The skull roof tracks the brain during the evolution and development of reptiles including birds

Matteo Fabbri*, Nicolás Mongiardino Koch†, Adam C. Pritchard‡, Michael Hanson¶, Eva Hoffman†, Gabriel S. Bever*, Amy M. Balanoff§, Zachary S. Morris∥, Daniel J. Field†, Jasmin Camacho‡, Timothy B. Rowe*, Mark A. Norell*, Roger M. Smith∥, Arhat Abzhanov* and Bhart-Anjan S. Bhullar†

Major transformations in brain size and proportions, such as the enlargement of the brain during the evolution of birds, are accompanied by profound modifications to the skull roof. However, the hypothesis of concerted evolution of shape between brain and skull roof over major phylogenetic transitions, and in particular of an ontogenetic relationship between specific regions of the brain and the skull roof, has never been formally tested. We performed 3D morphometric analyses to examine the deep history of brain and skull-roof morphology in Reptilia, focusing on changes during the well-documented transition from early reptiles through archosauromorphs, including nonavian dinosaurs, to birds. Non-avialan taxa cluster tightly together in morphology, whereas Archaeopteryx and crown birds occupy a separate region. There is a one-to-one correspondence between the forebrain and frontal bone and the midbrain and parietal bone. Furthermore, the position of the forebrain–midbrain boundary correlates significantly with the position of the frontoparietal suture across the phylogenetic breadth of Reptilia and during the ontogeny of individual taxa. Conservation of position and identity in the skull roof is apparent, and there is no support for previous hypotheses that the avian parietal is a transformed postparietal. The correlation and apparent developmental link between regions of the brain and bony skull elements are likely to be ancestral to Tetrapoda and may be fundamental to all of Osteichthyes, coeval with the origin of the dermatocranium.

The skull roof tracks the brain during the evolution and development of reptiles including birds

Matteo Fabbri*, Nicolás Mongiardino Koch†, Adam C. Pritchard‡, Michael Hanson¶, Eva Hoffman†, Gabriel S. Bever*, Amy M. Balanoff§, Zachary S. Morris∥, Daniel J. Field†, Jasmin Camacho‡, Timothy B. Rowe*, Mark A. Norell*, Roger M. Smith∥, Arhat Abzhanov* and Bhart-Anjan S. Bhullar†

Major transformations in brain size and proportions, such as the enlargement of the brain during the evolution of birds, are accompanied by profound modifications to the skull roof. However, the hypothesis of concerted evolution of shape between brain and skull roof over major phylogenetic transitions, and in particular of an ontogenetic relationship between specific regions of the brain and the skull roof, has never been formally tested. We performed 3D morphometric analyses to examine the deep history of brain and skull-roof morphology in Reptilia, focusing on changes during the well-documented transition from early reptiles through archosauromorphs, including nonavian dinosaurs, to birds. Non-avialan taxa cluster tightly together in morphology, whereas Archaeopteryx and crown birds occupy a separate region. There is a one-to-one correspondence between the forebrain and frontal bone and the midbrain and parietal bone. Furthermore, the position of the forebrain–midbrain boundary correlates significantly with the position of the frontoparietal suture across the phylogenetic breadth of Reptilia and during the ontogeny of individual taxa. Conservation of position and identity in the skull roof is apparent, and there is no support for previous hypotheses that the avian parietal is a transformed postparietal. The correlation and apparent developmental link between regions of the brain and bony skull elements are likely to be ancestral to Tetrapoda and may be fundamental to all of Osteichthyes, coeval with the origin of the dermatocranium.

