

Structural Constraints in the Evolution of the Tetrapod Skull Complexity: Williston's Law Revisited Using Network Models

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Abstract Ever since the appearance of the first land vertebrates, the skull has undergone a simplification by loss and fusion of bones in all major groups. This well-documented evolutionary trend is known as “Williston's Law”. Both loss and fusion of bones are developmental events that generate, at large evolutionary scales, a net reduction in the number of skull bones. We reassess this evolutionary trend by analyzing the patterns of skull organization captured in network models in which nodes represent bones and links represent suture joints. We also evaluate the compensatory process of anisomerism (bone specialization) suggested to occur as a result of this reduction by quantifying the heterogeneity and the ratio of unpaired bones in real skulls. Finally, we perform simulations to test the differential effect of bone losses in skull evolution. We show that the reduction in bone number during evolution is accompanied by a trend toward a more complex organization, rather than toward simplification. Our results indicate that the processes by which bones are lost or fused during development are central to explain the evolution of

the morphology of the skull. Our simulations suggest that the evolutionary trend of increasing morphological complexity can be caused as a result of a structural constraint, the systematic loss of less connected bones during development.

Keywords Morphological complexity · Constraints · Network theory · Tetrapod skull

Introduction

One of the best-documented trends in vertebrate evolution is the reduction in number of skull bones, also known as Williston's law (Gregory et al. 1935). For instance, the mammalian skull lacks bones that are characteristically present in ancestral forms, such as the pre- and post-frontals, postorbitals, and quadratojugals, and has also new bones that have appeared from the fusions of others, such as the occipital and the sphenoid (Sidor 2001). Similar patterns of bone loss have been reported in other lineages, including snakes, lizards, birds, and turtles (Goodrich 1958; Estes 1961; Gaffney 1979; Carroll 1988; Rieppel 1993; Laurin 1996; Sereno 1997; Kardong 2005).

This reduction of the number of skull bones in vertebrates has been interpreted as an evolutionary trend toward simplification of skull architecture, associated to a decrease in complexity (Hildebrand 1988, Chap. 8). Sidor (2001) argued that this reduction is phylogenetically sound in synapsids, interpreting that simplified, compact skulls are selectively advantageous. At the same time, developmental constraints that facilitate the loss and fusion of bones, and others that prevent the formation of new ossification centers, favor this trend. A constraint that can cause an evolutionary reduction in number of skull bones is related to

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changes in the developmental timing of suture formation (Depew and Griffin 2008); both losses and fusions are caused by either lack of formation of ossification centers or premature closures of suture joints.

The difficulty of measuring and comparing morphological complexity in the skull across lineages hampers the evolutionary study of complexity at large-scales. According to Gregory (1934), a greater complexity of individual bones compensates for the reduction in number. Gregory called this compensation “anisomerism”, a trade-off process that generates more specialized, different anatomical elements, as a result of this reduction in number. The opposite process, “polysomerism”, accounts for a pattern of less specialized, similar anatomical elements (Gregory 1934).

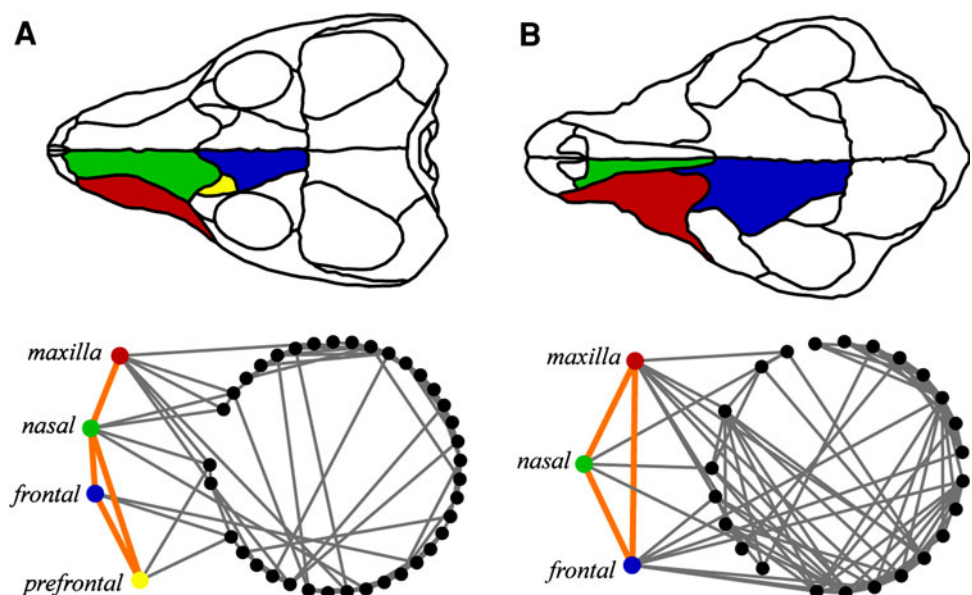
A simple and operative way to study this general trend in major groups of vertebrates is by defining skull complexity as a function of the number of bones (Sidor 2001); however, this approach is limited (for a thorough discussion, see McShea 1991, 1996, 1998). In order to circumvent these limitations, we represent each vertebrate skull as a network of connected bones using network analysis to detect changes in their structural arrangement (Fig. 1). This method provides an operative framework for the early comparative anatomy ideas of Geoffroy Saint-Hilaire’s principle of connections (Riedl 1978; Le Guyader 2004). Its output yields connectivity patterns among individual bones, within bone groups, as well as statistical signals of morphological complexity.

