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Evo-Devo insights from pathological networks: exploring craniosynostosis as a developmental mechanism for modularity and complexity in the human skull

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Summary – *Bone fusion has occurred repeatedly during skull evolution in all tetrapod lineages, leading to a reduction in the number of bones and an increase in their morphological complexity. The ontogeny of the human skull includes also bone fusions as part of its normal developmental process. However, several disruptions might cause premature closure of cranial sutures (craniosynostosis), reducing the number of bones and producing new skull growth patterns that causes shape changes. Here, we compare skull network models of a normal newborn with different craniosynostosis conditions, the normal adult stage, and phylogenetically reconstructed forms of a primitive tetrapod, a synapsid, and a placental mammal. Changes in morphological complexity of newborn-to-synostosed skulls are two to three times less than in newborn-to-adult; and even smaller when we compare them to the increases among the reconstructed ancestors in the evolutionary transitions. In addition, normal, synostosed, and adult human skulls show the same connectivity modules: facial and cranial. Differences arise in the internal structure of these modules. In the adult skull the facial module has an internal hierarchical organization, whereas the cranial module has a regular network organization. However, all newborn forms, normal and synostosed, do not reach such kind of internal organization. We conclude that the subtle changes in skull complexity at the developmental scale can change the modular substructure of the newborn skull to more integrated modules in the adult skull, but is not enough to generate radical changes as it occurs at a macroevolutionary scale. The timing of closure of craniofacial sutures, together with the conserved patterns of morphological modularity, highlights a potential relation between the premature fusion of bones and the evolution of the shape of the skull in hominids.*

Keywords – *Craniosynostosis, Evolution, Modularity, Morphological complexity, Networks, Human skull.*

Introduction

Craniosynostosis is the premature closure of one or more cranial sutures; this condition provokes a change in the normal growth pattern of the skull, resulting in skull malformations due to growth restrictions imposed by the fused suture (Cohen & MacLean, 2000; Rice, 2008). This pathology has been described in non-human primates (Corner & Richtsmeier, 1992) as well as extinct hominins (Gracia *et al.*, 2009, 2010), and has been extensively studied at a genetic level in model organisms such as mouse (Martínez-Abadías *et al.*, 2010, 2011; Motch Perrine *et al.*, 2014) or zebrafish (Laue *et al.*, 2011). In humans, craniosynostosis affects approximately 3-5 out of 10,000 live births either as part of a syndrome or as a nonsyndromic condition (Cohen, 2000), and can occur isolated or in combination with other bone fusions (Derderian & Seaward, 2012; Garza & Khosla, 2012). Each type of nonsyndromic craniosynostosis provokes characteristic

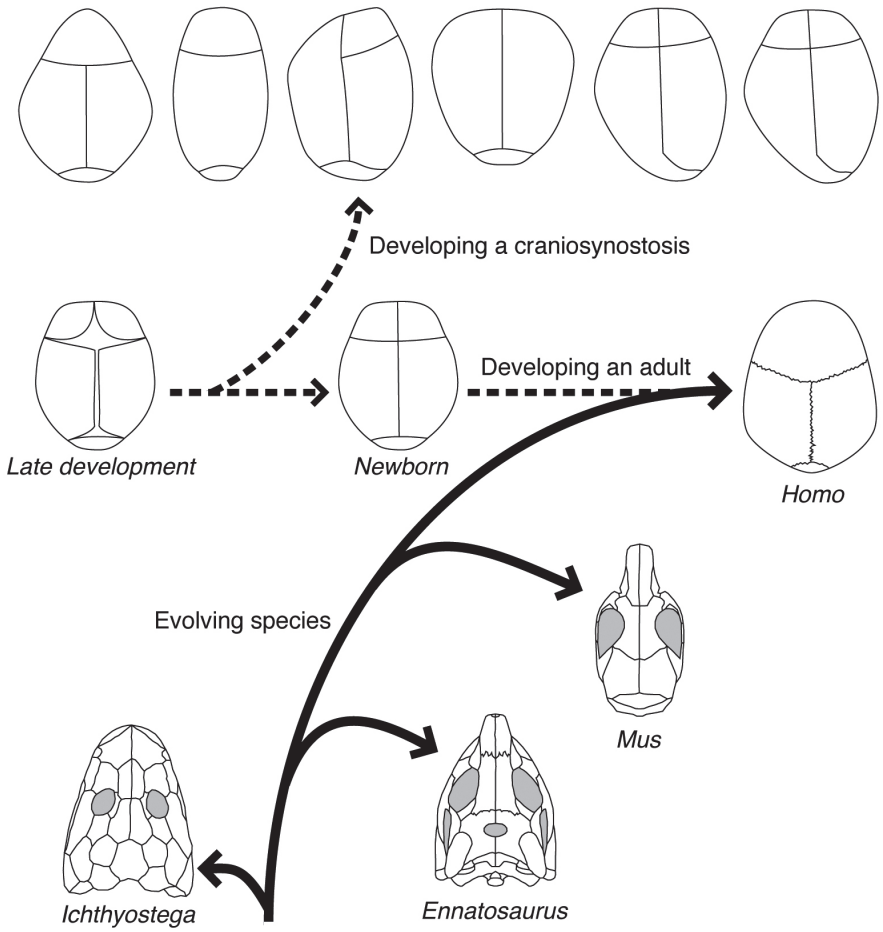

Fig. 1 - Craniosynostosis in an Evo-Devo framework. Bone number reduction correlates with shape changes in evolution and in normal and pathological development. Skull outlines not to scale.