The skull roof tracks the brain during the evolution and development of reptiles including birds

Matteo Fabbri*, Nicolás Mongiardino Koch†, Adam C. Pritchard‡, Michael Hanson¶, Eva Hoffman†, Gabriel S. Bever*, Amy M. Balanoff§, Zachary S. Morris∥, Daniel J. Field†, Jasmin Camacho‡, Timothy B. Rowe*, Mark A. Norell*, Roger M. Smith∥, Arhat Abzhanov* and Bhart-Anjan S. Bhullar†

Major transformations in brain size and proportions, such as the enlargement of the brain during the evolution of birds, are accompanied by profound modifications to the skull roof. However, the hypothesis of concerted evolution of shape between brain and skull roof over major phylogenetic transitions, and in particular of an ontogenetic relationship between specific regions of the brain and the skull roof, has never been formally tested. We performed 3D morphometric analyses to examine the deep history of brain and skull-roof morphology in Reptilia, focusing on changes during the well-documented transition from early reptiles through archosauromorphs, including nonavian dinosaurs, to birds. Non-avialan taxa cluster tightly together in morphology, whereas Archaeopteryx and crown birds occupy a separate region. There is a one-to-one correspondence between the forebrain and frontal bone and the midbrain and parietal bone. Furthermore, the position of the forebrain–midbrain boundary correlates significantly with the position of the frontoparietal suture across the phylogenetic breadth of Reptilia and during the ontogeny of individual taxa. Conservation of position and identity in the skull roof is apparent, and there is no support for previous hypotheses that the avian parietal is a transformed postparietal. The correlation and apparent developmental link between regions of the brain and bony skull elements are likely to be ancestral to Tetrapoda and may be fundamental to all of Osteichthyes, coeval with the origin of the dermatocranium.

The skull roof tracks the brain during the evolution and development of reptiles including birds

Matteo Fabbri*, Nicolás Mongiardino Koch†, Adam C. Pritchard‡, Michael Hanson¶, Eva Hoffman†, Gabriel S. Bever*, Amy M. Balanoff§, Zachary S. Morris∥, Daniel J. Field†, Jasmin Camacho‡, Timothy B. Rowe*, Mark A. Norell*, Roger M. Smith∥, Arhat Abzhanov* and Bhart-Anjan S. Bhullar†

Major transformations in brain size and proportions, such as the enlargement of the brain during the evolution of birds, are accompanied by profound modifications to the skull roof. However, the hypothesis of concerted evolution of shape between brain and skull roof over major phylogenetic transitions, and in particular of an ontogenetic relationship between specific regions of the brain and the skull roof, has never been formally tested. We performed 3D morphometric analyses to examine the deep history of brain and skull-roof morphology in Reptilia, focusing on changes during the well-documented transition from early reptiles through archosauromorphs, including nonavian dinosaurs, to birds. Non-avialan taxa cluster tightly together in morphology, whereas Archaeopteryx and crown birds occupy a separate region. There is a one-to-one correspondence between the forebrain and frontal bone and the midbrain and parietal bone. Furthermore, the position of the forebrain–midbrain boundary correlates significantly with the position of the frontoparietal suture across the phylogenetic breadth of Reptilia and during the ontogeny of individual taxa. Conservation of position and identity in the skull roof is apparent, and there is no support for previous hypotheses that the avian parietal is a transformed postparietal. The correlation and apparent developmental link between regions of the brain and bony skull elements are likely to be ancestral to Tetrapoda and may be fundamental to all of Osteichthyes, coeval with the origin of the dermatocranium.
Fig. 1 | Summary of skull roof evolution and relationship to soft tissue structures in Reptilia. a, To the left, a phylogenetic tree showing presence (orange) or absence (green) of separate postparietal. The postparietal is ancestral for Reptilia and was lost several times within it, notably at the base of crown Archosauria with one reversal in Gracilisuchus. To the right, dorsal views of the segmented skull roof and brain endocast of selected taxa, with the anterior to the left, demonstrating the uniformly small size of the postparietal and the gradual transformation of the skull roof towards the avian lineages. b, A sagittal cutaway through the skull of the stem crocodylian Gracilisuchus stipanicorum including brain endocast, showing the relationship of the skull roof bones to the endocranium and the separation of the postparietal from the brain by the skull roof, braincase and nuchal musculature. Anterior to the left. c, A sagittal cutaway as in b, but of near-hatching (E46) Alligator mississippiensis, showing the relation of contrast-stained brain and nuchal musculature to skull roof and braincase. d, A sagittal cutaway of contrast-stained chicken, Gallus gallus, showing the relation of contrast-stained brain and nuchal musculature to skull roof and braincase. The frontal bone is shown in fuchsia; parietal in green; postparietal in orange; and brain endocast in blue.
To test the hypothesis that there is a close relationship between brain and skull roof, we used 3D geometric morphometrics and comparative embryology to explore the association between these structures along the entire stem and crown reptile lineage, including Lepidosauria (lizards, snakes and tuatars), stem and crown Crocodylia and Aves, as well as stem-group Archosauria and stem-group Reptilia (Fig. 1a). This sample includes pivotal taxa whose endocranial spaces and surrounding bones have never been examined (see Supplementary Table 1 for a list of taxa included in this study). Extant taxa included embryonic series for alligator (Alligator mississippiensis) and chicken (Gallus gallus), which we stained to reveal soft tissues. When embryonic skull roofs were not sufficiently ossified, we used contrast-stained brains to extract brain shapes directly.

