Studying the skull and neuroanatomy of a Mesozoic bird from Argentina

Abstract – Fossils can preserve the physical remains of extinct taxa in exquisite detail, as well as traces of behavior, but they rarely provide direct evidence of the behaviors in which extinct organisms engaged. Behavior is ultimately governed by the brain, but the brain itself does not fossilize, so the brain **endocasts** of extinct species are often used to make inferences about their behavior. The brain endocast (referred to from here on as the endocast) is generated from the cavity in the skull in which the brain was located during life, and the inner surface of this bony cavity has been shown to be a relatively faithful representation of the external **morphology** of brains in birds. Thus, the endocasts of birds provide a good estimate of brain volume and of the surface areas of externally-visible brain structures. Unfortunately, the known correlations between the sizes of individual brain structures and behavior in **extant** birds are based on internal **neural** volume, whereas only the surface area of the externally-visible portion of a brain structure is available for extinct birds. Without a better understanding of the brains and behaviors of extinct members throughout the avian lineage, we have a very limited ability to understand the evolutionary pathways that led to the complex brain organization and specialized behaviors we see in these animals today. My dissertation aims to increase the interpretability of avian endocasts by determining which factors have influenced avian brain evolution as it is expressed in endocast morphology and by establishing statistical relationships between external surface area and internal volume in a few behaviorally relevant neural structures. Doing so will allow me to make predictions about behaviors in extinct birds based on overall endocast morphology and by using surface areas measurements of endocast structures to predict volumes of brain structures. My study of avian brain evolution will barely scratch the surface if I do not include extinct birds, as these allow me to observe the changes in **neuroanatomy** that occurred along the transition from **non-avian dinosaurs** to birds. The proposed project will fill in a significant gap in my dataset of extinct birds as well as in the field of paleontology's understanding of birds from early in the group's evolution by allowing me to study *Patagopteryx deferrariisi*, a bird from the **Mesozoic Era** of Argentina.

Narrative

Introduction – The brains of birds rival those of mammals in the complexity of their organization and the behaviors they mediate, but they have long been underestimated because they evolved through a completely separate pathway from mammals (Jarvis et al. 2005). Researchers are still determining the links between neuroanatomy and behavior in living avian species, but is generally accepted that an increase in the size of a brain region is caused by an increase in the number of **neurons** within it, which in turn should increase the ability of that region to process information (Jerison 1973). Studies based on this principle have focused on determining if enlargements of specific brain regions are tied to increases in the processing powers of these regions, which should be reflected in behaviors tied to the information processed therein (Wylie et al. 2015). Although these studies have not always shown clear correlations, as many brain regions have multiple connections with other regions, there have been some studies showing positive relationships between the volume of a neural structure and the behaviors mediated by that structure (Iwaniuk and Wylie 2006, Iwaniuk et al. 2008, Gutiérrez-Ibáñez et al. 2009, Walsh and Milner 2011a, Wylie et al. 2015).

To truly understand the evolutionary trajectory that led to the unique and complex organization of bird brains, we must study the brains of extinct birds, but these are only represented by brain endocasts, or casts of the size and shape of the bony cavity occupied by the brain. Unlike the endocasts of most of their non-avian dinosaur relatives, the endocasts of birds faithfully represent external brain structure (Iwaniuk and Nelson 2002). This fidelity has allowed paleontologists to study endocasts of extinct birds and combine their anatomical findings with the correlations between neuroanatomy and behavior established in extant birds to make inferences about behavior in extinct species (Elzanowski and Galton 1991, Burish et al. 2004, Ashwell and Scofield 2008, Milner and Walsh 2009, Walsh and Milner 2011b, Ksepka et al. 2012). These and other studies of the endocasts of extinct birds are motivated by a desire to elucidate when and how changes in neuroanatomy and behavior occurred and led to the diversity seen in modern birds. My dissertation shares this motivation and seeks to address a limitation of previous studies: they assumed that the surface area of an external brain structure, which can be seen on the endocast, is a fair proxy for the volume of the underlying internal neural structure, which is only available from brains. To test this hypothesis, I have collected data on the surface area of endocast structures and statistically compared these measurements to the volume of the corresponding brain structures in extant birds, which I compiled from the literature. I then use the resulting statistical relationship to infer the volume of the underlying brain structures based on the surface area of the relevant endocast structures measured on the endocasts of extinct birds. These volumes can then be used to describe the neuroanatomy of extinct birds and may allow behavioral inferences to be made with greater confidence.