malformations (Hukki *et al.*, 2008), whose severity can vary depending on fusion origin, direction, and speed (Heuzé *et al.*, 2010). These consequences are also meaningful in a broader Evo-Devo framework (Fig. 1), because craniosynostosis can be seen as an instance of the general morphogenetic process of bone fusion (Richtsmeier *et al.*, 2006; Esteve-Altava *et al.*, 2013b).

Bone fusion and morphological complexity

In general, early tetrapods had skulls with around 60 bones; this large number has been reduced by lineage-specific processes of bone fusion and bone loss in all tetrapod groups, resulting in skulls with less than 30 bones (Esteve-Altava *et al.*, 2013b). This reduction in the number of bones is a macroevolutionary trend known as Williston's Law (Williston, 1914; Gregory, 1935). Some authors have argued that the reduction in bone number entails a decrease in skull morphological complexity (Sidor, 2001; McShea & Hordijk, 2013). In contrast, in previous works using network analysis we have shown that Williston's Law is a trend toward increasing morphological complexity (Esteve-Altava *et al.*, 2013b, 2014). By modeling bones and suture joints as

the nodes and links of a network, morphological complexity can be quantified as a function of the pattern of organization of the skull bones (Rasskin-Gutman & Esteve-Altava, 2014). This quantification leads to a definition of morphological complexity as the presence and amount of multiple types of organizational patterns-including clusters, modules, and short distances-among anatomical parts (for alternative definitions of morphological complexity see, McShea, 1991; McShea & Brandon, 2010). We used three network parameters to quantify complexity in the skull: density of connections and clustering coefficient (showing significant positive correlation with bone number reduction), and characteristic path length (showing significant negative correlation). The density of connections is a raw estimate of complexity as number of connections realized as compared to the maximum possible; the clustering coefficient estimates complexity associated to the formation of correlated connections between skull bones; and the characteristic path length estimates complexity as effective proximity between bones, which it is translated into efficiency for transmitting biomechanical loads and biochemical signals throughout the skull (Esteve-Altava *et al.*, 2011, 2013b, 2014). For example, in synapsids, for a reduction from around 50 bones in basal forms to around 20 in modern forms, the density of connections increases from 0.11 to 0.30, the clustering coefficient from 0.42 to 0.63, and the characteristic path length decreases from 2.82 to 1.74 (Esteve-Altava *et al.*, 2013b). Several studies suggest that the decrease in number of skull bones during evolution is associated with an increase in their shape differences; this phenomenon has been called anisomerism (Gregory, 1934, 1935; for related studies on the vertebral column see McShea, 1991, 1993). Our own work has assessed anisomerism in anatomical networks by quantifying heterogeneity in the number of bone connections; additionally, we have used the relative number of unpaired bones as a proxy for the formation of modified, specialized bones (Esteve-Altava *et al.*, 2013b). In general, only the amount of unpaired bones correlated positively with bone number reduction in tetrapods, whereas heterogeneity showed a significant correlation only in synapsids (Esteve-Altava *et al.*, 2013b). Thus, during evolution, fusion of bones has generally resulted in the formation of new unpaired bones, such as the occipital, sphenoid, and frontal bones; in addition, the human skull has lost other typical mammalian bones such as prefrontals, postfrontals, or postorbital (Esteve-Altava & Rasskin-Gutman, 2014). In addition, during human skull ontogeny fusion events change the number of bones at birth from 25 to 21 in a typical adult skull; the timing of these bone fusions is significantly delayed as compared to those seen in the closest human relatives (Zollikofer & Ponce de León, 2010; Cray *et al.*, 2012; Falk *et al.*, 2012). Consequently, the same fusion patterns are observed both during evolution and development of the mammalian skull.