Recent analyses of complexity trends in many biological systems using network theory demonstrate that complexity can be quantified more accurately as a function of the relational properties of the system’s components than as the number of elements (Sporns 2002; Newman and

Forgacs 2005; Proulx et al. 2005; Newman et al. 2006; Mason and Verwoerd 2007; Dunne et al. 2008a; Knight and Pinney 2009). These methods can also be applied to the study of morphological complexity in anatomical systems (Esteve-Altava et al. 2011; see also Rasskin-Gutman 2003). Here, morphological complexity is quantified as a function of the pattern of organization of the skull, in which bones and suture joints are modeled as the nodes and links of a network. Using this framework, we have re-assessed Williston’s Law in the tetrapod skull.

Morphological complexity was quantified with three different well-established network statistics: the density of connections (D), the characteristic path length (L), and the cluster coefficient (C). These statics have been used before to approach complexity in other biological systems, in different ways. For example, D has been used in ecological network models to analyze complex functional responses (Dunne et al. 2008a, b). We used D as a direct measure of complexity; the more connected a network, the more complex its organization. L is often used to estimate the speed of information flow between the nodes of a network associated to complex organizations (Xu et al. 2011). This flow depends on the nature of each type of network; for instance, the Internet transmits data, a food web transmits biomass, and the brain transmits electric impulses. For instance, in skull networks this flow could be equated to the diffusion of stress forces acting on skull bones (Moazen et al. 2009). Accordingly, we used L as an estimate of complexity in terms of efficiency for spreading biomechanical forces as well as molecular signals between skull bones. Finally, C measures the presence of loops of connections between elements (triangular motifs), which promotes functional and structural correlations between connected

Fig. 1 Example of how connectivity relationships among bones can change between evolutionary related species in a schematic skull representation of **a** an extinct therapsid, *Thrinaxodon liorhinus*, and **b** a modern mammal, *Canis lupus*. The left frontal, maxilla, nasal, and prefrontal have been highlighted with colors; blue, red, green, and yellow, respectively, to show changes in the local connectivity pattern as a result of prefrontal bone loss. A new connection appears between the frontal and the maxilla as a consequence of the prefrontal loss



parts as a result of the formation of clusters (Dorogovtsev and Mendes 2003). We interpret the presence of cluster-coordinate responses in varying traits as an indication of morphological integration and modularity (Olson and Miller 1958; Chernoff and Magwene 1999; Magwene 2008); using C as an estimate of complexity as it relates to patterns of integration among skull bones. In summary, we have assessed changes in structural patterns in the tetrapod skull with three complementary qualities of morphological complexity captured by well-established network statistics: structural organization (D), functional efficiency (L), and integration (C).

The compensatory process of anisomerism proposed by Gregory (op. cit.) is far more complicated to capture than skull complexity by means of network statistics because its effects occur mainly at the level of bones, not across the entire skull. Gregory's basic definition of anisomerism refers to structural similarity among elements. In a network context, an easy way to estimate this similarity among bones is to compare the number of connections they have. Therefore, we quantified anisomerism as connectivity heterogeneity (H) according to Horvath and Dong (2008). In addition, we also estimated the relative number of unpaired bones (UBR) as a side-measure of anisomerism, for two reasons: (1) they appear in evolution from the fusion of two or more pre-existing bones, which is one of the proposed causes of Williston's law, and (2) they are among the most modified, specialized bones.

Here, we test whether the evolutionary trend toward reduction in number of skull bones simplifies the skull structure or rather makes it more morphologically complex. To do so, we first quantified skull complexity using network statistics. Then we tested if there is a correlation between the number of skull bones, morphological complexity, and anisomerism in a phylogenetic context. Finally, we analyzed different scenarios of bone number reduction: selective loss of most connected bones, selective loss of less connected bones, and random losses in order to check which scenario is more suitable to generate trends in complexity during evolution.

Materials and Methods

We built three-dimensional unweighted network models for each of a sample of 44 tetrapod skulls as they are described in the literature (see Table 1 for species names, references, and network statistic values). Taxa were selected to show the diversity of tetrapod skull morphologies, including extinct basal forms. The nodes and links of networks represent the bones and suture joints between them. Connectivity information of each skull is codified in a symmetric adjacency matrix of size equal to the number of bones, in

which 1 indicates presence of a suture connection and 0 absence (Esteve-Altava et al. 2011). All the following analyses were implemented by developing special purpose routines in MATLAB (2010), unless otherwise stated.

Estimation of Complexity and Anisomerism in Skull Networks

We measured skull complexity with the three network statistics proposed (see Newman 2003, for a more extensive mathematical description).

D is the number of existing connections with respect to the maximum possible (1),

$$D = \frac{2 \times K}{N(N-1)} \quad (1)$$

where K is the number of links in the network and N the number of nodes.

