oral-nasal and molar modules (table A2). The orbital group is recovered as a discrete cluster in 71 of the 104 examined therian taxa. In the other 35 taxa, landmarks of this module are often mixed with zygomatic and pterygoid landmarks. The landmark correlations within the orbit module are significantly greater than 0 in only 30 species.

The zygomatic-ptyergoid module also displays low levels of integration across taxa. This group includes the zygomatic arch, the sphenoid bones, the pterygoid region, and the anterior cranial base (table A1; fig. 3). The zygomatic-ptyergoid region is the most weakly integrated of the mod-

ules, occurring as a discrete cluster in 54 of the 104 therian species. Strikingly, the within-module landmark correlations are significantly greater than those between modules in only nine species (table A2).

Within the neurocranial skeleton, two distinct modules are recovered: the cranial vault and the basicranium. The basicranium is primarily composed of endochondral bones of mesodermal origin (table A1; fig. 3). The basicranial module forms as a discrete cluster in 86 of the 104 therian species and shows landmark correlations significantly greater than those between modules in 81 species (table A2).

In contrast to the basicranium, the cranial vault is a weakly integrated module, with landmarks clustering as a distinct group in 45 therian species and landmark correlations significantly greater than those between modules in only 38 taxa. In the 59 therian species in which the cranial vault does not form a discrete cluster, the cranial vault landmarks cluster weakly around, but not within, the basicranial landmarks in 16 species; cluster within the basicranial landmarks in five species; or simply do not cluster with any other landmarks in 38 species. Across Placentalia, primates display significantly lower correlations among cranial vault landmarks than observed in Carnivora (table A2; $P \ll .0001$). There are also differences in cranial vault integration across Marsupialia (table A2; $P = .0001$), with the order Peramelia displaying high mean correlations ($r = 0.62$), while the order Diprotodontia shows low mean correlations ($r = 0.09$).

As seen in figure 3, clustered landmarks are physically close, suggesting that the observed pattern may merely reflect distance. The proximity and correlation between pairs of landmarks are significantly correlated ($r = 0.53$, $P < .01$) within Theria, but this relationship does not explain all aspects of the observed pattern. Cranial modules are conservative in pattern, but individual modules vary in degree of landmark integration, with some modules displaying high landmark correlations (e.g., anterior oral-nasal module) and other modules showing much lower landmark correlations (e.g., zygomatic-ptyergoid module; table A2). This variation is not explained by differences in landmark distance but may be explained by functional or developmental models.

Monotremes

While therian mammals consistently display six distinct cranial modules, monotremes (*Ornithorhynchus* and *Tachyglossus*) display only two discrete clusters of landmarks, the anterior oral-nasal and basicranial groups, that exhibit high landmark correlations (fig. 4). While other landmarks are forced to cluster by cluster analysis (fig. 5), there is no clear regional, functional, or developmental pattern to

the clusters. More strikingly, the maximum correlation among landmarks within the clusters is 0.27, showing that there is not significant correlation between landmarks outside of the anterior oral-nasal and basicranial regions (table A2). Extant monotremes are edentulous, precluding examination of a molar group. *Ornithorhynchus* bears a keratinous pad in the place of teeth, but landmarks from this pad are not significantly correlated, in contrast to the well-integrated therian molar group. The orbital, zygomatic-pterygoid, and vault groups are not recovered in either monotreme.

Theoretical Correlation Matrices

One functional and three developmental models of cranial landmark integration were analyzed and compared. The functional model was significantly correlated with observed landmark correlation matrices for every species (table A2; $P < .01$). The tissue origin model was significantly correlated with landmark correlation matrices for every species (table A2; $P < .01$) except *Cebuella* and *Cerdocyon*. The mode of ossification model was significantly correlated with landmark correlation matrices for every species (table A2; $P \ll .01$) except *Cebuella* and *Cerdocyon*. The combined developmental model was significantly correlated with landmark correlation matrices for every species (table A2; $P < .01$) except *Cebuella* and *Cerdocyon*.