Results and discussion

Our data revealed an overall conservation of skull-roof architecture across Reptilia, as well as conservation in the relationship between the skull roof and the brain. The adult frontal bone always lies over the forebrain, whereas the adult parietal bone lies either over the midbrain or over a combination of midbrain and posterior forebrain (Figs. 1b–d and 2). The postparietal, when present in extinct reptiles, is a diminutive dermal element that does not directly override the brain. It is generally excluded from contacting the dura mater by the parietals and supraoccipital (Figs. 1b and 2). Its most consistent relationship is with the nuchal musculature, which can be reconstructed to have attached broadly to its posterior surface (Fig. 1b).

The postparietal is lost in Lepidosauria (Figs. 1a and 2). It is commonly present, with some exceptions, along the archosaurian stem, and then almost completely disappears before the divergence of Archosauria. We confirmed, however, one case of reappearance of the postparietal within the crocodile lineage: our CT scans of Gracilisuchus stipanicorum show clear sutures between the parietals and the element posited to be a secondarily acquired postparietal (Figs. 1a,b and 2). Previous work has questioned the identity of this element and its distinction from the parietal. The reacquired postparietal of G. stipanicorum is small and, as expected, does not border the endocranial space. In all the postparietals present in the extinct reptiles we examined, we could find neither anatomical nor topological points of similarity that could be used to suggest primary homology with dinosaurian (including avian) parietals, though it is possible that in some cases the small postparietal was absorbed by the parietals to form a minute posterior eminence.

Despite an overall conservation of organization in the reptile cranium, we also detected major evolutionary alterations to its architecture. Three-dimensional morphometric comparative analyses (Fig. 3, Supplementary Figs. 1 and 2 and Supplementary Table 1; see also the list of landmarks used in the Supplementary Information) yield a tight cluster that comprises non-avialan dinosaurs, crocodile-line (pseudosuchian) archosaurs, stem archosaurs, lepidosaurs and stem reptiles. This clustering arises despite an evolutionary divergence of 250 million years, an extremely wide range of apparent ecological niches, and a size range of several orders of magnitude, from small Anolis lizards (Squamata) to giant tyrannosaurs. Principal component 1 (PC1) captures the transverse expansion of the brain and skull roof as well as the posterior shift of the forebrain–midbrain boundary and the frontoparietal suture, while PC2 represents the reduction of the parietal relative to the frontal, the expansion of the frontal, and the relative inflation of the forebrain and the cerebellum (Supplementary Fig. 3). The presence or absence of a postparietal does not seem to influence the clustering of taxa in morphospace. One of the most divergent clusters is composed of the giant allosauroids Allosaurus and Acrocanthosaurus. Their aberrant position appears to be driven solely by the depth of the skull roof, possibly for the attachment of jaw muscles, as the deepening drastically increases the surface area of lateral adductor attachment sites on the parietal. Brain endocasts of the giant

Fig. 2 | Oblique dorsolateral views of skull roofs, braincases and brain endocasts of selected newly sampled fossil taxa, anterior to the lower left.