L is the average of the minimum distance between all nodes in the network (2); here, distance refers to the number of connections, each one having unit length,

$$L = \frac{1}{N(N-1)} \sum_{i,j} d_{i,j} \quad (2)$$

where $d_{i,j}$ is the distance in number of links to connect nodes i and j .

C is the average of the sum of connections between all neighbors of each node with respect to the maximum possible (3),

$$C = \frac{1}{N} \sum \frac{\sum \tau_i}{\sum k_i(k_i-1)} \quad (3)$$

where τ_i is the number of connections between neighbors of node i and k_i is the number of connections of node i . Notice that higher values of D and C mean greater degree of complexity, while L is inversely related to complexity; higher values mean lower complexity.

Finally, we estimated anisomerism as the connectivity heterogeneity (H) in each skull (4), which provides an estimate of the irregularity of the network; higher values of H indicate greater disparity in the number of connections among skull bones, whereas lower values indicate homogeneity, regularity (polysomerism).

$$H = \frac{\sqrt{\text{var}(k)}}{\text{mean}(k)} \quad (4)$$

The ratio of unpaired bones (UBR) in relation to the total number of bones (5) was quantified as a complementary estimation of skull anisomerism.

$$UBR = \frac{\text{Unpaired Bones}}{\text{Total Skull Bones}} \quad (5)$$

Table 1 Summary of network statistics values measured in this study

Species	N	K	D	L	C	H	UBR	Ref.
<i>Ichthyostega</i> sp.	56	148	0.10	2.94	0.39	0.38	0.07	Kardong 2005
<i>Seymouria baylorensis</i>	56	144	0.09	3.00	0.38	0.33	0.04	Laurin 1996
<i>Epicrionops petersi</i>	23	51	0.20	1.96	0.59	0.63	0.04	Trueb 1993 Nussbaum 1977
<i>Salamandra salamandra</i>	25	52	0.17	2.61	0.45	0.27	0.04	Trueb 1993
<i>Gastrotheca walkeri</i>	22	43	0.19	2.26	0.44	0.41	0.09	Trueb 1993
<i>Procolophon pricei</i>	45	120	0.12	2.69	0.45	0.41	0.07	Carroll and Lindsay 1985
<i>Proganochelys quenstedti</i>	43	111	0.12	2.66	0.43	0.40	0.07	Gaffney 1990
<i>Podocnemis unifilis</i>	34	90	0.16	2.41	0.34	0.32	0.12	Gaffney 1979
<i>Chelodina longicollis</i>	33	80	0.15	2.56	0.43	0.40	0.15	Gaffney 1979
<i>Kayentachelys aprix</i>	38	101	0.14	2.53	0.41	0.36	0.11	Sterli and Joyce 2007
<i>Chisternon</i> sp.	36	98	0.16	2.43	0.44	0.39	0.11	Gaffney 1979
<i>Chelydra serpentina</i>	36	91	0.14	2.47	0.44	0.38	0.11	Gaffney 1979
<i>Carettochelys insculpta</i>	36	92	0.15	2.50	0.39	0.43	0.11	Gaffney 1979
<i>Gopherus polyphemus</i>	36	90	0.14	2.46	0.44	0.43	0.11	Gaffney 1979
<i>Testudo graeca</i>	34	94	0.17	2.36	0.45	0.41	0.12	Gaffney 1979
<i>Petrolacosaurus kansensis</i>	55	132	0.09	3.06	0.47	0.43	0.05	Reisz 1981
<i>Younginia capensis</i>	53	122	0.09	3.12	0.38	0.43	0.06	Carroll 1988 Gardner et al. 2010
<i>Rhamphorhynchus</i> sp.	41	95	0.12	2.79	0.31	0.35	0.12	Padian 1984
<i>Crocodylus moreletii</i>	39	97	0.13	2.62	0.43	0.33	0.13	Goodrich 1958
<i>Stegosaurus armatus</i>	47	114	0.11	2.89	0.32	0.34	0.09	Gilmore 1914 Weishampel et al. 1993
<i>Corythosaurus casuarius</i>	33	77	0.15	2.62	0.43	0.34	0.12	Ostrom 1961
<i>Plateosaurus engelhardti</i>	49	112	0.10	3.08	0.34	0.42	0.10	Weishampel et al. 1993
<i>Dromaeosaurus albertensis</i>	41	99	0.12	2.73	0.29	0.28	0.07	Carroll 1988
<i>Anser anser</i>	18	27	0.18	2.18	0.38	0.62	0.33	Kardong 2005
<i>Sphenodon punctatus</i>	38	78	0.11	2.85	0.28	0.42	0.05	Goodrich 1958
<i>Iguana iguana</i>	42	122	0.14	2.48	0.47	0.43	0.14	Estes et al. 1988
<i>Python regius</i>	35	68	0.11	2.76	0.46	0.49	0.17	Estes et al. 1988 Kardong 2005
<i>Hemitheconyx caudicinctus</i>	34	72	0.13	2.70	0.33	0.39	0.18	Estes et al. 1988 Payne et al. 2011
<i>Tupinambis teguixin</i>	42	94	0.11	2.81	0.37	0.42	0.14	Estes et al. 1988
<i>Diplometopon zarudnyi</i>	26	57	0.18	2.52	0.61	0.44	0.15	Maisano et al. 2006
<i>Stenocercus guentheri</i>	44	97	0.10	2.90	0.34	0.45	0.14	Torres-Carvajal 2003
<i>Varanus salvator</i>	42	85	0.10	3.19	0.29	0.42	0.14	Estes et al. 1988 Rieppel 1993
<i>Ennatosaurus tecton</i>	52	124	0.09	2.87	0.36	0.51	0.08	Maddin et al. 2008
<i>Dimetrodon gigas</i>	45	111	0.11	2.71	0.44	0.39	0.07	Case 1904
<i>Jonkeria ingens</i>	51	130	0.10	2.75	0.46	0.50	0.14	Boonstra 1936
<i>Thrinaxodon liorhinus</i>	41	87	0.11	2.94	0.39	0.38	0.07	Estes 1961
<i>Ornithorhynchus anatinus</i>	26	65	0.20	2.13	0.55	0.45	0.27	Kardong 2005
<i>Phascolarctos cinereus</i>	31	87	0.19	2.26	0.47	0.34	0.16	Louys et al. 2009
<i>Didelphis virginiana</i>	26	66	0.20	2.15	0.43	0.39	0.23	Kardong 2005
<i>Homo sapiens</i>	21	64	0.30	1.74	0.63	0.49	0.24	Gray 1918
<i>Pteropus lylei</i>	21	47	0.22	2.03	0.53	0.55	0.14	Giannini et al. 2006
<i>Mus musculus</i>	28	80	0.21	2.10	0.46	0.40	0.21	Goodrich 1958