Without fossils, our understanding of how a given system or structure evolved is limited to only the most recent fraction of time during which that evolution occurred. In addition, sampling throughout "**deep time**" often reveals that evolution is not as unidirectional as it might appear if we had only looked at the earliest member of a group and the most recent member. For example, it is generally thought that one of the characteristics of avian brain evolution is the relative expansion of a midbrain structure called the **optic tectum**, but my own work on a recently-extinct bird has shown that its optic tecta were **secondarily** reduced (Early et al., in prep.). I have endocasts of a few fossils in my dissertation dataset already (Fig. 1, Appendix), including one from the Mesozoic Era, the time in which birds first evolved from non-avian dinosaurs. The endocasts of Mesozoic birds are critical to my dissertation because they simultaneously serve as a baseline to which I can compare the neural elaboration seen in extant birds and as a goal for behavioral predictions that can only be effectively grounded by the study of the relationships between endocasts, brains, and behavior in extant birds. Unfortunately, there are very few Mesozoic birds with three-dimensionally (3D) preserved skulls, which is a requisite for making virtual endocasts. One of the few fossils that meets these criteria, *Patagopteryx deferrariisi*, is housed in Argentina (Alvarenga and Bonaparte, 1992; Chiappe, 2002). The cranial anatomy of this specimen has not been described in detail, making it hard to study in comparison to its extant relatives, and national policies dictate that, due to its scientific importance and rarity, it cannot leave the country.

Objectives – I am requesting funds to go to Argentina to study and scan this specimen using **computed tomography (CT)** and add it to my extinct avian sample. I will generate an endocast from this extinct bird and make inferences about its neuroanatomy and behavior based on the statistical relationships I have established in extant birds. In addition, I will study the specimen in person to observe anatomical details which cannot be evaluated from published photographs and descriptions. Studying these aspects of external anatomy will allow me to include this specimen in chapters of my dissertation about other neuroanatomical traits of birds.

Materials and Methods – I have compiled brain volumes for the extant birds in my sample from the literature (Iwaniuk et al., 2004, 2008, 2006, 2010; Iwaniuk and Wylie, 2006, Gutiérrez-Ibáñez et al. 2013, 2014). With the support of other grants, I have borrowed and CT scanned the skulls of the same species for which I have brain structure volumes, and I have been generating endocasts from these scan data using the software programs available in my advisor's lab (Avizo, Maya). As I generate these endocasts, I measure the surface area of the endocast structures and regress the volumes of the brain structures on these values. This regression yields an equation which can be used to predict the volumes of these brain structures in fossil specimens, for which surface area is the only metric available. These predicted volumes will allow fossil taxa to be incorporated into the analyses of comparative studies of these brain structures that have linked brain structure size with behavioral specializations. This portion of data collection for my dissertation has already been funded and the processing and analysis of the data is underway, but it is directly tied to the work for which I am requesting funding, and the endocast generation methods will be applied to the specimen studied in the proposed project.

The present proposal requests funding for study and CT scanning of a Mesozoic bird from Argentina to generate an endocast of and make predictions about the behavior of this extinct species. *Patagopteryx deferrariisi* (MACN-N-11), a bird from the **Cretaceous Period**, is housed in the collections of the Museo Argentino de Ciencias Naturales (MACN) in Buenos Aires, Argentina. MACN-N-11 is the most complete skull known for this species, and for this reason it cannot leave Argentina. Currently, there are fewer than ten published endocasts of Mesozoic birds, and fewer than five of these are based on CT scans and thus available for true 3D study and comparison, so CT scanning and publishing the endocast of even one Mesozoic bird is hugely impactful to the field of paleontology. Although MACN-N-11 is not perfect, it is still a remarkably complete 3D-preserved braincase for a Mesozoic bird and will yield important insights into the endocranial anatomy of this group. To study it, I will need to fly to Buenos Aires, borrow the specimen (the loan of which has already been granted; see Appendix), hand-carry it to a CT scanning facility within Buenos Aires province, and return it to the collections. There is a microCT scanner at YPF Tecnología (https://y-tec.com.ar/Paginas/caracterizacion-digital.aspx), a collaborative enterprise between an Argentinian energy company and CONICET, the latter being the main organization in charge of promoting funding and science (equivalent to the National Science Foundation). My colleague at CONICET, Dr. Federico "Dino" Degrange, has been working to reserve time for us to use this equipment (see Appendix).

Timeline – I am planning this trip for early December, when I will have a break in my teaching commitments. If I am not able to visit then, I will try for a later date in the spring. I plan to spend a week studying this specimen and potentially others in the MACN collections, and I will process the CT scan data in my advisor's lab when I return.