Skull elements are cut along the sagittal plane leaving only the right sides, but endocasts are entire. Shown are the brain endocasts of early stem reptile Youngina, the stem archosaur Proterosuchus, the near-crown stem archosaur Euparkeria, the stem crocodileian Gracilisuchus, and the early-diverging dinosaur Herrerasaurus. The postparietal, where present, is uniformly small and restricted to a superficial, posterior position on the skull. The skull roof in Zanabazar is characteristic of coelurosaurians in that the frontoparietal suture is shifted backwards, closer to the forebrain–midbrain boundary. Scale bars, 1 cm.
allosauroids fall out with those of the other conservative taxa (Fig. 3c and Supplementary Fig. 2). *Archaeopteryx* and crown birds diverge from the more conservative cluster along PC1 (Fig. 3 and Supplementary Fig. 2); *Archaeopteryx* is closer to crown birds than to non-avialan maniraptorans in brain and skull-roof shape despite having a plesiomorphic endocranial volume.

The inclusion of ontogenetic series for chicken and alligator revealed that, relative to alligator ontogeny, chicken ontogeny is morphologically short. Brains and skull roofs of chicken embryos are similar to those of adults, despite our having sampled chicken ontogeny from early embryonic to late adult stages. The ontogenetic trajectory of alligator is longer, traversing a distance in morphospace equivalent to 134% that of chicken. Alligator embryos clustered with crown birds and *Archaeopteryx* in the combined and skull-roof analyses, whereas they fell within the cluster of more conservative taxa when we included only the brain (Fig. 3b,c). We noted a negative allometry between the brain and skull during the development of alligators: the brain is relatively large in the early stages of development and becomes smaller with respect to the skull during growth. On the other hand, birds have a very large brain at hatching relative to the skull, and the brain continues to expand during ontogeny, growing with positive allometry. We suggest that the brain in Aves should be considered peramorphic in recognition of earlier onset of growth, faster sustained growth, and absolutely larger adult size in comparison with all but the most crownward non-avian avialans.

Given the generally consistent clustering of taxa on the brain and skull-roof morphometric plots, we expected to find a correspondence between regions of the brain and bony elements of the skull roof. First, we tested and failed to reject the hypothesis of integration (similar levels of covariation for morphological traits between and within modules) between the skull roof and brain across the evolutionary history of reptiles (covariance ratio (CR) = 0.982, \( P = 0.092 \)), which was contrary to previous studies. This result reveals that, despite great morphological changes throughout the evolutionary history of the main reptile lineages, the general
morphologies of the skull roof and brain regions are integrated across the entire clade. Second, as we wanted to test for a direct relationship between frontal and forebrain and parietal and midbrain, we compared the anteroposterior positions of the forebrain–midbrain boundary and the frontoparietal suture, and demonstrated a significant correlation between the two ($P = 0.014$), but with an unexpected pattern (Fig. 4 and Supplementary Tables 2 and 3). The best-fitting linear regression model also included a categorical variable subdividing sampled taxa into two groups ($P = 8.0 	imes 10^{-5}$). The first group includes non-coelurosaurian reptiles, stem reptiles and non-coelurosaurian archosaurs. In this sample, some of the taxa have a frontoparietal contact positioned anterior to the forebrain–midbrain boundary. The second group consists of coelurosaurian dinosaurs, including tyranosaurs and crown birds. In these taxa, the forebrain–midbrain boundary and the overlying suture are nearly aligned (Supplementary Fig. 4). Conspicuously, alligators shift from a bird-like configuration, with the brain and skull boundaries aligned, toward a non-coelurosaurian configuration, with an offset between the two, during their ontogeny.