Table 1 continued

Species	N	K	D	L	C	H	UBR	Ref.
<i>Canis lupus</i>	29	97	0.22	2.05	0.60	0.50	0.17	Mead and Fordyce 2009
<i>Tursiops truncatus</i>	32	99	0.20	2.19	0.51	0.49	0.19	Mead and Fordyce 2009

N number of nodes, *K* total number of connections, *D* density of connections, *L* characteristic path length, *C* network clustering coefficient, *H* network connectivity heterogeneity, *UBR* unpaired bone ratio

Results were shown along with the quantification of complexity in circular, random (Erdos and Renyi 1959), and Gabriel networks (Gabriel and Sokal 1969) of the same size in order to offer a visual contrast between the values of complexity in skull networks and those estimated for common theoretical models (Fig. 3).

Phylogenetic Analysis

We assessed the correlation between the skull bone number and morphological complexity in a phylogenetic context (Fig. 2) with a phylogenetic independent contrast FIC4 (Felsenstein 1985; Laurin 2004; for an extensive explanation of this test see Laurin 2010); performed using the DPAP: PDTREE module for Mesquite (Midford et al. 2008).

The phylogeny used is a supertree assembled from various published phylogenies (Meylan 2001; Springer et al. 2003; Hugall et al. 2007; Cannatella 2008; Pace et al. 2008; Phillips et al. 2009; Okajima and Kumazawa 2010; Laurin 2011; Laurin and Gauthier 2011, 2012; Laurin and Reisz 2011). Principal clades were assembled following consensus phylogenies discussed in The Tree of Life Web Project (Maddison and Schulz 2007). The phylogeny branch lengths were calibrated in millions of years of evolution for the minimal divergence time of the crown group according to the Paleobiological Database (available at <http://paleodb.org>) and published phylogenies (op. cit.), according to recent geologic time scales (Gradstein et al. 1995; Roscher and Schneider 2006; Gibbard et al. 2010). The branch lengths were set to 3 when dichotomies seemed to occur in the same age as suggested by Laurin (2004). Time calibration was performed in Mesquite (Maddison and Maddison 2011) using the Stratigraphic Tool (Josse et al. 2006).

Tests of Robustness

We studied the response of skull complexity to the loss of bones with a simulation that iteratively removed network nodes, measuring complexity after each node removal. This kind of simulation has been used to study network resilience to selective versus random deletions (Albert et al. 2000).

We performed node removal under three different likely evolutionary scenarios: (1) random loss of bones, (2) selective loss of highly connected bones, and (3) selective

loss of poorly connected bones. In a scenario of random losses all bones can be lost with same probability. In selective loss scenarios there is a bias in favor of losing either highly connected or poorly connected bones (ties were solved at random). We simulated 10,000 iterations of sequential losses for each skull and scenario.

Results

Complexity and Anisomerism

The morphological complexity in the tetrapod skull showed a significant correlation with the number of bones: Negative in *D* and *C*, and positive in *L* (Table 2). This indicates that the reduction in bone number correlates with an increase in complexity. Figure 3 shows scatter plots for *D*, *L*, and *C* along with the estimations of circular, random, and Gabriel simulations. Considering the three statistics together, the organization of skull networks clearly differs from the three theoretical models.

Whereas bone heterogeneity, *H*, did not show a significant correlation with bone number reduction, the Unpaired Bone Ratio, *UBR*, did (Table 2). Thus, there is no evidence of increase in heterogeneity for all skull bones; in this respect skull networks do not differ from Gabriel networks (Fig. 4a). However, as predicted by the anisomerism hypothesis, if we consider only the relative amount of unpaired bones, the reduction in the number of bones occurs simultaneously with an increase in complexity and specialization of individual bones, (Fig. 4b). In other words, bone number reduction is linked to skull specialization as a result of the appearance of new unpaired bones, which occur from the fusion of ancestral paired ones.

