RESEARCH ARTICLE

Grist for Riedl's Mill: A Network Model Perspective on the Integration and Modularity of the Human Skull

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ABSTRACT

Riedl's concept of burden neatly links development and evolution by ascertaining that structures that show a high degree of developmental co-dependencies with other structures are more constrained in evolution. The human skull can be precisely modeled as an articulated complex system of bones connected by sutures, forming a network of structural co-dependencies. We present a quantitative analysis of the morphological integration, modularity, and hierarchical organization of this human skull network model. Our overall results show that the human skull is a small-world network, with two well-delimited connectivity modules: one facial organized around the ethmoid bone, and one cranial organized around the sphenoid bone. Geometric morphometrics further support this two-module division, stressing the direct relationship between the developmental information enclosed in connectivity patterns and skull shape. Whereas the facial module shows a hierarchy of clustered blocks of bones, the bones of the cranial modules show a regular pattern of connections. We analyze the significance of these arrangements by hypothesizing specific structural roles for the most important bones involved in the formation of both modules, in the context of Riedl's burden. We conclude that it is the morphological integration of each group of bones that defines the semi-hierarchical organization of the human skull, reflecting fundamental differences in the ontogenetic patterns of growth and the structural constraints that generate each module. Our study also demonstrates the adequacy of network analysis as an innovative tool to understand the morphological complexity of anatomical systems. *J. Exp. Zool. (Mol. Dev. Evol.) 9999B: XX–XX, 2013.* © 2013 Wiley Periodicals, Inc.

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The morphological integration and modularity of the adult human skull is the result of a mosaic evolution of embryonary parts with diverse developmental mechanisms (Cheverud, '82; Bastir and Rosas, 2005; Bastir et al., 2008; Klingenberg, 2008; Bastir and Rosas, 2009; Lieberman, 2011; Martínez-Abadías et al., 2012). Studies of the morphological integration and modularity of the human skull start by establishing a developmental or functional hypothesis; this is then tested by means of patterns of covariation and correlation using different morphometric tools (Chernoff and Magwene, '99; Bastir, 2008; Mitteroecker and Bookstein, 2008). Even though this approach has proven very successful, it uses morphological information only as datasets to test a priori biological hypotheses. In contrast, few efforts have been devoted to articulate theoretical and mechanistic models to quantify integration and describe modules at a morphological level without functional or developmental assumptions (but see Rasskin-Gutman and Buscalioni, 2001; Eble, 2005; Rasskin-Gutman, 2005). Such an approach can be carried out in the skull using network models of bone connectivity patterns (Rasskin-Gutman, 2003; Rasskin-Gutman, 2005; Esteve-Altava et al., 2011, 2013). Within this framework, modules are recognized based exclusively on morphological organization without a priori assumptions. However, the number and pattern of connections for each bone can be seen also as developmental and functional dependencies, providing a quantitative estimate of Riedl's burden rank (Riedl, '78; Schoch, 2010; Esteve-Altava et al., 2013) and allowing, in turn, an a posteriori direct measure of integration and modularity.

We build these network models formalizing each bone and suture of the skull as nodes and links in an adjacency matrix. This type of analysis provides a new modeling framework to understand evolutionary patterns, developmental constraints, and morphospace occupation (Rasskin-Gutman, 2005; Dera et al., 2008; Esteve-Altava et al., 2011). Following this approach, we have previously studied Williston's Law in a broad sample of tetrapod skulls, including all major phylogenetic groups (Esteve-Altava et al., 2013). Our results suggested that the loss of poorly connected bones constitutes a mechanism that underlies a general trend toward an increase in morphological complexity and variation in the degree of integration. In addition, the human skull network showed the highest degree of morphological complexity in terms of structural organization, integration, and biomechanical or functional efficiency. This prompted us to further investigate the network structure of the human skull as a null model to provide new insights on its integration and modularity in an evo–devo context.

Here we show that the human skull is a small-world network with two differently organized connectivity modules, *cranial* and *facial*. The facial module has a hierarchical sub-modular structure in blocks, which we have named *frontonasal*, *left maxillary*, *right maxillary*, and *ethmoidal* blocks. The cranial module lacks this kind of internal organization; rather, its structure resembles that of a regular network. The significance of these results is discussed together with the morphogenetic processes involved in skull development and evolution within a general trend of bone loss and fusion in the evolution of tetrapod skulls (Esteve-Altava et al., 2013). In the following sections, we extend the conceptual framework introduced in Esteve-Altava et al. (2011, 2013) to analyze morphological networks, providing the necessary background to put our results in context.

Integration and Biological Burden

Morphological integration is generally defined as the covariation among morphological structures due to common developmental and functional causes (Olson and Miller, '58). Given the role of craniofacial sutures in bone growth (Opperman, 2000; Rice, 2008), intracranial movements of bones (Jaslow, '90), and strain sinks (Rafferty et al., 2003; Moazen et al., 2009), it is reasonable to expect that bones with more suture connections have central structural and functional roles affecting the morphology of the entire skull; in other words, the higher the number of connections, the stronger the functional and developmental dependencies (structural constraints). This association between the number of connections and the intensity of constraints, due to acquired developmental and evolutionary compromises, immediately resonates with "biological burden" (Riedl, '78; Wimsatt, 2007; Schoch, 2010). The concept of burden neatly links development and evolution (Wagner and Laubichler, 2004) and underlies the evolutionary pattern of skull bone reduction in Williston's Law (Esteve-Altava et al., 2013).