Thus, in coelurosaurs, compared with their successive sister taxa, the adult frontoparietal suture shifts posteriorly relative to brain compartmental boundaries in a paedomorphic retention of the original embryonic relationship (Figs. 1a, 2 and 4, and Supplementary Fig. 4). We propose that the maintenance of the posterior suture position in adults is accompanied by the incorporation of additional and more posteriorly located sources of skeletal precursor cells into the frontal, as suggested by cell-lineage labelling experiments in chicken embryos. The exact germ layer origin of these cells is unknown, but is most likely to be mesodermal, and may correspond to mesodermal precursors that contribute to the parietal bone in non-avian reptiles. We note, however, that if this is the case, there was no intrinsic morphological information contained in these precursors: the back of the crown-avian frontal does not begin to look like the front of the ancestral dinosaurian parietal during the evolutionary transition. Instead, despite altered proportional proportions, it flares outwards and has the same proportional shape and articulations as other reptilian frontals (Fig. 1). The alternative explanation is a novel contribution of more posteriorly located mesenchymal precursor cells, also resulting in a largely mesodermal but potentially mixed composition of the ‘new’ posterior part of the avian frontal. This configuration could explain the ongoing confusion and debate regarding the exact developmental origin of this structure. Some studies still suggest that the entire avian frontal is derived from neural crest cells, a migratory population of neuroectodermal derivation; other experiments suggest a mesodermal contribution. Germ layer origins have been used to argue that the avian frontal must be a fused frontoparietal because it contains cells derived from both neural crest and mesoderm, and that the avian parietal, generally accepted as being mesodermally derived, is a postparietal. However, the central part of the interparietal of mammals, which is homologous to the ancestral amniote postparietal, has been described as being derived from neural crest. This means that the avian frontoparietal hypothesis must invoke a shift in germ layer origin, the avoidance of which was its core inspiration. Moreover, the germ layer origins of cranial roof bones are more varied than previously reported. The parietal in mammals, for instance, is also reported to be of dual origin, with its central part derived from neural crest and its more lateral portions from mesoderm. In amphibians, the parietal is reportedly either mesodermally derived (in the axolotl) or of mixed neural crest and mesodermal origin (in the fused frontoparietal element of Xenopus frogs). More notably, still, the frontal of zebrafish, like that of chicken, is reported to be of composite neural crest and mesodermal origin. This raises the possibility that a composite frontal is in fact the ancestral osteichthyan condition. Data from additional groups, especially from reptiles such as representatives of Crocodylia and Squamata, and from other non-tetrapod vertebrates, are needed to establish both the ancestral osteichthyan condition and the polarity of change.

The topology of all other structures in the avian head remains consistent with our conclusion that the entire enlarged avian frontal bone is homologous to the smaller frontal in more conservative groups, and that the constricted and rotated avian parietal is nonetheless homologous to the ancestral reptilian parietal. The positional relations of these two elements to other cranial structures,
This bone-to-brain relationship had not previously been examined following what we hypothesized to be an early close association of the frontal and parietal with the brain, which is anything other than a structure homologous to the ancestral reptilian parietal. The novel and dramatic posterior expansion of the avian frontal may be stimulated by the contribution of additional skeletal progenitor cells, most likely of mesodermal origin. Overall evidence indicates that this expansion of the frontal is not a product of fusion with the parietal. Finally, we have shown a one-to-one correspondence in embryos between major parts of the brain and the early ossifications of the skull. It also highlights the fact that developmental data by itself is not sufficient to determine homology and must be interpreted within a phylogenetic framework provided by the fossil record and comparative morphology. Finally, it raises the question of whether the intimate association of the frontal and parietal with the brain, which is known to act as a major signalling centre at least in terms of facial development, is the reason for their universal conservation in bony vertebrates; and whether the repeated losses of posterior cranial elements such as the postparietal, tabulars and supratemporals are related to their more peripheral positions with respect to an influential source of molecular developmental patterning information.

**Fig. 6** | Ossification of the frontal and parietal bones in embryonic alligator, chicken and lizard. a. Ossification of the frontal (pink) and parietal (green) over the brain (blue) in embryonic alligator (top; sample size = 6) and chicken (bottom; sample size = 6), with the eyes (white/grey) and nuchal musculature (orange) shown in place. b. Ossification of the frontal and parietal in an embryonic *Chalcides* lizard (Squamata) (sample size = 2: YPM R 15062). In all of these taxa, bracketing Reptilia, the frontal (Fr) first forms over the forebrain (fb) and the parietal (Pa) first over the midbrain (mb). Scale bars, 2 mm.