Bone fusion and modularity

The premature closure of sutures changes the growth patterns of skull bones modifying the final shape of the skull (Heuzé *et al.*, 2012). In addition, morphological integration and modularity suffer subtle changes, which vary in magnitude but that do not modify the general pattern (Martínez-Abadías *et al.*, 2009, 2011). Thus, some studies have shown that patterns of morphological integration among humans and primates are very similar (González-José *et al.*, 2004; Mitteroecker & Bookstein, 2008; Singh *et al.*, 2012). In the human skull, modularity has been extensively studied and modeled using complementary functional, developmental, and morphological approaches (Bastir, 2008). Functionally, the skull has been typically divided in braincase (vault and base) and facial skeleton (Gray, 1918; see also Richtsmeier & DeLeon, 2009); in addition, it has been further subdivided according to the functional matrix hypothesis (Moss & Young, 1960) to accommodate more specific functional units (e.g., Ackermann & Cheverud, 2004). On the other hand, developmental studies have either focused on the cellular or tissular origins of the skull bones (Morriss-Kay, 2001) or the quantitative genetics of morphometric modules (Martínez-Abadías *et al.*, 2012). Thus, depending on the developmental focus, the skull has been divided

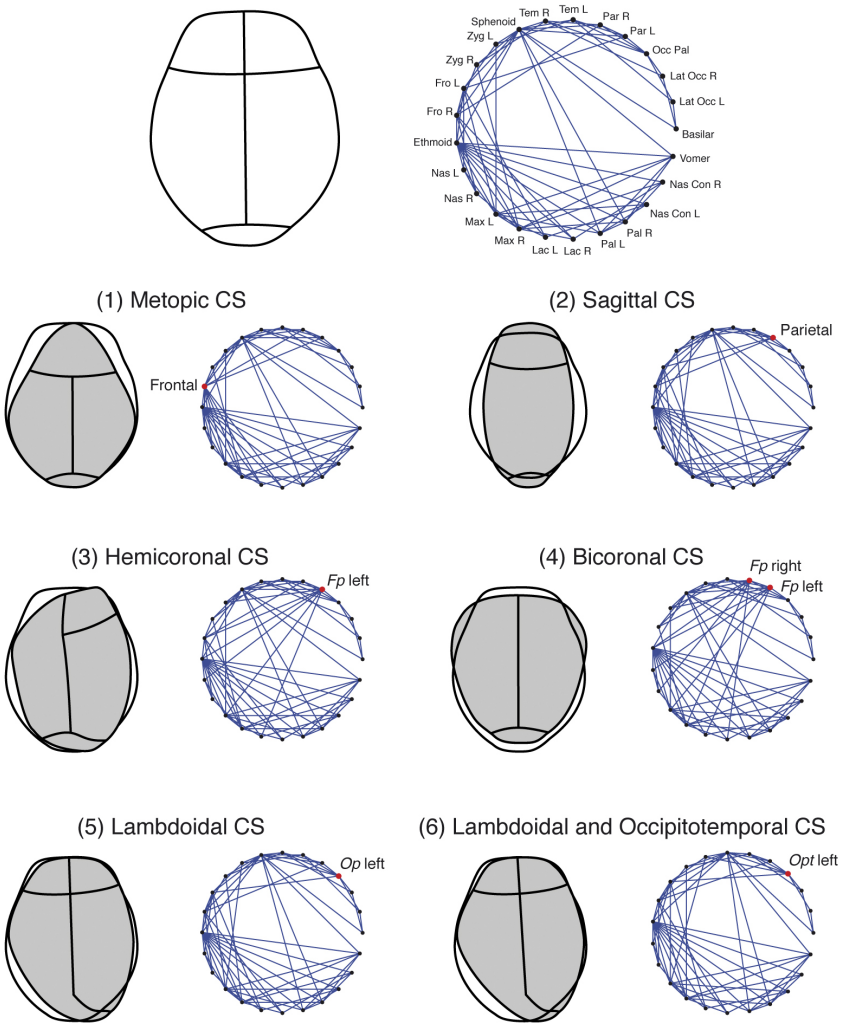

Fig. 2 - Skull networks analyzed and schematic outlines showing typical shape changes due to each nonsyndromic craniosynostosis in dorsal view. Red dots indicate the new bone formed by the fusion event. **Labels: Con, concha; Fro, frontal; Fp, frontoparietal; Lac, lacrimal; Lat, lateral; Max, maxilla; Nas, nasal; Occ, occipital; Op, occipitoparietal; Pal, palatal; Opt, occipitoparietotemporal; Par, parietal; Tem, temporal; Zyg, zygomatic; L, left; R, right.** ***The colour version of this figure is available at the JASs website.***

in different partitions, such as: chordal and prechordal (Couly *et al.*, 1993), neural crest and mesodermal (Santagati & Rijli, 2003), endochondral and dermal (recently reviewed in Lieberman, 2011). Furthermore, the human skull has been also partitioned according to the temporal pattern of suture closure during late growth and senescence in six divisions: cranial vault, cranial base, circum-meatal, palatal, facial, and cranio-facial (Krogman, 1930); this type of division stresses the relationship between modularity in the skull and heterochrony in the fusion

BOX: Network concepts used in this analysis (see Supplementary information for more details)

Node: element in the network model (bones)
Link: connection in the network model (contacts)
Density of Connections: number of existing links with respect to the maximum possible.
Clustering Coefficient: arithmetic mean of the number of existing links connecting the neighbors of each node with respect to the maximum possible.
Characteristic Path Length: arithmetic mean of the number of connections required to connect any two nodes in the network.
Heterogeneity: ratio between the variance and the mean number of connections per node.
Topological Overlap: number of connections to common neighbors between two nodes in relation to the maximum possible.
Q-Value: index comparing the distribution of connections of all nodes within and between modules with respect to the expected at random.

of bones (Goswami, 2007; Wilson & Sánchez-Villagra, 2009; Wilson, 2013).