"Small-Worldness" in Morphological Networks

Network structures can be assessed in different ways. While the number of connections for each bone defines its burden rank (Esteve-Altava et al., 2013), there are other network parameters that quantify morphological integration for the entire skull, such as the clustering coefficient and characteristic path length (Esteve-Altava et al., 2011). These parameters capture information about the degree of integration of the entire skull, the former by quantifying short-range feedback loops, and the latter by

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quantifying effective proximity. Together, by comparing them with random networks, they can be used to detect the presence of a special kind of network configuration that is known as small-world (Watts and Strogatz, '98). Small-world networks are more clustered than random ones (sometimes even more than regular networks), and yet the effective proximity between elements is as small as it is in random networks. One consequence of this order in small-world networks is the emergence of modularity because of the heterogeneous pattern of connections (Pereira-Leal et al., 2006; Gallos et al., 2012). Correcting for network size, small networks (as in a skull) can also be tested for "small-worldness" (see Methods Section). In addition, this type of organization in a skull would indicate that bones connect to each other following a certain order, one that lies between regularity and randomness. Riedl ('78) already recognized that morphological systems have this dual organization and defined this as "a region of unspecified probability, a no-man's-land between accident and necessity."

Skull Modularity

Morphological integration and modularity are strongly linked concepts; modularity emerges as a consequence of the presence of heterogeneous patterns of integration. Indeed, we are able to perceive parts in a system only because these parts are integrated differently within the system (Klingenberg, 2008)—that is why regular systems lack sub-divisions. To identify the parts of the system (modules) and the strengths of their interaction (integration) we need a precise and operative definition of module and modularity as it relates to integration (for general reviews of the modularity concept see Schlosser and Wagner, 2004; Callebaut and Rasskin-Gutman, 2005). In the context of network analysis, quantifying connectivity patterns readily accomplishes this. In a skull network, a connectivity module is a highly connected group of bones (Rasskin-Gutman, 2005; Esteve-Altava et al., 2011) allowing a precise detection of modules by using general network analysis tools. It is important to note that datasets to infer connectivity modules are totally different from the ones used to infer other morphological modules, such as variational ones (e.g., Mitteroecker and Bookstein, 2007; Wagner et al., 2007; Klingenberg, 2010). In connectivity modules raw data is taken from connections between morphological units, whereas in variational modules it is taken from the shapes of these units.

Skull Bone Hierarchy

Various studies reported different shape and growth rates for different skull regions, suggesting that the human skull is organized hierarchically (reviewed in Bastir, 2008). In networks, there is a hierarchical organization when nodes within modules tend to group in highly clustered sub-modules or blocks (Ravasz et al., 2002). In many biological networks, this type of analysis suggested that some network elements specialize in different roles related to the maintenance of the network architecture and function (Guimerà and Nunes-Amaral, 2005). For instance, in metabolic networks, nodes with few connections tend to cluster into blocks, while highly connected nodes integrate those blocks into modules (Jeong et al., 2000); this is the case also in brain networks (Meunier et al., 2010). Finding a hierarchical organization in the network model would suggest that along with shape and growth, connectivity patterns are also involved in the hierarchy of the human skull.

Skull Bone Connectivity Role

In modular structures that exhibit a hierarchical organization, each component has a connectivity role, based on which level it occupies in the hierarchy (Guimerà and Nunes-Amaral, 2005). Network analysis tools allow a quantitative definition of these roles (see Methods Section). The relationship of individual bone connectivity pattern within and between modules gives each bone a specific structural role. Bones that are above in the hierarchy are those that contribute greatly to integration between blocks or modules. Some bones are keystones that hold together all the bones in a module by having a high number of connections within the module (*local hubs*); some bones are also highly connected but their connections are shared between modules (*connector hubs*); and some are scarcely connected within or between modules (*local and connector non-hubs*). As we will show, each role has different theoretical relevance for integration and modularity in the development and evolution of the human skull.

MATERIALS AND METHODS

The Skull Network Model

We built a 3D network model of the human skull based on bone articulation as described in the literature (Gray, '18). Accordingly, the human skull has been modeled as an undirected/unweighted network composed of 21 nodes (bones) and 64 links (sutures). Since the bones and the connecting sutures are of very different sizes in the human skull, it can be argued that weighted connections, for example, by estimating the contact area between bones, will produce a more realistic model. However, results using a weighted network model were very similar (not shown); consequently, we kept the model to its simplest, unweighted form. We used MATLAB to implement all methods unless otherwise noted.

Analysis of the Small-World Effect

We identified the presence of a small-world organization in the human skull by comparing its clustering coefficient, C, and its characteristic path length, L, to those of random equivalent networks (Watts and Strogatz, '98). We quantified C as the average of the sum of connections between all neighbors of each bone with respect to the maximum possible (1),

$$C = \frac{1}{N}\sum \frac{\sum \tau_i}{\sum k_i(k_i - 1)} \quad (1)$$

where τ_i is the number of connections between the neighbors of bone i and k_i the number of connections. Parameter L was quantified as the average of the minimum distance between all bones in the network (2),

$$L = \frac{1}{n(n-1)} \sum_{ij} d_{ij} \quad (2)$$

where d_{ij} is the distance in number of links to connect bones i and j, and n the number of bones.