Skull Robustness to Bone Losses

The robustness test yielded different results depending on the way we removed bones (Fig. 5). A sequential removal of bones at random did not cause a net change in *D*, *L*, and *C*. Only after removal of the 15 % of bones there was a slight loss of complexity. In contrast, a selective removal of the most connected bones generated a rapid loss of complexity, while a selective removal of the less connected

Fig. 2 Calibrated phylogeny of the 44 analyzed species used for the statistical analysis (see text for description)

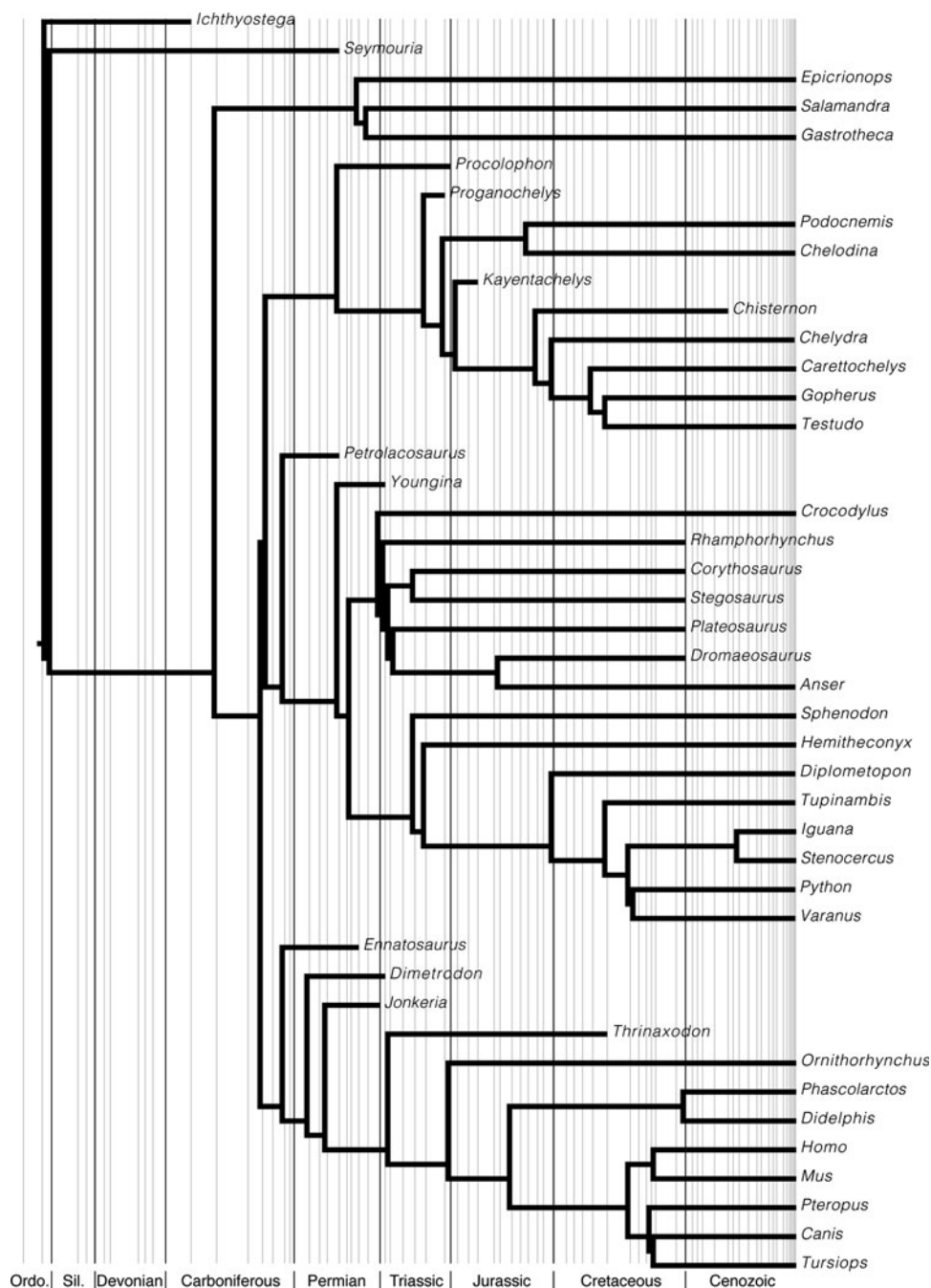
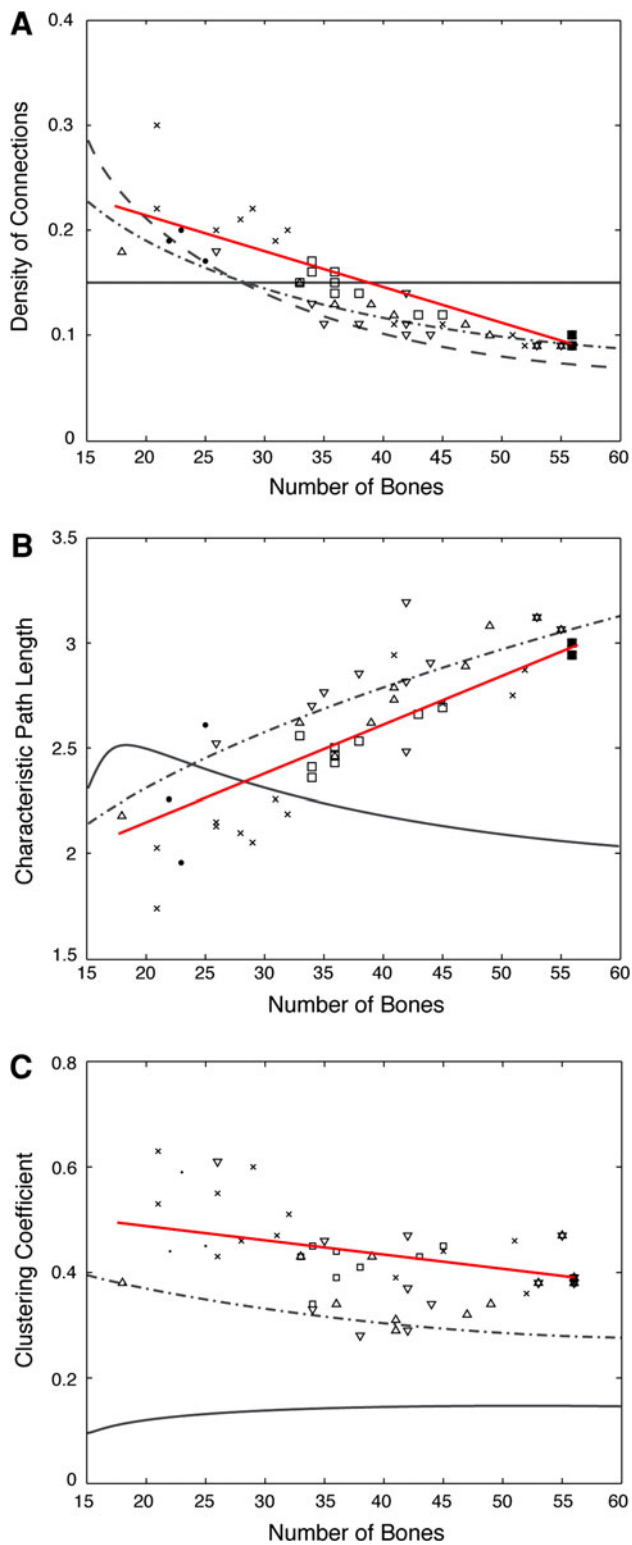


