The Phenotypic Interplay Between the Brain and Skull in Developing Chicken (*Gallus gallus*) and American Alligator (*Alligator mississippiensis*)

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Background

- The brain and skull are spatially adjacent and share important developmental pathways influenced by biological and environmental factors.[2, 3]
- Studies suggest that developmental mechanisms, rather than ecological and functional factors, explain a major proportion of craniofacial variation in birds.[1, 4, 6]
- Most phenotypic studies so far have focused on analyzing brain and skull development and evolution separately.
- As birds have proportionally large brains comparable to mammals, understanding their brain-skull integration can elucidate the consequences of large brains on skull formation, including in humans.
- In line with the tenets of osteopathic medicine, we gain a holistic view of how one region of the body influences another by analyzing high-resolution datasets from multiple tissue types.

Hypotheses

- **Does brain shape correlate significantly with skull shape during development in chickens?** If so, which regions of the brain and skull undergo coordinated changes?
- **Do chicken and alligators (closest living group to birds) exhibit the same pattern of correlated brain-skull development?** A similar pattern would suggest a shared, conserved relationship between brain and skull development across distantly-related species.
- **Do these developmental patterns mirror the correlated brain-skull shape variation that we observe across modern birds?** If the developmental patterns of brain-skull integration are similar, this suggests that the interaction has had profound influence on the way diverse skull shapes have evolved in birds.

Materials & Methods

Experimental Design: Observational (Cross-Sectional) of developing chickens and alligators.

High-Resolution 3D Brain & Skull Models: We used high-resolution computed tomography (CT) scans of unstained and iodine-stained chicken (*Gallus gallus; n* = 9) and alligator skulls (*Alligator mississippiensis; n = 5*) in several different stages of development. 3-D skulls and brains reconstructed using the CT and 3D model programs VG Studio and Geomagic Wrap.

High-Density Landmark-Based Shape Data: We placed 3D coordinate points on the skull and brain reconstructions. In the skull, we demarcated the shape of 16 cranial regions, mostly at the level of individual bones. For brain shape data, we characterized the morphology of major developmental and functional regions: cerebrum, optic lobe, medulla, and cerebellum (*Fig. 1*).

Analysis in R Programming Language: We used two-block partial least squares analysis to determine how the brain, skull, and their major regions covary during development. Then we compared the patterns of brain-skull covariation in chickens and alligators to modern birds.