Then, we compared the values of the human skull network with those of 10,000 random equivalent networks, simulated with the same number of bones and connectivity distribution as the human skull, but with all connections randomly shuffled. A network is said to be a small-world network if $L \geq L_{\text{rand}}$ and $C \gg C_{\text{rand}}$ (Watts and Strogatz, '98). We assessed the presence of a small-world organization quantifying an index of "small-worldness" S^{ws} (3) proposed by Humphries and Gurney (2008),

$$S^{\text{ws}} = \frac{C/C_{\text{rand}}}{L/L_{\text{rand}}} \quad (3)$$

A network is small-world if $S^{\text{ws}} > 1$. However, a common problem in the analysis of the small-world effect in networks with few nodes (i.e., $n < 100$) is that the value of C cannot be sufficiently higher than for random networks; hence, the detection of a small-world is unreliable. To circumvent this problem, the parameter test can be corrected so that for any number of bones, a network is small-world if $S^{\text{ws}} \geq 0.012n^{1.11}$ (Humphries and Gurney, 2008).

Analysis of the Hierarchical Organization of the Skull

The hierarchical organization of the human skull network was evaluated by goodness of fit tests of the connectivity distribution, P(k), and the clustering coefficient distribution, C(k), to four different theoretical distributions: Poisson, uniform, exponential, and power-law. The human skull is hierarchical if both P(k) and C (k) fit a power-law distribution function (Ravasz and Barabási, 2003; Wuchty et al., 2006).

Analysis of Modularity

We identified connectivity modules using a hierarchical cluster analysis, in which our measure of similarity was the topological overlap, TO (Ravasz et al., 2002; Solé et al., 2006; Yip and Horvath, 2007). Topological overlap is a normalized measure of similarity that quantifies pair-wise common neighbors between bones (4),

$$\text{TO}(v_i, v_j) = \text{TO}(v_j, v_i) = \frac{J(v_i, v_j)}{\min(k_i, k_j)} \quad (4)$$

where $J(v_i,v_j)$ is the number of connections to the same other bones (i.e., neighbors in common) and $\min(k_i,k_j)$ the lowest connectivity of both bones. Two bones that share all their connections with the same other bones have a TO of 1, whereas two bones without any neighbor in common have a TO of 0. It is expected that bones that belong to the same module share connections to the same bones, which actually are also in the same module. Notice that after each match the topological overlap matrix changes due to new calculations, that is, grouped bones will act as new elements in establishing similarities of neighbors. The hierarchical cluster analysis groups together bones with higher TO in single branches until all bones form one single group. This process of grouping generates many possible nested modules. We evaluated all of them with the modularity Q-value index, Q, which measures the extent to which a network is organized into clearly delimited modules (Newman and Girvan, 2004). We measured Q for each cut-off bifurcation in the dendrogram; the highest value indicated the best module partition. We chose not to apply additional restrictions to this grouping method (e.g., linkage preference for symmetric bones) because we wanted to identify modularity without imposed biological constraints. Symmetry is a key property of the skull morphology, but introducing it into the grouping method would prevent the possibility of it emerging directly from the raw connectivity patterns.

Analysis of Bone-Role in Skull Modules

Two complementary indexes characterized the role of each bone within a given modular structure: the within-module connectivity coefficient, Z, and the participation coefficient to other bones outside its module, P (Guimerà and Nunes-Amaral, 2005). Parameter Z measures the normalized number of connections of one bone to others in its module (5),

$$Z_i = \frac{k_i - \overline{k}_{si}}{\sigma_{k_{si}}} \quad (5)$$

where k_i is the total number of connections of bone i and k_{si} is the number of connections within-module s. Parameter P measures the degree of uniformity of the distribution of connections of one bone in relation to others in different modules (6),

$$P_i = 1 - \sum_{s=1}^{N_{\text{M}}} \left(\frac{k_{is}}{k_i} \right)^2 \quad (6)$$

where $P = 0$ if a bone has all its connections within its module and $P = 1$ if the distribution of all bone connections is uniform to all other existing modules.

The Z and P indexes are better indicators of the biological role of each element in a network than the number of connections (Guimerà and Nunes-Amaral, 2005; Horvath et al., 2006). Thus, we classified skull bones within this ZP space in four categories: (1) local hub, when Z is high and P is low; (2) connector hub, high Z and P; (3) connector non-hubs, low Z and high P; and (4) local non-hubs, low Z and P. Thus, a bone is a hub if it has a value of Z