**Conclusions**

We have shown that across the great change in brain size and shape in the evolution of birds from the reptile ancestor, the skull roof tracks the brain early in ontogeny and then becomes decoupled, with a truncation of this decoupling occurring in Coelurosauria. Calvarial architecture is remarkably conserved across reptiles, and we found no evidence that the avian parietal is anything other than an ancestral reptilian condition. There is no evidence that the avian frontal bone is a derived condition from the ancestral reptilian condition. In particular, the parietal is always broadly flanked by the squamosals and contacts the supraoccipital posteriorly. The orbits remain encircled by the frontals despite the greater size of eyes in crown birds, and the pseudotemporals or adductor internus group of the jaw muscles remains attached to the side of the parietal, even as this bone becomes smaller and rotates to assume a more vertical orientation (Supplementary Fig. 5). The midbrain, including the optic lobes, remains subjacent to the parietal, though it is shifted ventrally in crown birds. This conservation of topology stands in contrast to recent claims that spatial relationships of the avian frontal and parietal are modified from the ancestral reptilian condition. Thus, in terms of adult anatomical homology, there is no evidence that the avian frontal bone is a composite frontoparietal. Also, there is no evidence that the parietal is either a ‘semiparietal’ or a reacquired and radically transformed postparietal. A proposed novel contribution to the posterior part of the avian frontal and a potential shift in germ layer contribution appear to have occurred without violating the anatomical homology of the bone.

The ontogenetic shift in developing alligator from close alignment of the forebrain–midbrain boundary and frontoparietal suture to a displacement of those structures suggests that a decoupling between the brain and skull roof occurs later during development, following what we hypothesized to be an early close association of the initial ossifications of the skull roof with the divisions of the brain. This bone-to-brain relationship had not previously been examined in embryos, so we used contrast-stained computed tomography (CT) scanning to simultaneously visualize the developing brain and skull roof in *Alligator mississippiensis* and *Gallus gallus* (Fig. 6). We supplemented these data with a developmental series of the lizard *Chalcides chalcides*. In all three taxa examined there is a direct, one-to-one correspondence between the developing forebrain and the frontal bone primordium and the developing midbrain and the parietal bone primordium. Moreover, this relationship can be seen to exist in mouse and opossum, though it has attracted little comment. Contrary to a recent report based on 1937 data, the initial parietal ossifications in chicken and mammals, as well as in non-avian reptiles, appear in the same topological position relative to the brain, the other bones of the skull, the parietal bone primordium and the chondrocranion (Fig. 5). Our results therefore support the notion that the brain plays an important role in patterning the skull roof.

**Methods**

Original CT scan data of the taxa included in this analysis were acquired at the University of Texas High-Resolution X-Ray Scanning Facility (UTCT), at the University of the Witwatersrand Bernard Price Institute for Palaeontological Research (BP), and at the Harvard Center for Nanoscale Systems (CNS). Endocasts of the brain and the skull elements were segmented using the software VGStudio. The taxa stem-ward of *Proterosuchus* do not ossify the anteroventral portion of their braincases, such that endocasts were necessarily less complete. However, we only analysed those parts of the endocasts that were directly in contact with the skull roof (Supplementary Fig. 1). The braincases of the coelurosaur *Garudimimus* and of the stem archosaur *Euparkeria* were partially disarticulated and required digital re-articulation. The restoration was performed in VGStudio. Normal developmental series for *Gallus gallus* (four stages, including E12, E15, E17 and E19), *Alligator mississippiensis* (four stages, including E32, E40, E46 and hatching) and *Chalcides chalcides* (YPM R 15063) were included in the analyses for investigating the pattern of skull ossification and to test the one-to-one correlation between frontal and forebrain on the one hand and parietal and midbrain on the other. Embryos were stained in 5% (by mass) phosphomolybdic acid, before CT scanning.

© 2017 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.
The 3D configurations of landmarks were digitized with VGStudio on the CT scan data for all the taxa included in this study. Only one side of the braincase was landmarked. The right or left side of the braincase was chosen on the basis of the preservation of the braincase, quality of the landmarks and of the specimen. In the case of *Gaudryimimus*, we placed the landmarks on the right side of the braincase, because it was less deformed as suggested by the more rounded morphology of the orbit and orbitosphenoid. Generalized procrustes analysis (GPA) was applied to remove information relating to the location, size and orientation of the landmark configurations. The 3D coordinates of landmarks were subjected to a full GPA, given the reduced sensitivity to outliers of this approach.