Morphological approaches have typically focused on the validation of functional or developmental hypotheses using morphometrics (Olson & Miller, 1958; Cheverud, 1982; Klingenberg, 2008; Mitteroecker *et al.*, 2012), rather than using information on organismal form to define morphological modules (Eble, 2005). Using network models to identify modules in the skull (Esteve-Altava *et al.*, 2011) is an example of the latter type of morphological approach. Using a network model, we have shown that the bones of the human skull are organized in two well-delimited connectivity modules, one facial and one cranial (Esteve-Altava *et al.*, 2013a). These two modules have different internal organization: the facial module is hierarchical, an arrangement in which bones are grouped in smaller blocks or sub-modules that further group together into the final module; in contrast, the cranial module has a regular structure, an arrangement in which bones are equally connected to their closest neighbors. There are four facial blocks: the frontal (frontal and nasal bones), the left maxillary and the right maxillary (maxilla, lacrimal and inferior nasal concha for each side), and the ethmoidal (ethmoid, vomer and palatines). The cranial connectivity module groups the sphenoid, occipital, parietal, temporal, and zygomatic bones, without an internal substructure in sub-modules. We also demonstrated, using an independent geometric morphometric analysis, that these two connectivity modules are units of allometric growth. To explain this finding, we suggested that, since sutures act as primary sites of bone growth, bone connections in skull network models enclose growth co-dependences, which are translated into allometric shape correlations. As a consequence, it is expected that changes in bone growth co-dependences, such as those due to craniosynostosis, would also affect the modular organization of the skull network.

Given that craniosynostosis is an abnormal instance of the general process of bone fusion that occurs during the ontogeny of the skull, and that skull bone fusion is a common event in evolution, the questions we want to address here are: (1) to which extent changes in the morphological complexity that result from craniosynostosis in human skulls resemble those seen in evolution and (2) how craniosynostosis modifies the arrangement of bones in connectivity modules. Our starting hypothesis is that early fusion of bones in newborns would affect the organizational patterns of the human skull, increasing its complexity parameters as well as modifying its modular organization. To answer this, we have compared the network model of the normal human skull at birth with those of six different nonsyndromic craniosynostosis conditions. To complete this comparison of changes

Tab. 1 - Network parameters describing morphological complexity and anisomerism in each network model in this study.

Skull	N	K	D	C	L	H	UBR
Newborn	25	75	0.24	0.51	1.99	0.49	0.20
Metopic CS	24	70	0.25	0.53	1.97	0.51	0.25
Sagittal CS	24	70	0.25	0.51	1.98	0.49	0.25
Hemicoronal CS	24	71	0.26	0.52	1.92	0.51	0.21
Bicoronal CS	23	68	0.27	0.53	1.88	0.49	0.22
Lambdoidal CS	24	70	0.25	0.52	1.93	0.51	0.21
Lambdoidal and Occipitomastoid CS	23	67	0.26	0.52	1.91	0.51	0.22
Adult Human[1]	21	64	0.30	0.63	1.74	0.49	0.24
Placental Mammal RLCM[1]	30	87	0.20	0.51	2.20	0.46	0.18
Synapsid RLCM[1]	48	124	0.11	0.42	2.82	0.44	0.08
Tetrapod RLCM[1]	52	133	0.10	0.40	2.90	0.39	0.06

N, number of bones; K, number of connections; D, density of connections; C, network clustering coefficient; L, characteristic path length; H, heterogeneity; UBR, relative amount of unpaired bones; CS, craniosynostosis; RLCM, reconstructed last common ancestor.

[1] Network models included for comparison (Esteve-Altava *et al.*, 2013b).

in morphological complexity and modularity, we have included information of the adult human skull as well as network parameters for reconstructed common ancestors of placental mammals, synapsids, and tetrapods from other studies.

Material and Methods

Network models of craniosynostosis

Anatomical network models are codified in a square adjacency matrix of skull bones, in which their binary values are 1s for presence and 0s for absence of bone contacts (Esteve-Altava *et al.*, 2011). We have built seven newborn skull network models: one normal and six showing different nonsyndromic craniosynostosis conditions (Fig. 2). The network model for the anatomically normal child at birth was built by using detailed suture descriptions from the literature (Gray, 1918; Rice, 2008). Then, each craniosynostotic skull network model was derived from the anatomically normal one by modifying the adjacency matrix; accordingly, when bones are fused the corresponding columns and rows are merged, modifying the corresponding pattern of 1s and 0s in the matrix. Thus, while the anatomically normal child at birth has paired frontals and occipital bones not fused, each craniosynostotic skull is affected in one or more specific suture, as follows: (1) metopic, paired frontals fused at the metopic suture forming an unpaired frontal bone; (2) sagittal, parietals fused at the sagittal suture forming an unpaired parietal bone; (3) left hemicoronal, left parietal and left frontal fused at the coronal suture forming one frontoparietal bone; (4) bicoronal, parietals and frontals fused at the coronal suture forming two frontoparietal bones; (5) lambdoidal, occipital plate and left parietal fused at the lambdoidal suture forming an unpaired occipitoparietal bone; and

(6) lambdoidal plus occipitomastoid, occipital plate, left parietal, and left temporal fused at the lambdoidal and occipitomastoid sutures forming an unpaired occipitoparietotemporal bone. Henceforth, each network model will be named according to its fused suture.