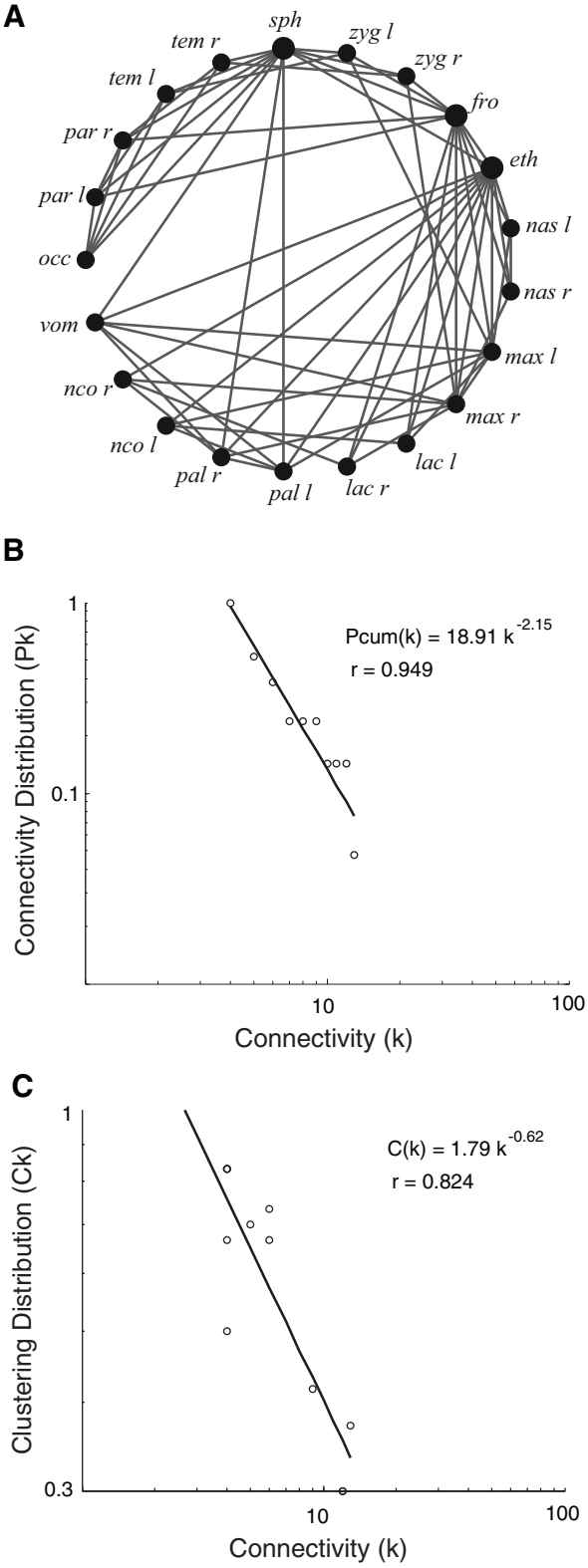

Figure 1. Continued.

higher than 1, with a number of connections within its module higher than the mean plus one standard deviation; and a bone is a connector if it has a $P \geq 0.4$, which means that its connections are almost equally spread to all modules.

Morphometric Analysis of Network Modules

We tested the correspondence between connectivity and variational modules, using a geometric morphometric analysis. We used growth allometries to characterize different developmental units; with this method, a module is taken as a developmental unit if it shows a specific allometric growth pattern with the expectation that the best modularity hypothesis is the one that, summing the variance of both modules, explains most of the overall skull variance (Rosas and Bastir, 2004). We used a total of 51 landmarks and semi-landmarks digitized on lateral radiographs from a full ontogenetic sample (n = 225) of 28 individuals of the Denver Growth Study (see Bastir et al., 2006, for a detailed description of the sample, technical information, and landmarks location). These landmarks capture information from external and internal structures of the human skull projected on the sagittal plane. Although a full 3D dataset could bring more information, major portions in human craniofacial growth occur antero-vertically (i.e., sagittally) in the skull (Enlow, '90; Enlow and Hans, '96); this has been demonstrated in both 2D and 3D growth studies (Bastir and Rosas, 2004; Bastir et al., 2006, 2007). Hence, given the nature of our sample, we are capturing most of the relevant variation needed to test the modularity hypothesis.

We performed multivariate regressions of shape on size. We tested for overall skull centroid size and partition-specific centroid size. Each shape consisted of combinations of landmarks that represented different modules according to different modularity models: one based on network modules (Model A) and three alternative ones (Models B, C, and D) to further test the results of the connectivity hypothesis. We based the composition of these four models on the results of the connectivity analysis, which yielded two distinct modules, facial and cranial. Since two bones—the frontal and the zygomatics—were shown to act as connectors

Figure 1. Connectivity pattern of the human skull network model represented as a circular graph (**A**). The cumulative connectivity distribution shows that the frequency of bones decays with the number of connections as a power-law (**B**). The clustering coefficient distribution also follows a power-law function, showing an inverse relationship between the number of connections and the clustering coefficient (**C**). The fit of both distributions to a power-law function indicates a hierarchical organization of connections in the human skull network (see Methods Section).

between both modules, we further tested alternative modularity hypotheses to explore the validity of the connectivity hypothesis. Thus, Model A represents the result of the network analysis; model B considers the zygomatics as part of the face; in model C the frontal is in the cranial module; and in Model D the zygomatics are in the facial and the frontal in the cranial module. We performed these multivariate regression analyses in MorphoJ (Klingenberg, 2011).

RESULTS

Network Parameters

We modeled the human skull as a network (Fig. 1A) and analyzed its structure. Table 1 summarizes the values of all calculated parameters for the human skull network and each single bone. The clustering coefficient is 0.634 and the characteristic path length is 1.741. These values exceed by more than two times the SD of those observed in the random equivalent networks simulated (C_{rand} = 0.444, SD = 0.036; L_{rand} = 1.678, SD = 0.019). Accordingly, parameter S^{ws} is 1.3762, which is also higher than that expected for a random network of the same size as the human skull (S^{ws} = 0.35). This indicates with confidence that the human skull network is small-world. In addition, both P(k) and C(k) distributions fit a power-law function (Fig. 1B and C), which indicates a hierarchical organization of connections. The ethmoid, the frontal, and the sphenoid bones show the highest burden-rank estimated by their significant above-average number of connections (13, 12, and 12).