Analysis were performed using MorphoJ v. 1.03b, which automatically reflects specimens that were digitized on alternative sides. Major patterns of morphological variability were then extracted using principal component analysis (PCA) on the procrustes-aligned coordinates. The proportion of variability in a given shape set that is, groups of landmarks (or modules) was done using k-means clustering. The method aims at partitioning all observations into k groups such that the sum of squares from all observations to their assigned cluster centers is minimized. Given the herbivorous nature of the method, results shown derive from initiating the process from 1,000 randomly located cluster centers. Number of clusters in each case was determined using R package *NbClust* that provides 30 metrics to evaluate the optimal number of clusters in a dataset. In all cases (Fig. 3 and Supplementary Fig. 2), clustering was performed using all principal components that explained a variance >1%, and the number of clusters chosen was that supported by the majority of metrics.

We used the coefficient of modularity (CR) to test the hypothesis of modularity between skull roof and brain. The covariance ratio (CR) was chosen as a measure to characterize the degree of covariance between these two a priori defined modules. CR was preferred over the widely used RV coefficient because it is not influenced by attributes of the data such as the sample size and the number of variables. Briefly, this metric represents the overall covariation between defined modules relative to the null covariation of the sets of variables, the CR coefficient has an expected value of one; significant departures towards lower values are indicative of modularity. Significance was tested using a permutation test that randomly reassigns landmarks into groups of equal size as the original partitions, and calculates the CR value for the generated subdivisions of landmarks. The degree of modularity between the skull roof and the brain was tested using: (a) the full set of digitized landmark configurations (*n* = 21), and (b) only those corresponding to adult specimens (*n* = 17). The sampling employed in the first set leads to difficulties interpreting the results, since they are determined by a mix of ontogenetic and evolutionary signals of integration across structures. Therefore, results in the main text correspond to those of set B, which only assesses the degree of evolutionary integration. In both cases, we performed 10⁴ permutations, and *P* values were empirically calculated as the fraction of permutations with CR values lower than the original. The analysis of set A (that is, including the landmark configurations of the chicken and crocodile embryos), results in a significant degree of modularity between skull roof and brain (CR = 0.967, *P* = 0.031). This might provide further evidence for the morphological decoupling between the two structures during ontogeny as discussed in the main text, but once again caution should be taken when interpreting this result.

To further explore the morphological covariance between skull roof and brain regions, we measured the relative positions of the frontoparietal and forebrain-midbrain-basal ganglia. Sagittal sections of the braincase and brain of all adults were extracted from the CT scans using VGStudio. Images were then imported into tpsDIG v.2.22, where the distance between the anterior tip of the olfactory bulbs and the forebrain was measured as the curved line joining those points along the internal side of the skull. This measure was used as a proxy for the overall skull roof length. The relative positions of both frontoparietal and fore–mid–brain structures along the same line was normalized as their distance to the tip of the olfactory bulbs divided by the overall length, thus eliminating differences due to specimen size.

A relationship between the positions of the fronto-parial suture and forebrain-midbrain suture was explored using a combination of ordinary and phylogenetic linear models. For this purpose, a phylogenetic super-tree was created in the software Mesquite v 3.04. The topography of the tree was based on previous studies for early diapsids and Archosauromorpha, Squamata, Archosauriformes and Archosauria, Tethyae, Coelurosauria and Aves. A time-scaled version of the phylogenetic tree was built using the calibration method described previously. First and last appearances of all fossil taxa were recorded from the literature (see Supplementary Table 1 for ages of the taxa (corresponding citations) and used to estimate the time of divergence of the clades represented in the analysis. This was done using the timePaleoPhy function in the R package *poleotree*. Molecular estimates for the divergence of crown clades were drawn from previous studies, and used to constrain minimum ages. To account for uncertainty in both branch length estimates and phylogenetic relationships among paravian lineages, 100 different trees were generated by assigning an age for each taxon through random sampling between its first and last appearance in the fossil record, as well as randomly resolving the polytomy at the base of Paraves. The resulting 100 trees were subsequently used in all following analysis.