Discussion

There are many hypotheses on the evolutionary significance of modularity, from circumventing the constraints of genetic pleiotropy and developmental canalization to facilitating the transformations of functional groups. Un-

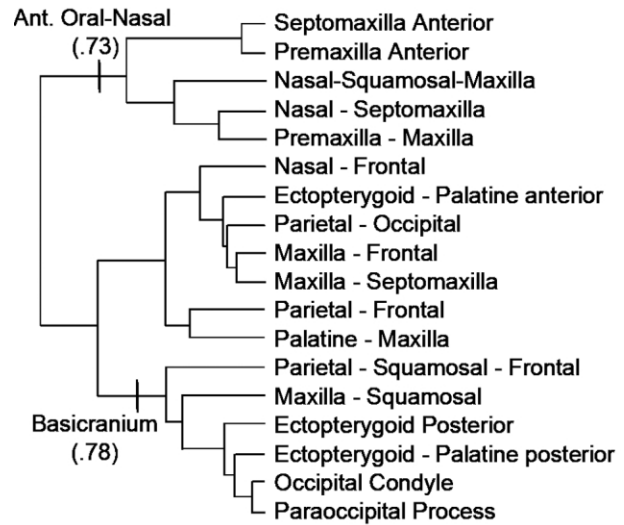


Figure 5: Cluster analyses of cranial traits for a monotreme, *Tachyglossus aculeatus* (short-beaked echidna), with mean within-group correlations for identified modules.

derstanding the significance of modularity to morphological evolution first requires an assessment of how modularity varies across taxa. If modules vary across all taxa, then it may be possible to determine how modularity relates to functional and developmental influences on morphology and to differences in patterns of morphological variation, evolution, and diversity. However, few studies have assessed variation in modularity across taxa, with the broadest studies limited to the suborder Anthroproidea (Mammalia: Primates; Marroig and Cheverud 2001, 2005; Ackermann and Cheverud 2004b). This quantitative study

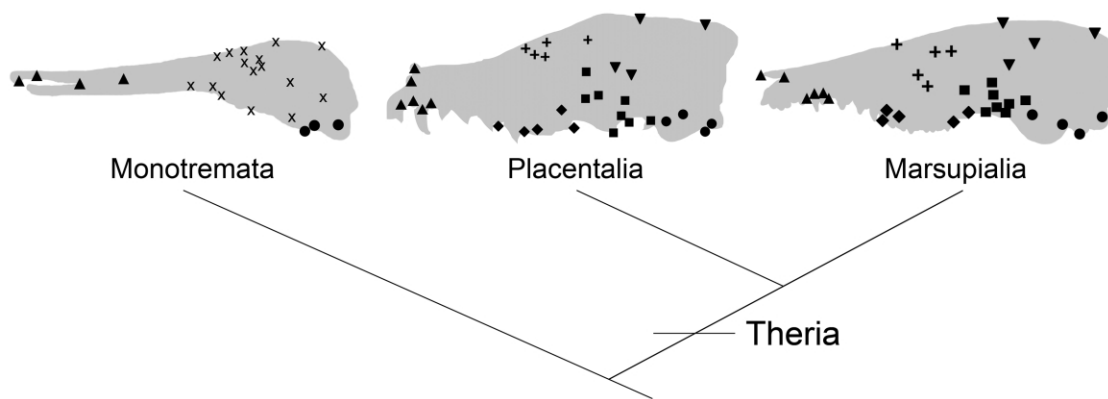


Figure 4: Skull outline of a monotreme, *Ornithorhynchus anatinus* (duck-billed platypus); a placentar, *Vulpes vulpes* (red fox); and a marsupial, *Isodon obesulus* (southern brown bandicoot). Symbols represent landmark associations with modules: triangle, anterior oral-nasal; diamond, molar; plus sign, orbit; square, zygomatic-pterygoid; inverted triangle, vault; circle, basicranium; and cross, none.

of the mammalian cranium is the broadest assessment of phenotypic modularity yet undertaken. This study verifies that the cranium is modular and that modularity is indeed an evolving character. Modularity shifts during mammalian evolution, as demonstrated by the marked differences in cranial modularity between monotremes and therians. These differences between monotremes and therians, as well as differences within these clades and across the skull, can be used to assess the relationships among function, development, modularity, and morphology.