Table 2 Pearson product-moment correlation coefficient for the five network statistics

	<i>D</i>	<i>L</i>	<i>C</i>	<i>H</i>	<i>UBR</i>
Pearson's <i>r</i>	−0.827	0.797	−0.302	−0.078	−0.558
<i>p</i> value: 2-tailed	4.46·e ^{−12}	9.8·e ^{−11}	0.046	0.613	8.24·e ^{−5}

bones had the opposite effect; a slow increase of network complexity. This indicates that skull networks are robust to random losses, but fragile to selective losses of highly

connected bones. Moreover, we found that a selective loss of poorly connected bones promoted an increase of complexity; *D* and *C* increase, and *L* decreases.



Discussion

Largely inspired by Williston's work (1914), Gregory et al. (1935) showed that the vertebrate skull has undergone a general process of bone number reduction along with a

◀ **Fig. 3** Correlation of bone number reduction with morphological complexity measured with different network statistics. *Red line* indicates regression slope, *gray lines* indicate the estimations for theoretical models: *solid*, Erdős, & Renyi random network; *dashed* regular circular network; *dot-dashed* Gabriel network. **a** The density of connections shows a clear increasing trend as the bone number diminishes. In contrast, density does not vary with bone number in the random model. The circular and Gabriel model show a similar behavior than skull networks, but underestimate density. **b** The characteristic path length shows a decreasing trend with bone number reduction. The random model has a completely different behavior, while the Gabriel model shows a similar decay but mostly overestimate L . The circular model has L values out of range and is not shown here. **c** The clustering coefficient shows a slightly increasing trend with bone number reduction. Both the random and the Gabriel models strongly underestimate C when comparing with real skull networks. The circular model has a constant C equal to 0 and it is not shown. Legend: Crosses for synapsids, squares for anapsids, *up-triangles* for archosaurs, *down-triangles* for lepidosaurs, and dots for amphibians

functional specialization of each individual bone. This evolutionary trend was incorporated and accepted into general anatomical knowledge under the name of Williston's law. This law has been entrenched with the idea that the vertebrate skull has suffered an evolutionary simplification (Williams 1966 p. 43; Sidor 2001) according to the classical assessment of morphological complexity as the number of distinct anatomical elements (Bonner 1988; McShea 1991; Valentine et al. 1994).