Anatomical network analysis

For each network model we have quantified its structural complexity and modular organization. The structural complexity was measured using the following network parameters: density of connections, mean clustering coefficient, mean shortest path length, heterogeneity, and ratio of unpaired bones (Esteve-Altava *et al.*, 2013b, 2014). The modular organization was assessed using a hierarchical cluster analysis of the topological overlap similarity matrix (Esteve-Altava *et al.*, 2013a). We define a connectivity module as a group of bones with more connections among them than to other bones outside the module; consequently, bones sharing connections to the same neighbors will fall within the same module. The Box shows a brief summary of the network concepts used in the analysis; for a detailed description of each network parameter and method used to analyze skull network models, see Supplementary methods information (for further details, see also Esteve-Altava *et al.*, 2011, 2013a, 2013b).

Complexity measures of the newborn were compared with those quantified in the synostosed skulls, the adult human skull, and the hypothetical skulls of the last common ancestors of tetrapods, synapsids, and placental mammals, reconstructed using parsimony optimization (Esteve-Altava *et al.*, 2013b). Connectivity modules in the newborn were compared with those identified in the adult human skull (Esteve-Altava *et al.*, 2013a) and with the synostosed models.

Results and Discussion

Increase of morphological complexity in development and evolution

The results show that all skulls with craniosynostosis have higher morphological complexity than the anatomically normal newborn skull for all network parameters. Thus, while the density of connections and the clustering coefficient increase, the characteristic path length decreases (Tab. 1). In contrast, a net increase in anisomerism is only observed for the relative amount of unpaired bones, and not for heterogeneity of connections. Table 1 also shows the parameters of the adult skull network as well as those of recent ancestors of placental mammals, synapsids, and tetrapods, which highlight the occurrence of the same increase in morphological complexity in ontogeny and phylogeny: (1) during human ontogeny, the skull reduces the number of bony elements by fusing up to 11 sutures; (2) during evolution, a large reduction in number of bones along with the fusion of many sutures have occurred in all tetrapod linages (Esteve-Altava *et al.*, 2013b; 2014; Esteve-Altava & Rasskin-Gutman, 2014). In all instances, bone fusion reduces the number of skull elements as well as their overall suture relation in the normal ontogenetic process, in the pathological process, as well as during the evolution of the tetrapod skull (see also, Richtsmeier *et al.*, 2006). However, it is worth noting that a reduction in the number of bones not always leads to an increase in morphological complexity, this only occurs when losses occur at random and highly connected bones are fused (Esteve-Altava *et al.*, 2014).

Premature bone fusions are a source of phenotypic variation and novelty in skull evolution (Morriss-Kay, 2001; Richtsmeier *et al.*, 2006; Koyabu *et al.*, 2012; Esteve-Altava *et al.*, 2013b). These shifts in the timing of bone fusions during skull development are examples of heterochrony in skull evolution, which are particularly common in mammals (Schoch, 2006; Goswami, 2007; Hallgrimsson & Lieberman, 2008; Zollikofer & Ponce de León 2008; Wilson & Sánchez-Villagra, 2009). For this reason, the premature closure of bone sutures in the skull that results in craniosynostosis can also be seen as a putative developmental process involved in the formation of evolutionary trends in bone reduction in the vertebrate skull (Richtsmeier *et al.*, 2006; Esteve-Altava *et al.*, 2013b; 2014). The