Modularity and Bone-Role

The analysis of modularity yields two modules (Fig. 2). The first module (*facial*) groups together the frontal, ethmoid, inferior nasal conchas, vomer, maxillas, lacrimals, nasals, and palatines. The second module (*cranial*) groups together the sphenoid, occipital, parietals, temporals, and zygomatics. The hierarchy test shown in the previous section indicates that the human skull has a hierarchical structure. However, looking at each module separately, we observe that only the facial module shows a clear hierarchical structure further sub-divided into four blocks. In contrast, the cranial module shows no hierarchical structure, as a consequence of a more regular pattern of connections. We name each block in the facial module after the most connected bone present in it. Accordingly, the four blocks are: (1) frontal, composed of frontal and nasal bones; (2) left and (3) right maxillary, composed of the respective left and right maxilla, lacrimal, and nasal concha bones; and (4) ethmoidal, composed of ethmoid, vomer, and palatine bones. The length of the dendrogram branches for each block indicates that they are highly consistent (see Jain and Dubes, '88).

The values of parameters Z and P within this modular organization classify each bone into one specific structural role (Fig. 3): the ethmoid has its connections within the facial module (*local hub*); the sphenoid spreads its many connections between the facial and cranial modules (*connector hub*); the frontal and zygomatics are more involved in connecting the facial and cranial modules than in participating in their internal integration (*connector non-hubs*); and the vomer, the occipital, maxillas,

Table 1. Human skull whole network and single bone parameter values.

	Node connectivity	Clustering coefficient	Shortest path length	Participation index	Z score
Human skull	–	0.63	1.74	–	–
Ethmoid[a,b]	13	0.37	1.35	0.14	2.6
Frontal[b,c]	12	0.30	1.40	0.49	0.54
Inf. nasal concha[c]	4	0.83	2.1	0	−0.70
Lacrimal[a]	4	0.83	1.95	0	−0.70
Maxilla[c]	9	0.42	1.65	0.20	0.95
Nasal[c]	4	0.83	1.95	0	−0.70
Occipital[a,b]	5	0.7	2.15	0	0.62
Palatine[c]	6	0.67	1.7	0.28	−0.28
Parietal[a]	5	0.7	1.85	0.32	0
Sphenoid[a,b]	12	0.30	1.4	0.49	1.87
Temporal[c]	4	0.67	2.15	0	0
Vomer[a,b]	6	0.73	1.7	0.28	−0.28
Zygomatic[a]	4	0.5	1.85	0.5	−1.25

[a]Cranial connectivity module.
[b]Unpaired bone.
[c]Facial connectivity module.