As seen in "Results," all mammals display well-integrated anterior oral-nasal and basicranial regions. Therians also exhibit a well-integrated molar region. In contrast, the orbit, zygomatic-pterygoid, and vault regions are poorly integrated in most therians and are not observed in either monotreme studied here. There are some exceptions to the therian pattern that deserve some additional consideration.

An interesting exception to this pattern is displayed by the extinct saber-toothed felid cat *Smilodon fatalis*, in which the facial skeleton and the enlarged canines form two separate modules rather than a combined anterior oral-nasal group. In addition, correlations among anterior oral-nasal and molar landmarks are lower than in most other therians. Although one might expect this deviation from the normal therian pattern to be a consequence of saber toothery, the ability to measure phenotypic modularity in fossil species allows for examination of species with a convergent canine morphology, such as the false saber-tooth nimravids. Nonfelid carnivorans in this study that display a similar saber-toothed morphology are the Eocene nimravids *Dinictis* and *Hoplophoneus*, and neither shows a similar separation of the anterior oral-nasal module (table A2). Therefore, by using this comparative approach to studying phenotypic modularity, one can reject the hypothesis that saber toothery necessitates the fragmentation of the anterior oral-nasal module and instead identify that the pattern observed in *Smilodon* is more specific, perhaps unique to felid saber-toothed species, or to just *Smilodon*. One could use these observed differences in modularity between felid and nimavid saber-toothed species to further study the consequences of modularity for morphological evolution and diversity.

Another difference among taxa is observed in the cranial vault, and these differences can be used to address hypotheses posed by previous researchers. Cheverud (1996a) and Ackermann and Cheverud (2004b) have suggested that the weak integration of the cranial vault in primates may allow for the great expansion of the brain during primate evolution. The primate cranial vault is significantly less integrated than the carnivoran vault. Therefore, the results of this study are supportive of this hypothesis, as primates are significantly less integrated in the vault and also more

encephalized than carnivorans. Additional, explicit testing of this hypothesis is necessary to establish the relationship between encephalization and cranial vault integration, but this example demonstrates the need for comparative analyses to test hypotheses of the evolutionary significance of integration and modularity.

Another example is provided by the cranial base, which bridges the skull and neck and provides brain support. Because of the role of the cranial base in multiple functions, it has been suggested that the cranial base may be well integrated with other parts of the skull (Lieberman et al. 2000). These analyses do not support that hypothesis because the cranial base, as it is recognized by Lieberman et al. (2000), overlaps two modules, the basicranium and zygomatic-pterygoid, identified in this study. Taken individually, the basicranium is strongly integrated but generally shows little integration with other parts of the skull, including the zygomatic-pterygoid region. Thus, while analyses do not support a functional module comprising the traditional cranial base region, they do support a strongly integrated but more limited module.

Because functional sets often incorporate a number of traits and because morphological integration has been hypothesized to facilitate the transformations of these sets, functionally related traits should be highly correlated and, thus, should comprise a single module. As discussed above, some hypothesized functional modules may be supported by this study. In addition, there may be a number of developmental interactions that can influence the correlations among traits. This study tested four models of functional and developmental integration with analysis of theoretical correlation matrices. Unfortunately, it was not possible to distinguish between functional and developmental hypotheses because all four models were significantly correlated with observed landmark correlation matrices for individual species and orders, with the exceptions of *Cebuella* (Primates) and *Cerdocyon* (Carnivora). This result may simply reflect a coincidental correspondence between the functional and developmental history of cranial structures, or it may suggest a strong relationship between function and development. Despite the ambiguity of these quantitative analyses, it is also possible to qualitatively assess the possible relationships among function, development, and modularity.