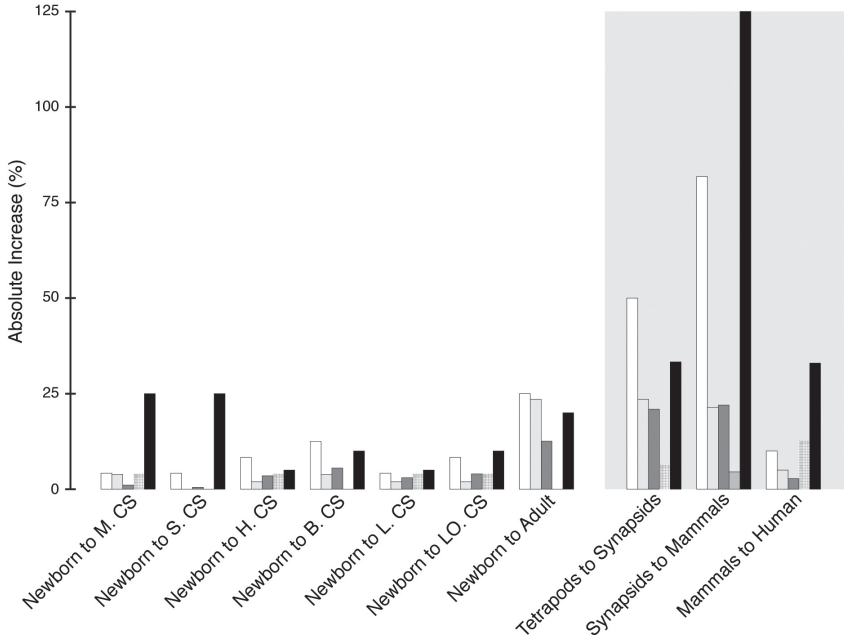

Fig. 3 - Variation in morphological complexity and anisomerism in each developmental (white background) and evolutionary transition (grey background). Variation is shown as the percentual absolute increase calculated from values in Table 1. Increases are higher in evolutionary transitions and normal development than in craniosynostosis; however, all increases are within a comparable scale. Bar code: white, density of connections; light grey, network clustering coefficient; dark grey, characteristic path length; hatched grey, heterogeneity; black, relative amount of unpaired bones. Craniosynostosis (CS): M, metopic; S, sagittal; H, hemicoronal; B, bicoronal; L, lambdoidal; LO, lambdoidal plus occipitomastoid.

examples of craniosynostosis analyzed here show analogous change patterns as it has taken place at a macroevolutionary scale (Fig. 3). Thus, bone fusion produces an increase in morphological complexity and anisomerism of the skull during normal and pathological development, as well as during evolution. However, there are quantitative differences as a consequence of the different amount of bones involved: 2 o 3 in craniosynostosis, 6 in normal adult development, and many more (up to 40) in evolutionary transitions. It is worth noting that craniosynostosis also increases the probability to develop wormian bones in the skull (Sánchez-Lara *et al.*, 2007; Wu *et al.*, 2011). However, wormian bones occur at the sutures between two bones, thus, barely affecting the overall connectivity pattern of the network (e.g., the density of connections would decrease, but the clustering coefficient would increase, and the shortest path length would not be affected). To which extent wormian bones could have significance at a macroevolutionary scale has not been yet explored extensively and warrants further research (but see, Di Ieva *et al.*, 2013).

Conservative connectivity modules in craniosynostosis

At different ontogenetic stages the skull shows different number of bones and different suture patterns; as a consequence, the newborn and the adult skull show different arrangement of bones, which results in differences in their modularity patterns (Fig. 4). Thus, the overall modular organization of the newborn skull resembles that of the adult: two separated connectivity

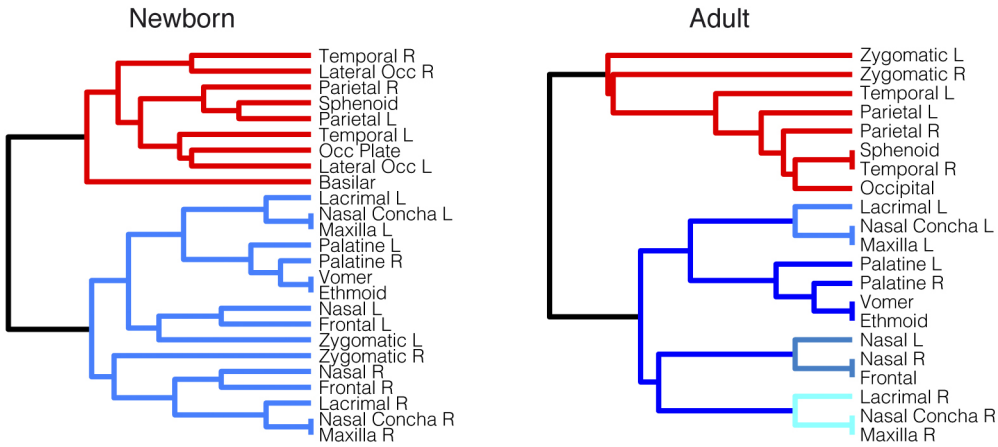

Fig. 4 - Comparison of connectivity modules found in the anatomically normal newborn skull and the adult. In both ontogenetic stages, we find the same two connectivity modules, one facial (blue) and one cranial (red). The main difference between the newborn and the adult skull is the internal structure of each module: regular for the cranial and hierarchical for the facial in the adult (blue shades), but not in the newborn. Modularity of the adult skull network has been modified from Esteve-Altava et al., 2013c (Newborn: Q2 = 0.277; Qmax = 0.299. Adult: Q2 = Qmax = 0.274.). The colour version of this figure is available at the JASs website.