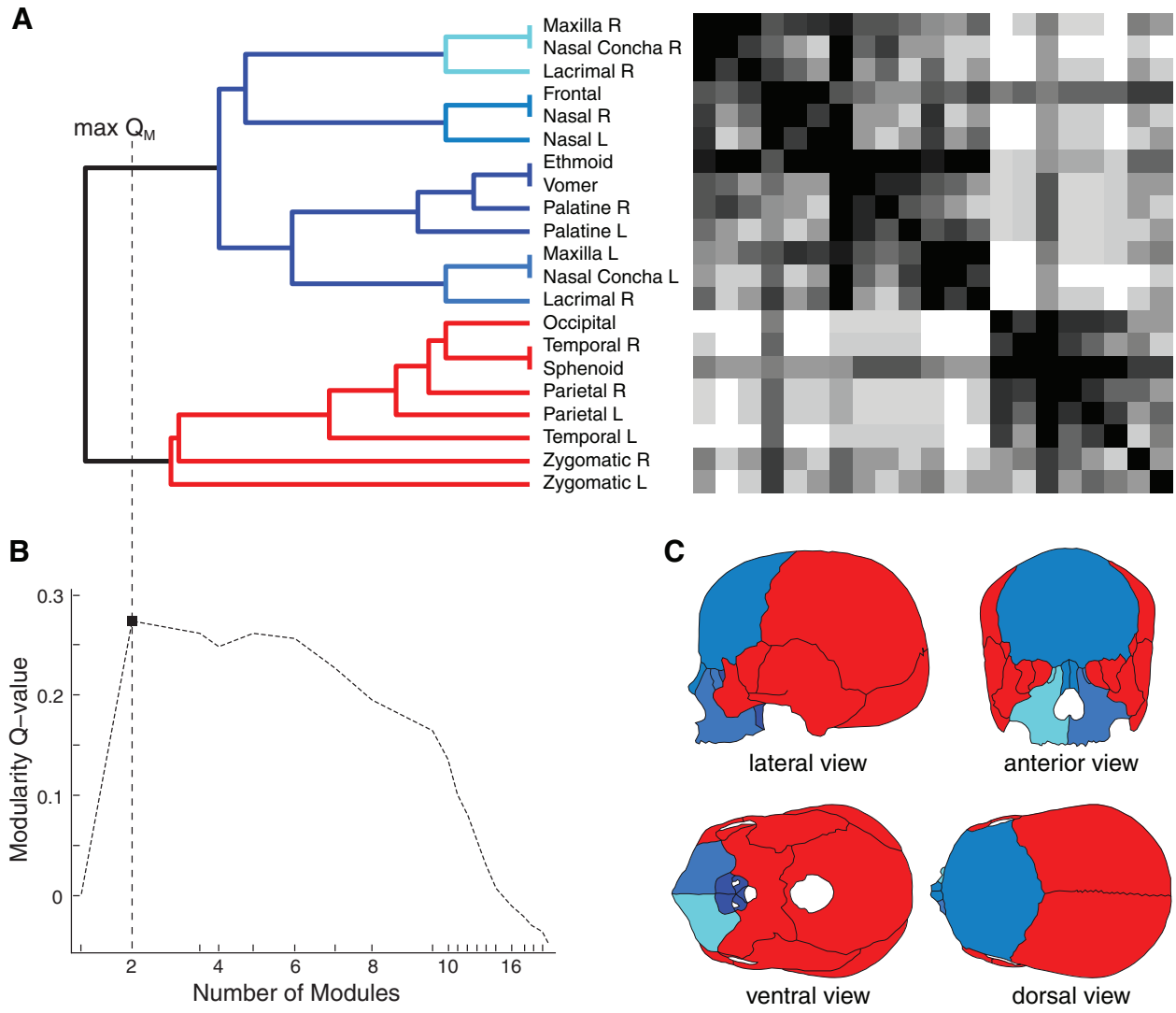

Figure 2. Analysis of modularity in the human skull network. According to the topological overlap matrix of similarity (**A** right), the cluster analysis shows two modules (**A** left) based on the highest *Q*-value (**B**). The dendrogram also shows that only the facial module has a hierarchical internal sub-organization in four blocks of spatially related bones. Each module and block is shown in different views: the cranial module is colored in red and the four blocks that compose the facial module are colored in different shades of blue (**C**).

temporals, parietals, lacrimals, nasals, nasal conchas, and palatines just contribute their few connections to their own module (*local non-hubs*).

Network Modules and Morphometrics

All regressions were highly statistically significant at $P < 0.001$ assessed from 1,000 randomizations. The amount of explained variance varied slightly according to whether we used the same overall skull size for both partitions or each partition-specific centroid size. Figure 4 shows the sum of total variance explained by both modules (cranial in red and facial in blue) for four alternative models. Model A explains most of the total variance, and performs slightly better than Model B; both of them perform better than Model C and Model D, thus supporting the result of the modularity hypothesis based on the network analysis.

DISCUSSION

We have shown that the pattern of connections between bones in the human skull is neither regular nor random. Instead, it follows a small-world organization that promotes the formation of highly integrated connectivity modules: (1) an anterior *facial* module, related to face and palate; and (2) a posterior *cranial* module,

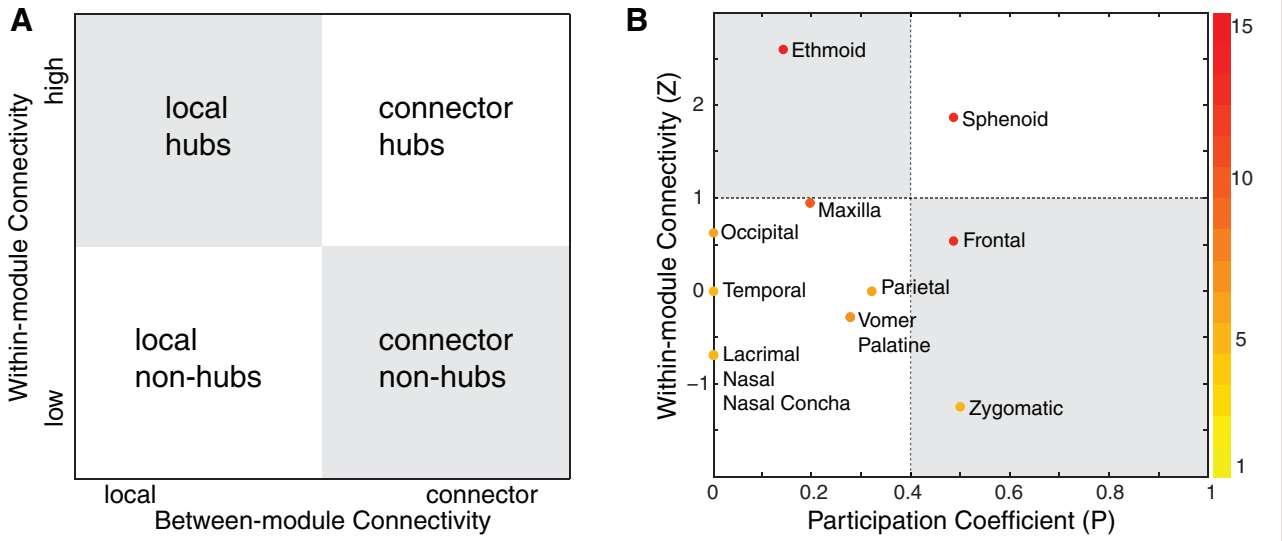

Figure 3. The role of each bone in the modular organization of the human skull is given by its position in the *ZP* space. According to the number of connections within and between modules, four categories of bones can be defined: local hubs, connector hubs, local non-hubs, and connector non-hubs (**A**). For the human skull the *P*-value that discriminates between local and connector role is 0.4, while the *Z*-value that discriminates between hubs and non-hubs is 1 (**B**). Notice that burden-rank alone, indicated by the colored bar, cannot discriminate between hubs (ethmoid and sphenoid) and non-hubs (all other bones) as classified in the *ZP* space.