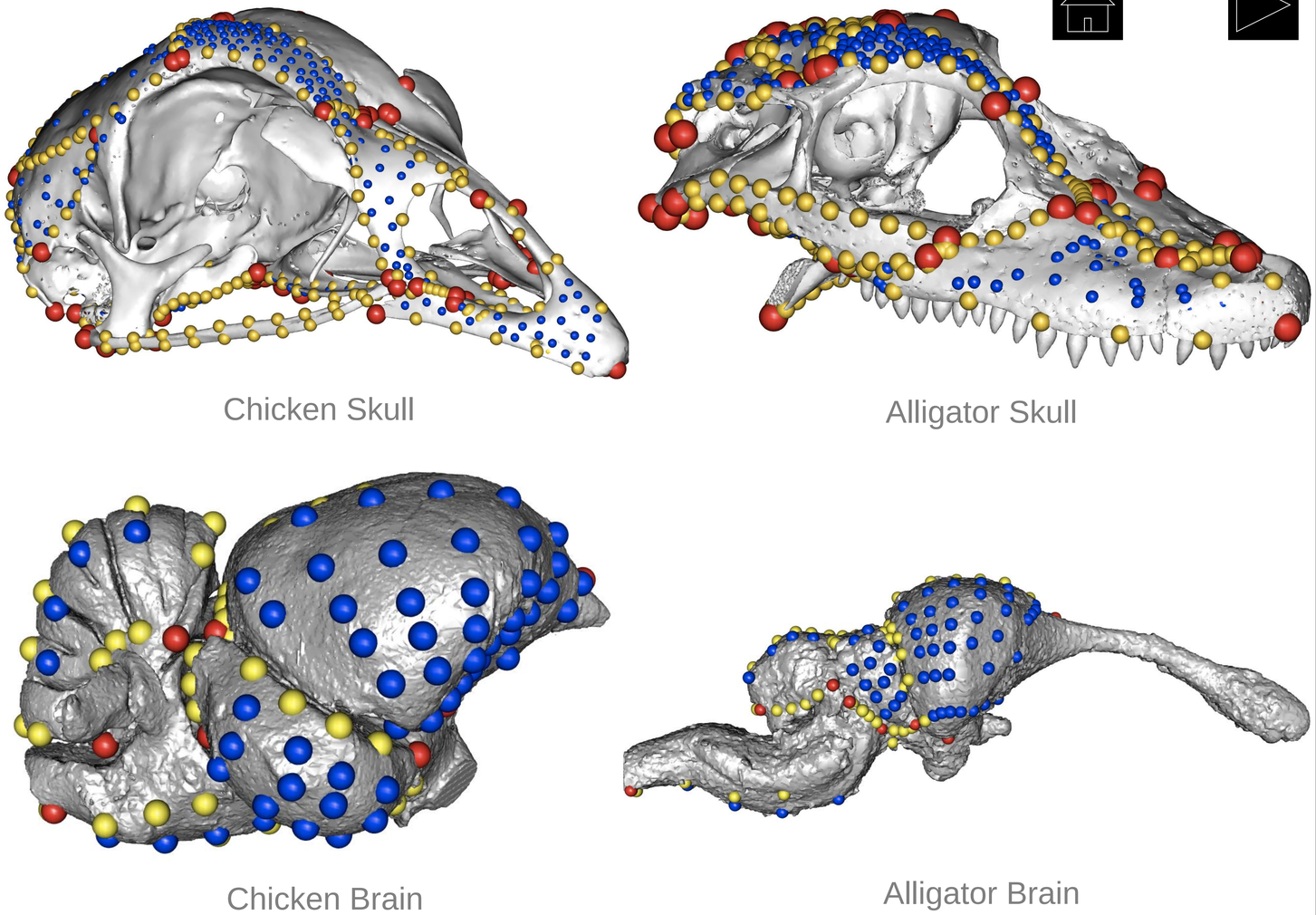

Fig. 1. 3D shape data used in this study. Fixed (red), curved (yellow) and template (blue) landmarks of skull (top) and brain (bottom) reconstructions of chicken (left) and alligator (right). Images not to scale.

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1. Does brain shape correlate significantly with skull shape during development in chickens?

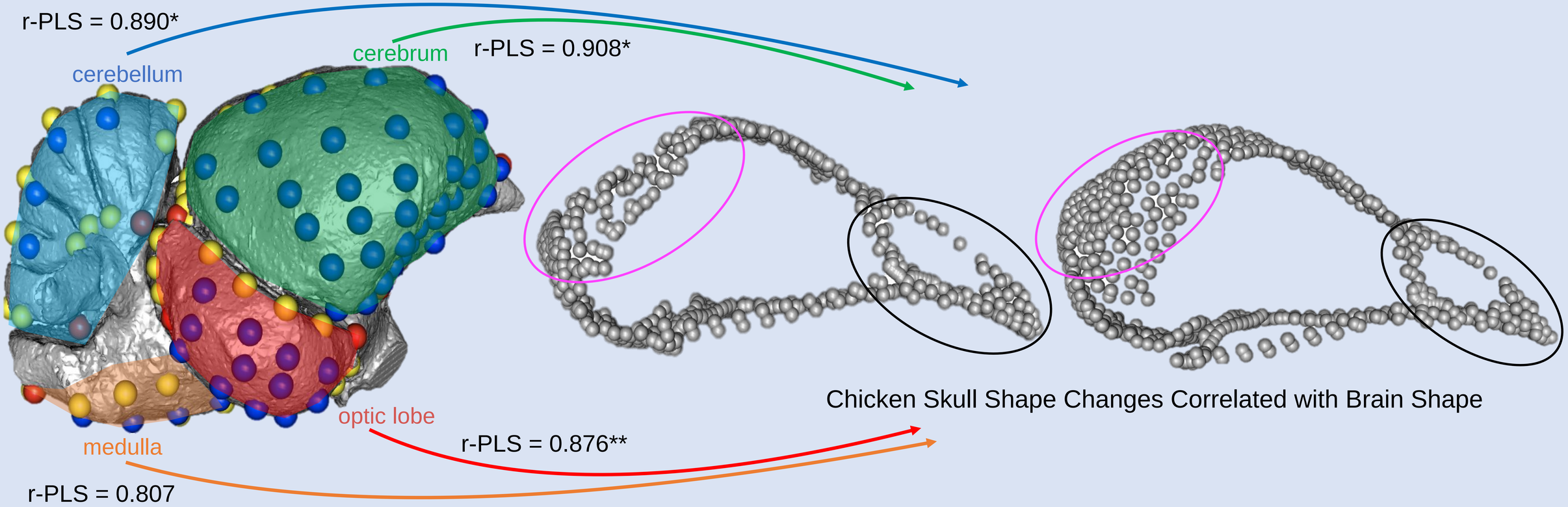

Fig. 2. Correlated skull shape (right) changes shown in lateral view in developing chicken associated with brain shape (left). The magnitude of brain-skull correlation for each region is measured by correlation coefficients (r-PLS values). * and ** denote P-values < 0.05 and <0.01.

***Yes*. Brain and skull shapes in developing chickens are statistically significantly correlated.** Specifically, brain shape is strongly correlated with skull roof (magenta circle) and anterior part of the skull (black circle), including the beak based on correlation coefficients *(Fig. 2)*. The strongest brain-skull correlations in chickens were observed for the cerebrum (r-PLS = 0.908, P = 0.02), as well as for the optic lobe (r-PLS = 0.890, P = 0.04).