Our results indicate that there is an increase of morphological complexity in the tetrapod skull associated with the reduction in number of skull bones during its evolution. An evolutionary pattern of skull complexity increase is even clearer if we carry out pairwise comparison of basal and modern forms (see Supplementary Material), such as from *Seymouria* and *Ichthyostega* to all living species, from *Procolophon* and *Proganochelys* to modern turtles, from *Petrolacosaurus* and *Younginia* to archosaurs and lepidosaurs, or from basal synapsids to modern mammals. In all, there is a reduction in the number of bones that is correlated with a relative increase of the number of suture relations (D), an increase in the structural proximity of bones (L), and proliferation of triangular motifs of integration (C).

The behavior of complexity estimates might be a consequence of differential rates of losses and fusions during skull evolution. For instance, an over-fusion of bones, especially along the midline, generates redundant connectivity patterns. In basal synapsids the palate is composed of three paired elements (vomeres, palatines, and maxillae); each one connects to its contralateral pair, as well as to the other bones of the same side, generating rectangular motifs. After the fusion of vomeres, rectangular motifs lead to triangular motifs; hence, the new unpaired vomer connects to two bones already connected (paired palatines and maxillae), and C increases in the skull network.

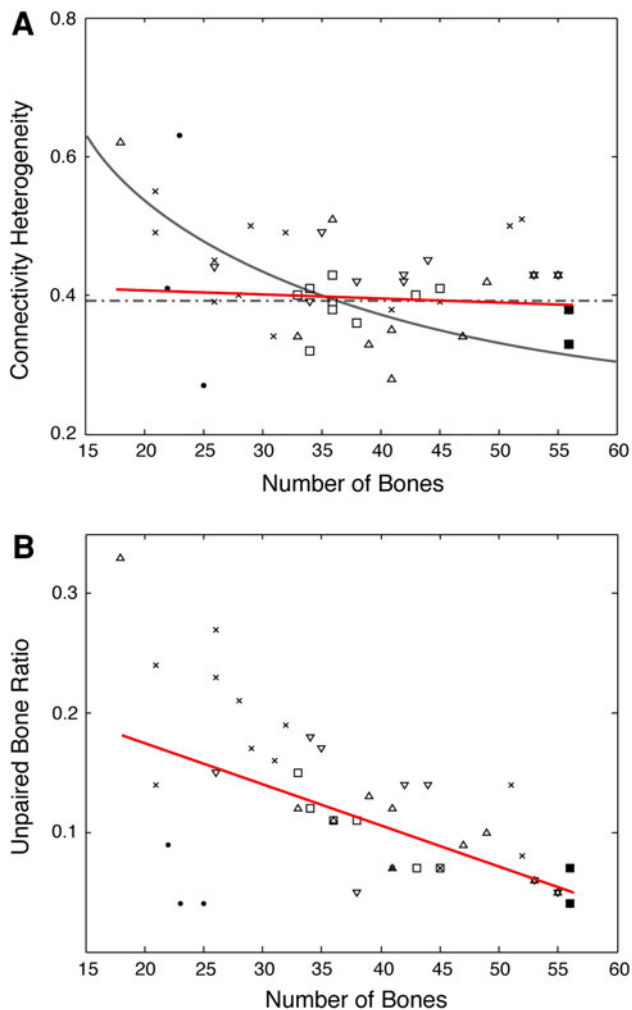


Fig. 4 Relationship between bone number reduction and anisomerism. **a** The heterogeneity of skull networks does not show correlation with bone number and behaves as the Gabriel model. In contrast, the random model shows an increase in H as the number of bones decrease. In the circular model H is constant. **b** The relative number of unpaired bones is reduced as the number of bones increases. This suggests a relationship between fusion events and bone number. Legend and symbols as in Fig. 3

On the other hand, it is known that the loss of bones provokes the reoccupation by other bones of the space left open (Girgis and Pritchard 1958; Mabbutt and Kokich 1979; Hall 2005); thus, new connections can form within this space by bones that, otherwise, were not previously connected. Because of this mechanism, a reduction in the bone number increases the density of connections provoking that distant bones now get closer; in contrast to fusions, this does not generate more triangulations. Therefore, the way in which the connections of the skull are reorganized after losses and fusions is what causes the observed pattern of complexity; which is different from that expected in theoretical models.