modules, one for the posterior bones of the vault and one for the anterior bones of the face; being the main difference the placement of zygomatic bones, facial in the newborn and cranial in the adult. In addition, bones that later will fuse to form the occipital appear together in the cranial module, while the paired frontals are within the facial module. However, the internal structure of each module changes from the newborn to the adult, reflecting a specific growth pattern for each module. While the cranial module grows from a non-regular to a regular organization, the facial module grows from a loose hierarchical organization to a well-defined hierarchy of modular blocks (as defined in Esteve-Altava *et al.*, 2013a). This finding suggests (1) a developmental transition from less to more organized structures and (2) that the growth processes involved in the late development of each part of the skull might be mechanistically and/or ontogenetically different--an evidence of morphological modules (Eble, 2005). Indeed, we have demonstrated that the facial and cranial connectivity modules are morphological units of allometric growth (Esteve-Altava *et al.*, 2013c). Furthermore, the human braincase is often cited as an example of peramorphosis, while the face is an example of paedomorphosis (Shea, 1989; also reviewed in Lieberman, 2011, chapter 5).

In contrast to what was expected, skulls with craniosynostosis show the same general pattern of connectivity modules than the anatomically normal newborn skull (Fig. 5). Thus, the cranial modules show a non-regular structure, while the facial modules do not show yet a definite hierarchical organization. Again, the main difference between modules is the placement of zygomatic bones, which, due to their role as between-modules connector and their similar connectivity patterns to both modules, can be grouped in any of the two modules with minor differences (Esteve-Altava *et al.*, 2013a). A notorious exception to this common pattern occurs in the metopic craniosynostosis. Here, the fusion of the frontal bones produces an adult-like organization of the facial module: a hierarchical structure composed of four blocks, maxillary left, maxillary right, frontal, and ethmoidal (Fig. 5A). Thus, it seems that the mere fusion of the paired frontals forming an unpaired, highly connected