We considered three increasingly complex least-squares regression models: a model of simple allometry, a model incorporating different intercepts for coelurosaur and non-coelurosaur diapsids but with same slope, and a model with both different intercepts and slopes. Division of the included taxa into a coelurosaur and a non-coelurosaur group was applied on the base of the shift in the organization of braincase-brain found through the analyses performed in this study. As already stated, ordinary least squares (OLS) and phylogenetic generalized least squares (PGLS) approach of these three models were performed (for the latter, we used the R package *paleotree*). A dummy variable representing clade membership was coded and included as a categorical factor. Given the sensitivity of methods to deviations from a strict Brownian motion model, branch length modifying parameters (*δ, ω* and *κ*) were simultaneously estimated along the regression parameters, following the recommendations of Revell. For each of the three models, the fit of OLS with simplified phylogeny (set A) was analysed using log-likelihood ratio tests (LRT). The model with the best fit was considered to be the one with the lowest AIC value overall.

Several lines of evidence favoured the OLS model including different intercepts but equal slopes for the two clades. As the result, we performed a regression of the form: 

\[ Y = \beta_0 + \beta_1 X + \epsilon \]

where *Y* is the log-braincase-brain, *X* is the log-braincase rostrum, and *ε* the error term. The model had an overall smallest AIC value, and represented a significant improvement with respect to the OLS simple allometry (*P* = 0.000); while the addition of different slopes for each clade was not considered to further improve the model (*P* = 0.394). This was also confirmed through the use of a partial F-test (*F* = 0.57, *P* = 0.464). In fact, the regression residuals were found to lack phylogenetic signal, with a value of *K* = 0.20 and *λ* = 0.73 (*P* = 0.64 and 0.37, respectively, using 100 simulations with function phylosig of R package phytools). Under such situation, OLS approaches have an estimation accuracy substantially higher than PGLS. Allowing for the simultaneous estimation of branch length modifying parameters also confirmed this result. The second best model including a clade effect with equal slopes is a PGLS approach that includes a parameter *λ* = 0 and *δ* = 0.019, transforming the tree into a star phylogeny and (almost completely) homogenizing terminal branch lengths. This transformation effectively eliminates residual correlation due to shared evolutionary history from the variance-covariance matrix of the linear model (analogous to the PGLS, approach discussed by Revell, with the addition of a parameter *δ* = 0 to eliminate differences in terminal branch lengths), resulting in an approach equivalent to an OLS.

Data availability. CT data are in part publicly available in the *digimorph.org*. The remnant CT data are available through the corresponding author, upon reasonable request. Landmarks are available in the Supplementary Information. 

Received: 31 May 2017; Accepted: 24 July 2017; Published online: 11 September 2017

References


© 2017 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.
ARTICLES


Acknowledgements

We are grateful to E. J. Rayfield, A. R. Cuff, Y. Kozasa, S. Chatterjee, A. Turner and L. Witmer for providing CT data; to G. Watkins-Colwell for assisting with cleared and stained squamate embryo specimens; and to D. Smith for imaging. F. A. Jenkins, Jr. J. A. Gauthier, G. Wagner and G. Navalón provided useful discussion. E. M. Selton, H. Maddin and J. Hanken commented on the early stages of the work as it was underway. All Yale authors are supported by funds from Yale University. D.I.F. is supported in part by NSF DDDG DEB 1507098 to B.-A.S.B and D.I.F. A.P. is supported by an NSF Postdoctoral Research Fellowship in Biology. J.C. was partially funded by an NSF Graduate Research Fellowship and by NSF DDDG DEB 150690. A.A. was supported by NSF grant 1257122, the Templeton Foundation grant RFP-12-0 and by funds from Imperial College London. G.S.B. and M.A.N. are supported in part by NSF DEB 1457181.

Author contributions


Competing interests

The authors declare no competing financial interests.

Additional information

Supplementary information is available for this paper at doi:10.1038/s41559-017-0288-2. Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to M.E. or A.A. or B.-A.S.B.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.