related to the cranial vault and base. The internal structure of each module is different: the facial module shows a hierarchical pattern sub-divided in blocks (*frontonasal*, *left maxillary*, *right maxillary*, and *ethmoidal*), whereas the cranial module exhibits a non-hierarchical, regular structure. Within these modules each bone has a distinctive connectivity pattern that allowed us to identify their structural role within the skull. In particular, three bones turn out to have key roles: the ethmoid, the sphenoid, and the frontal. The ethmoid bridges the blocks of the facial module; the sphenoid gives more cohesion to the regular structure of the cranial module and, along with the frontal, connects both modules together (a task shared with the zygomatics). It is worth noting that the connectivity modules resemble the classical, intuitive division of the human skull in an anterior face and a posterior cranial vault. However, our results support the adscription of the frontal and ethmoid bones to the facial module (Hofer, '65; Bastir et al., 2006). Furthermore, the ethmoidal block resembles the nasal capsule, an embryological, morphological, and evolutionary unit with a distinctive pattern of integration within the face (Bastir and Rosas, 2011). As noted, the zygomatics also play a key structural role in the skull by connecting both modules together, although their connectivity pattern make them part of the cranial module. However, this integrative role influences both cranial vault and facial growth and shape (e.g., orbits and zygomatic arch; reviewed in Lieberman, 2011), functionally redistributing tensile and compressive forces between skull regions (Witzel et al., 2004). Along with the other connector bones (frontal and sphenoid), the zygomatics provide inter-module integration, which could partly explain why it is so difficult to identify variational modules in the human skull (Martínez-Abadías et al., 2012).

Growth Correlates of Connectivity Modules

Traditionally, the human skull is divided in three modules—an anterior face, and posterior neurocranium and basicranium—a division which has long been accepted based on genetic, developmental, and phenotypic shape variation criteria (reviewed in Bastir, 2008). However, recent work by Martínez-Abadías et al. (2012) has challenged this general modularity hypothesis showing that morphometric modules cannot be clearly delimited (i.e., they show a stronger co-variation within modules than between them), highlighting the weakness of these criteria to delimit "true modules" in the human skull. In other words, any a priori assumptions depend on genetically and environmentally determined factors that overlap in such an intricate way as to make it impossible to discern modules with certainty. These difficulties have been extensively reviewed under the concept of the palimpsest model of covariation structure, which precisely argues that covariation factors influence each other over time, making the reverse analysis of trying to decipher those factors from phenotypic data a daunting task (Hallgrímsson et al., 2009).

Our approach tackles the problem from a completely different perspective, using the information encapsulated in connectivity

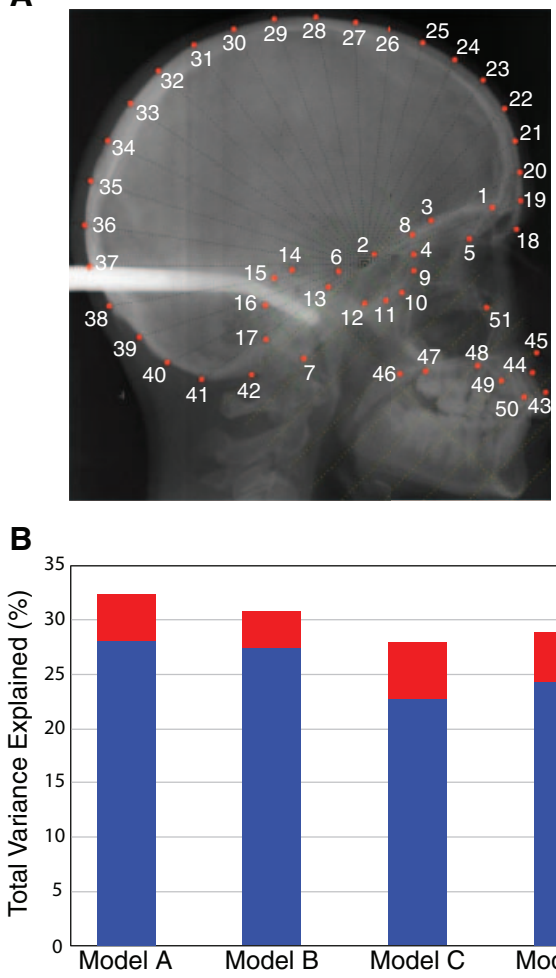

Figure 4. Maximum allometric growth variation test of connectivity modules using multivariate regression of shape on size. Landmarks used in the analysis (**A**); mandibular landmarks have been excluded (landmarks 43, 44, 45, 46, 47, 48, 49, 50, and 51 in the figure correspond to landmarks 58, 59, 60, 61, 62, 63, 64, 65, and 66 in Bastir et al., 2006). Results indicating the sum of total variance explained for the four alternative models of two partitions (blue, facial module; red, cranial module) for partition-specific centroid-size adjustment (**B**). Model A represents modularity as defined by the network analysis. Facial module: frontal, ethmoid, inferior nasal conchas, vomer, maxillas, lacrimals, nasals, and palatines; cranial module: sphenoid, occipital, parietals, temporals, and zygomatics. In Model B, the zygomatics have been shifted to the facial module. In Model C, the frontal has been shifted to the cranial module. In Model D, the zygomatics have been shifted to the facial module whereas the frontal has been shifted to the cranial module. Note that Model A explains the maximum amount of total variance; hence it supports the network modularity hypothesis.

patterns from which the modules are obtained. This allowed us to make a morphological a priori hypothesis of modularity and then to test it with morphometric tools based on independent landmark data. This test is based on maximum allometric growth variation; thus, the results suggest that there are growth patterns at play that determine connectivity patterns in the skull. Furthermore, the different internal structure of each connectivity module—hierarchical for the facial and regular for the cranial—also points in the same direction. As a consequence, our connectivity modules resemble, to a great extent but not completely, the ethmomaxillary and neurobasicranial complexes proposed as developmental units with different maturation timing (Enlow, '90; Enlow and Hans, '96; Bastir et al., 2006). Why this should be the case is neither trivial nor expected, since there is no need for one-to-one correlation between modular network organization and modular allometric variation (Eble, 2005; Hallgrímsson et al., 2009). We think this correlation occurs in the human skull because the allometric mechanisms of growth determine connectivity patterns, which, in turn, influence the individual shape of each skull bone. If this is true, skull networks could be interpreted as shape correlation maps (for a related approach, see Chernoff and Magwene, '99, p. 334–337; Magwene, 2001; Magwene, 2008).