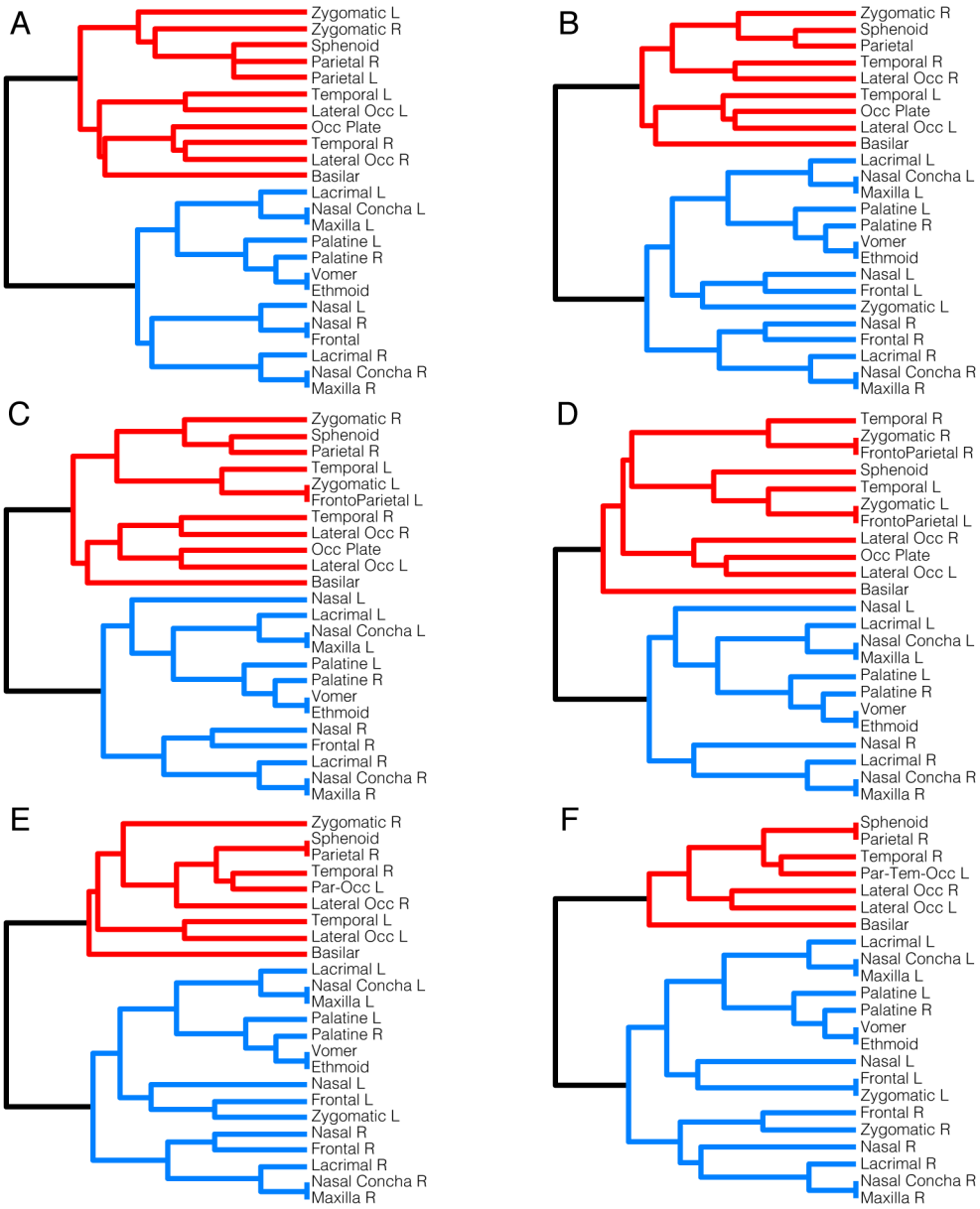

Fig. 5 - Connectivity modules identified in each craniosynostosis condition analyzed. All skull networks show a division into two connectivity modules: facial and cranial, grouping mostly the same bones than in the newborn skull. Differences arise due to variable placement of zygomatic bones according to local changes in connectivity patterns in the region between modules (see text). As in the anatomically normal skull, newborns with craniosynostosis show a different internal structure of connectivity modules than the adult form (Metopic CS: Q2 = Qmax = 0.319. Sagittal CS: Q2 = Qmax = 0.269. Hemicoronal CS: Q2 = Qmax = 0.291. Bicoronal CS: Q2 = Qmax = 0.290. Lambdoidal CS: Q2 = 0.275; Qmax = 0.269. Lambdoidal and Occipitomastoid CS: Q2 = 0.214; Qmax = 0.288.). The colour version of this figure is available at the JASs website.

bone is sufficient to reach the adult morphological organization in the facial module. Finally, it is also worth noting that the ethmoidal block, which comprises the ethmoid, vomer, and palatines, is already present in the newborn skull--and it is conserved in all human skull networks as an invariant aggrupation of bones. This block comprises the marginal palate bones that form the roof of the nasal capsule, an embryological, morphological, and evolutionary unit with a distinctive pattern of integration within the face (Schoch, 2006; Bastir & Rosas, 2013; Holton *et al.*, 2014). All in all, the similarity in modular organization between normal and abnormal skulls indicates that, even after a pathologic fusion of bones, growth co-dependences are not extremely affected at this level. Thus, reinforcing the idea of skull morphogenesis as an intrinsically robust process (Goodwin *et al.*, 1993); in fact, nonsyndromic craniosynostosis is, in many instances, phenotypically viable for individuals (e.g., Marchac *et al.*, 2008). We think this robustness could be the reason underlying stable patterns of morphological integration reported for shape correlations between skull regions after craniosynostosis as well (Richtsmeier & DeLeon, 2009; Martínez-Abadías *et al.*, 2011).

The link between modularity and complexity in the human skull

Ever since Herbert Simon's seminal work on the architecture of complexity (Simon, 1962), modular organization has been taken as a causal agent for the possibility to increase complexity in a system (Schlosser & Wagner, 2004; Callebaut & Rasskin-Gutman, 2005). By comparing modules in the newborn and the adult skull, we have shown that the cranial module changes from non-regular to regular, while the facial module changes from a loose hierarchy to a well-defined hierarchy of modular blocks. Thus, from the newborn to the adult, the internal structure of both modules changes considerably, and so their complexity increase. On the other hand, changes in connectivity patterns associated to craniosynostosis cause little change with respect to the modular structure found in the anatomically normal newborn skull. Hence, changes in morphological complexity of newborn-to-synostosed skulls are two to three times less than in newborn-to-adult; and even smaller if we compare them to the increases among the reconstructed ancestors in the evolutionary transitions (Fig. 3). Fusions of bones have occurred repeatedly during tetrapod evolution, resulting in the formation of new unpaired bones in the midline such as the occipital, sphenoid, and frontal bones (Esteve-Altava & Rasskin-Gutman, 2014), whose formation increased morphological complexity (Esteve-Altava *et al.,* 2013b, 2014) and modified the modular architecture of the skull (Esteve-Altava, 2013). In summary, our results support Simon's suggestion that, indeed, modularity and complexity are related in the evolution of a system. In the case of the human skull, fusion of paired bones from the newborn to the adult changes the internal structure of the modules, with a concomitant increase in its morphological complexity; the same pattern occurs among the synostosed skulls, but at a smaller scale. In the case of the evolutionary transitions, the same mechanism generates changes in modularity big enough to provoke big changes in complexity.

Concluding remarks

Shifts in the timing of closure of craniofacial sutures, together with the conserved patterns of morphological integration and modularity observed in primates (Singh *et al.*, 2012), provides a potential mechanism for the evolution of the human skull. Thus, a human pathology that can be studied at several levels of detail--genetic, cellular, and above--can be also efficiently used as a developmental explanation behind changes of the organization of suture connections among bones at a macroevolutionary scale. We have shown here that craniosynostosis reproduces at an ontogenetic scale the evolutionary patterns found in Williston's Law: an increase in morphological complexity and anisomerism. The subtle changes in skull complexity at the developmental scale can change the modular substructure of the

newborn skull to more integrated modules in the adult skull, but is not enough to generate radical changes as it occurs at a macroevolutionary scale.

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