Each of the observed modules corresponds easily to a functional group. The anterior oral-nasal and molar groups combined are the primary masticatory apparatus of the skull. The zygomatic-pterygoid group included attachment sites for jaw musculature and experiences the highest strains in the skull during mastication (Ross and Metzger 2004). The orbit houses the visual sensory organs. The cranial vault provides support and protection for the brain, and the basicranium serves as the attachment point

between the skull and axial skeleton, as well as provides support for the braincase. Three of these functional groups are strongly integrated in therians and two in monotremes. In contrast, three functional groups are poorly integrated. This result suggests that there may be some discrepancy between function and modularity, despite the significant correlation between the theoretical functional correlation matrix and the observed landmark correlation matrices.

In the analyses of the developmental models, the tissue origin, mode of ossification, and combination models were all significantly correlated with observed landmark correlation matrices in all but two species examined here, similar to the results for the functional model. However, the developmental models may better fit the observed patterns of modularity, although they lack the direct quantitative data that aid the analyses of cranial functions (strain data; Ross and Metzger 2004). Qualitatively, it is clear that the three strongly integrated modules (anterior oral-nasal, molar, and basicranium) are derived from a single tissue origin, and all constituent bones use a single mode of ossification (neural crest-dermal, neural crest-dermal, and mesoderm-endochondral, respectively; Couly et al. 1993; Kuratani 2005).

In contrast, the orbital region is predominantly composed of dermal, neural-crest-derived bones, but it also includes endochondral and possibly paraxial mesoderm-derived bones (Couly et al. 1993; Kuratani 2005). The zygomatic-pterygoid module encompasses the most developmentally complex region of the skull, including dermal and endochondral bones of both neural crest and paraxial mesoderm origin (Couly et al. 1993; Kuratani 2005). Last, the cranial vault is primarily dermal bone, but the exoccipital bone is endochondral. In addition, the boundary between neural crest and mesoderm is uncertain, but bones derived from both are certainly involved in the vault. Thus, there appears to be a clear difference in developmental complexity between the strongly integrated and the weakly integrated cranial modules. Without quantitative data from developing cranial bones, it is difficult to further test this hypothesis of developmental complexity, but it presents an interesting avenue for future study.

Of the variation in modularity discovered in this study, that between monotremes and therians is the most striking. Without sufficient and undistorted specimens of fossil taxa closely related to this group of extant mammals, it is impossible to determine whether the pattern of modularity observed in extant monotremes represents a primitive or derived condition for mammals (fig. 5). However, these results clearly demonstrate that significant changes in cranial modularity have occurred during mammalian evolution, regardless of which (if either) pattern is primitive for Mammalia. Although there are several landmarks that

are not homologous across therians and monotremes, therian mammals, with six distinct and consistent modules, exhibit greater cranial modularity than monotremes, which display only two distinct modules and low correlations among all other cranial landmarks.

Modularity and integration have often been linked to morphological variation and evolution, and the identification of significant variation in modularity across Mammalia provides avenues for further exploration of its evolutionary significance. This survey of phenotypic modularity across mammals demonstrates that modularity does evolve and vary across large clades and tests developmental and functional models of cranial integration. Moreover, these analyses of the developmental and functional factors that may influence patterns of phenotypic modularity are also applicable to other major vertebrate clades, which differ from mammals in developmental patterns (Jeffery et al. 2002), functional morphology, and cranial mechanics (Ross and Metzger 2004). Further analysis of the relationship of modularity to morphological evolution, in combination with comparative studies of functional and developmental influences on modularity, may ultimately elucidate the processes underlying evolutionary patterns of morphological diversity.

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