Bones within the same connectivity module share the same allometric growth pattern. Therefore, the best modularity hypothesis has to be the one that explains most of the total variance of the skull shape during ontogeny. We used the morphometric analysis to compare Model A (based on our connectivity hypothesis of modularity) to three alternative models, which were constructed by shifting connector bones (zygomatics and frontal) to a different module. Results indicated that Models A and B explain better the allometric patterns than Models C and D. Furthermore, Model A explains better the total variance than Model B, in which zygomatic bones are part of the face. This result supports the placement of the frontal bone as a facial element and of the zygomatic bones as cranial elements, as the analysis of connectivity patterns revealed.

Bone's Burden-Rank

The integration of modules and blocks in the human skull relies on three main bones: the ethmoid, the sphenoid, and the frontal; by themselves, they account for more than half of all connections in the skull network. The three bones have developmental and evolutionary origins that fit nicely within the concept of evolutionary burden: their formation during development is the result of many fusions of different ossification centers (Opperman, 2000; Rice and Rice, 2008); and the evolution of each can be traced back at least to the origin of early mammals as the fusion of several distinct bones (Sidor, 2001; Depew et al., 2008). Both the sphenoid and the ethmoid bones are the evolutionary result of the fusion of an original unpaired bone with several neighboring paired (e.g., pterygoids, orbitosphenoids, and cribriform plates) and unpaired (e.g., basisphenoid, parasphenoid,

and presphenoid) bones (Goodrich, '58; Romer and Parsons, '77). The link between development and evolution is paradigmatic for the frontal bone. The frontal bone develops as two paired bones early in the ontogeny of the human skull; these paired frontals will fuse totally during the first years of life, giving rise to the unpaired condition of the adult frontal (Weinzweig et al., 2003). Evolutionarily, frontal paired bones are a primitive condition in primates; the closure of the metopic suture (interfrontal) occurred several times independently within this group and before the origin of anthropoids (Rosenberger and Pagano, 2008). The morphogenetic process underlying this pattern relates to different timing in the closure of skull bone sutures at an evolutionary scale (Morriss-Kay, 2001; Richtsmeier et al., 2006; Esteve-Altava et al., 2013), which can sometimes cause severe pathologies in the human skull, known as craniosynostosis (Heuzé et al., 2010; Percival and Richtsmeier, 2011).

As a consequence of multiple fusions, these evolutionarily new unpaired bones have a higher number of connections, increasing their functional and developmental dependencies with other bones. A high number of dependencies (i.e., connections) and being above in the hierarchy of the structure are two of the characteristics that identify anatomical elements with high burden-rank (Riedl, '78; Schoch, 2010). Given the multiple tasks of sutures—sites of skull growth, intracranial movements, and strain sinks (Jaslow, '90; Opperman, 2000; Rafferty et al., 2003; Rice, 2008; Moazen et al., 2009)—it is reasonable to expect that bones that participate in many sutures have central developmental and functional roles as well, possibly affecting the entire skull morphology. This observation carries with it a general evolutionary implication: some bones (those with higher burden-rank: sphenoid, ethmoid, and frontal in the case of the human skull) will be more difficult to be lost than those that are less connected (Esteve-Altava et al., 2013). Indeed, it is known that bones that have few connections, such as the jugal, postfrontal, postorbital, prefrontal, and supratemporal, have been repeatedly lost in many tetrapod lineages (Hildebrand, '88; Benton, '90; Kardong, 2005). This has been additionally confirmed by computer simulations, within a thorough phylogenetic analysis for all major groups in tetrapods, including mammals, and has been suggested as the basis for the reduction in skull bone number as seen in Williston's Law (Esteve-Altava et al., 2013).

How bones are connected to each other will directly affect their possibility for shape change, as they form ontogenetic growth units during development; conversely, changes in shape bone proportions will directly affect the overall skull connectivity pattern (Rasskin-Gutman, 2003). To further explore this claim, a strong effort has to be made to study pair-wise bone shape-covariation that can be related to skull connectivity patterns of organization. To our knowledge, there is a lack of modern studies systematically analyzing the relationship between shape and connectivity in the entire skull, which is the type of information needed to test our claim that connection dependencies impose structural constraints on shape bone proportions. However, Pearson and Woo ('35) carried out a pioneering study analyzing craniometrical measures on single bones in human skulls, concluding that adjacency (i.e., connectivity) was the second most important factor of shape correlation after symmetry.

All in all, connectivity relations can be directly interpreted as correlations of changes in size and shape due to their developmental role as sites of bone growth. In sum, connections are a fundamental source of morphological integration and modularity in the human skull. This cannot be otherwise, since the interplay between development and evolution has determined the co-dependencies among the skull bones, burdening those with more sutural connections while freeing the remaining ones to undergo independent variation. And that is more grist in Rupert Riedl's mill!

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