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2. Do chickens and alligators (comparative outgroup to chickens) exhibit the same pattern of correlated brain-skull development?

Fig. 3. Correlated skull shape (right) changes in developing alligators associated with brain shape (left). The magnitude of brain-skull correlation for each region measured by correlation coefficients (r-PLS values). Inset shows the 3D skull model of a hatchling American alligator in lateral view as reference.

Yes, but… **results are not significant likely due to low sample size.** However, alligators show similar patterns in brain-skull integration to chickens where brain shape influences the skull roof and the snout morphology *(Fig. 3)*. Also, among brain regions, the cerebrum is most associated with skull shape changes, but unlike chickens, the cerebellum is tightly correlated with skull shape.

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3. Do these developmental patterns mirror the correlated brain-skull interplay that we observe across modern birds?

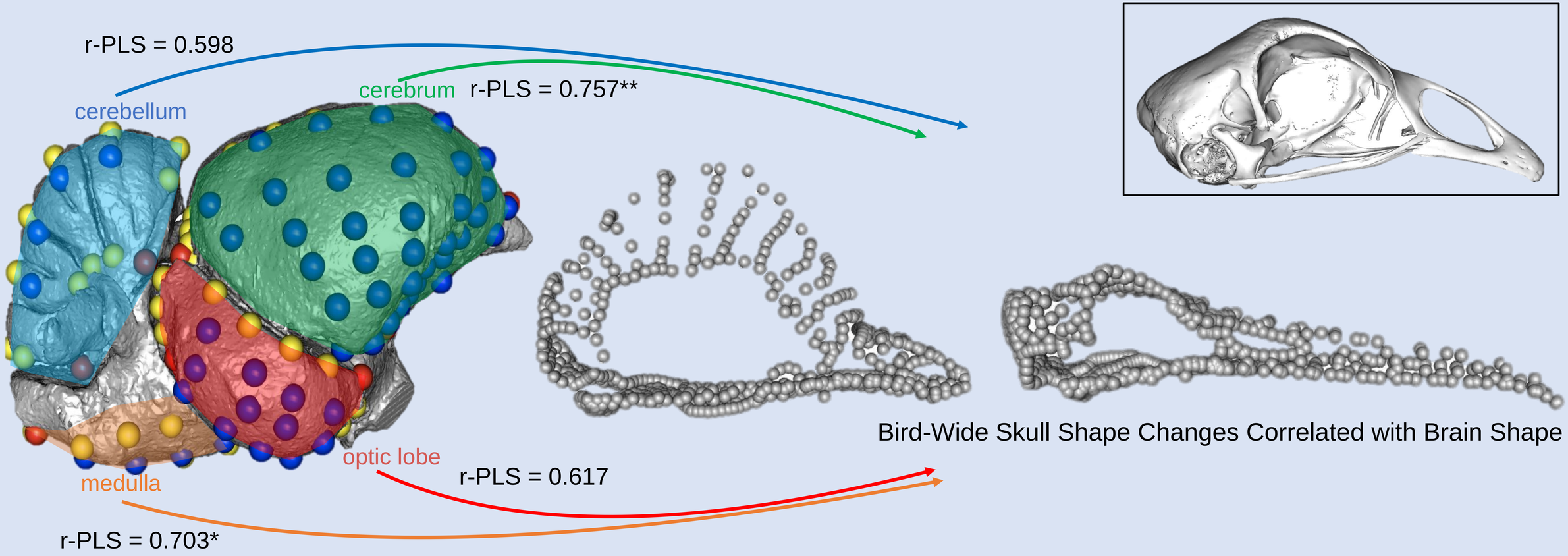

Fig. 4. Correlated skull shape (right) changes in lateral view associated with brain shape (left) across modern birds. The magnitude of brain-skull correlation for each region is measured by correlation coefficients (r-PLS values). * and ** denote P-values < 0.05 and <0.01. Inset shows the 3D skull model of an adult chicken in lateral view.

Yes. When compared to our earlier study, the developmental association between the brain and skull in chickens mirrors the evolutionary pattern of how brain shape correlates with skull shape across the avian tree of life, including skull roof and beak shape *(Fig. 4)*.

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Conclusions

- In both chickens and alligators, **the cerebrum (forebrain) has the greatest influence on skull shape** development, particularly in the skull roof and the beak. This result agrees with known molecular signaling pathways that originate from the cerebrum and drive beak formation[6].
- The patterns of brain-skull integration are similar between chickens and alligators, implying a **conserved pattern of brain-skull interplay.**
- This developmental interplay between the brain and the skull mirrors the evolutionary pattern in brain-skull integration. Therefore, the **influence of the developing brain on skull formation** was likely an essential **catalyst underlying diverse skull shapes seen in birds today**.

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Acknowledgments

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