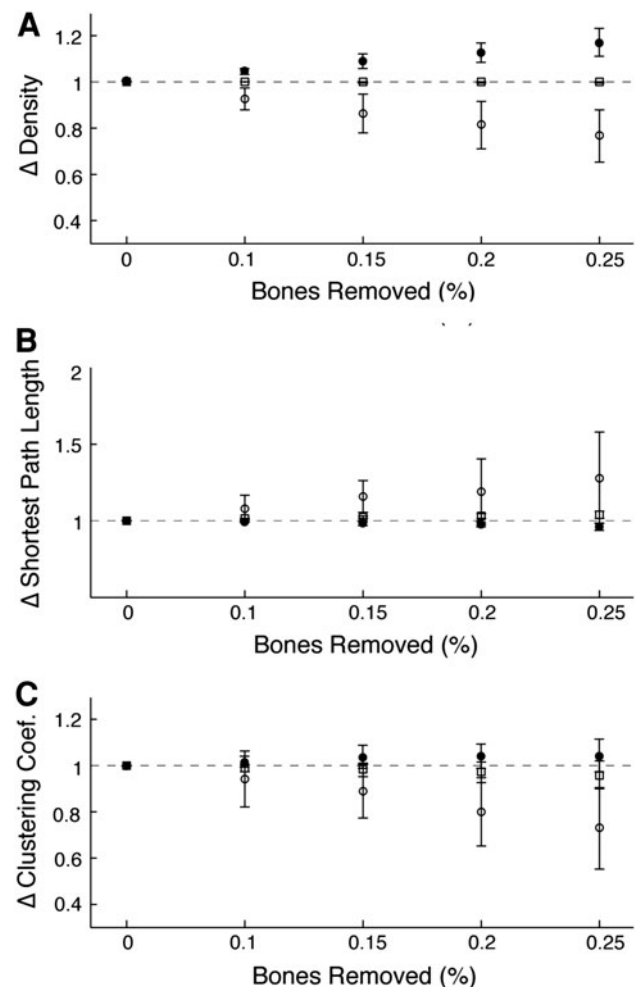


Fig. 5 Simulation of skull network complexity response after bone losses. Bones were removed sequentially according to three different scenarios: random removal of bones (*squares*), selection of more connected bones (*circles*), and selection of less connected bones (*dots*). The three complexity parameters: **a** density of connections, **b** characteristic path length, and **c** clustering coefficient shows the same behavior in each scenario. Skulls show a high robustness to losses of bones at random (complexity parameters barely change until more than the 15 % of bones are lost). In contrast, skulls are very sensitive to connectivity-selective losses. The loss of a highly connected bone weakens skull structure and makes complexity measures drop. Accumulation of such losses eventually destroys the complexity of the network. On the other hand, the loss of a poorly connected bone increases skull complexity. Of the three scenarios, selective loss of poorly connected bones (along with fusions) is the only scenario that explains the observed evolutionary pattern

Morphological Complexity and Anisomerism

Originally proposed by Gregory (1934), anisomerism is the process that guides reduction in number of skull bones by controlling developmental growth rates akin to heterochrony. For Gregory, reduction in number and differentiation of parts was one and the same evolutionary and developmental process. We find no support for a relationship between the

reduction of the number of bones and anisomerism (bone's individual complexity). This could be due to the difficulty of capturing anisomerism with a parameter (H) that does not describe individual bones.

However, the relative amount of unpaired bones does increase with bone number reduction suggesting that, indeed, there is an emergence of more specialized and differentiated bones after fusions, as predicted by the anisomerism hypothesis. This observation clearly stresses the relationship between fusion events during development and the evolutionary trend in skull bone number reduction (Aldridge et al. 2002; Richtsmeier et al. 2006).

Structural Constraints in Bone Loss and the Increase of Skull Complexity

The robustness simulation indicates that skull morphological complexity might vary after bone losses, according to the number of connections of each of the lost bones. This is a biased process; other things being equal, the loss of less connected bones will be more likely than the loss of highly connected ones, and indeed it is (Benton 1990 p. 297). Moreover, losses of less connected bones cause a net increase of morphological complexity in the skull (D and C increase, and L decreases).

This prompts two conclusions: (1) that connectivity, indeed, matters, as daringly pointed out by Saint-Hilaire, and (2) that not all the bones are equally important in maintaining skull structure. Thus, the structural stability of the skull against externally driven (environmental) or inherited bone losses varies according to the connectivity of the affected bones. Therefore, highly connected bones might have a primary role in shaping the skull with a robust internal organization. The structure would tend to collapse if these bones are lost.

Less connected, small bones often develop from single ossification centers (Rice 2008); and when these centers are lost, entire bones also disappear. These losses have minor effects upon skull architecture, because the compensatory growth of other bones can fill the space of the lost bone (Hall 2005). In contrast, more complex, specialized bones are seldom lost because they originated from fusion of several (sometimes many) centers of ossification (Koyabu et al. 2012), and would be hard to replace completely. The way most skull bones develop, by iterative fusion of ossification centers, would prevent the loss of complex, and more connected bones, whereas it would facilitate the loss of simple, less connected bones.

The observation that the developmental losses of less connected bones are responsible for this evolutionary trend in skull complexity emphasizes the relationship between connectivity of bones and their structural importance, or burden-rank (Riedl 1978). The concept of burden was

originally proposed as an organismic developmental constraint as a result of an increase in hierarchically nested constraints on traits during evolution (Schoch 2010); more connections entail more developmental dependences with other bones. In this context, the concept of burden explains the relationship between structural robustness and connectivity and the evolutionary trend in skull morphological complexity.

Conclusions

The reduction in the number of skull bones during vertebrate evolution has been interpreted as an evolutionary trend toward simplification as a consequence of selective advantages for more simplified, compact skulls. However, our results show that the reduction in bone number is not accompanied by a simplification of the skull; rather, there is an increase in the complexity of the connectivity patterns that organize the skull architecture as a consequence of how skull development buffer the harmful effects of bone losses and fusions. Our network simulations strongly suggest that a possible cause behind Williston's Law is a structural constraint by which less connected bones are more likely to be lost, shaping a general evolutionary trend toward higher skull complexity.

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