Significance – We cannot fully understand the behaviors we see in birds today without knowing how and when these behaviors evolved. This study will enhance my attempts to put the evolution of behavior on a solid foundation that extends into "deep time" by adding a specimen from a critical point in avian evolution to the sample of endocasts available to paleontologists. It directly impacts our understanding of the neuroanatomy and behavior of Mesozoic birds and the evolution of brains in extant birds. As endocasts of Mesozoic birds are very rare, this project will likely merit its own publication to make the findings available to other paleontologists as soon as possible, and being able to make such an important contribution to the field so early in my career would be highly beneficial to me. The act of CT scanning the specimen is itself meritorious, as it will result in a 3D record of a scientifically significant specimen that will serve as a virtual "backup" in case of damage, loss, or destruction. The data collected with this funding will also be presented alongside the rest of my dissertation research at conferences, at which I have an excellent track record of attending and presenting.

Literature Cited

Alvarenga HMF, Bonaparte JF. 1992. A new flightless landbird from the Cretaceous of Patagonia. *Papers in Avian Paleontology: Natural History Museum of Los Angeles County, Science Series* 36:51-64.

Ashwell KWS, Scofield RP. 2008. Big birds and their brains: paleoneurology and the New Zealand moa. *Brain, Behavior and Evolution* 71(2):151-166.

Burish MJ, Kueh HY, Wang SS-H. 2004. Brain architecture and social complexity in modern and ancient birds. *Brain, Behavior and Evolution* 63(2):107-124.

Chiappe LM. 2002. Osteology of the flightless *Patagopteryx deferrariisi* from the Late Cretaceous of Patagonia (Argentina). In: Chiappe LM, Witmer LM, editors. *Mesozoic Birds: Above the Heads of Dinosaurs*. Berkeley: University of California Press. p 281-316.

Early CM, Porter WR, Cerio DG, Ridgely RC, Witmer LM. in prep. The endocranial anatomy of *Dinornis robustus* (Aves: Dinornithiformes): a comparison to other palaeognaths and consideration of behavioral implications.

Elzanowski A, Galton PM. 1991. Braincase of *Enaliornis*, an early Cretaceous bird from England. *Journal of Vertebrate Paleontology* 11(1):90-107.

Gutiérrez-Ibáñez C, Iwaniuk AN, Lisney TJ, Wylie DRW. 2013. Comparative study of visual pathways in owls (Aves: Strigiformes). *Brain, Behavior and Evolution* 81(1):27-39.

Gutiérrez-Ibáñez C, Iwaniuk AN, Moore BA, Fernández-Juricic E, Corfield JR, Krilow JM, Kolominsky J, Wylie DRW. 2014. Mosaic and concerted evolution in the visual system of birds. *PLoS ONE* 9(3):e90102.

Gutiérrez-Ibáñez C, Iwaniuk AN, Wylie DRW. 2009. The independent evolution of the enlargement of the principal sensory nucleus of the trigeminal nerve in three different groups of birds. *Brain, Behavior and Evolution* 74(4):280-294.

Iwaniuk AN, Dean KM, Nelson JE. 2004. A mosaic pattern characterizes the evolution of the avian brain. *Proceedings of the Royal Society B* (suppl.) 271:S148-S151.

Iwaniuk AN, Gutiérrez-Ibáñez C, Pakan JMP, Wylie DRW. 2010. Allometric scaling of the tectofugal pathway in birds. *Brain, Behavior and Evolution* 75(2):122-137.

Iwaniuk AN, Heesy CP, Hall MI, Wylie DRW. 2008. Relative Wulst volume is correlated with orbit orientation and binocular visual field in birds. *Journal of Comparative Physiology A* 194(3):267-282.

Iwaniuk AN, Nelson JE. 2002. Can endocranial volume be used as an estimate of brain size in birds? *Canadian Journal of Zoology* 80(1):16-23.

Iwaniuk AN, Wylie DRW. 2006. The evolution of stereopsis and the Wulst in caprimulgiform birds: a comparative analysis. *Journal of Comparative Physiology A* 192(12):1313-1326.

Jarvis ED, Güntürkün O, Bruce L, Csillag A, Karten H, Kuenzel W, Medina L, Paxinos G, Perkel DJ, Shimizu T, Striedter G. Avian brains and a new understanding of vertebrate brain evolution. *Nature Reviews Neuroscience* 6(2):151-9.

Jerison HJ. 1973. *Evolution of the Brain and Intelligence in Vertebrates*. New York: Academic Press.

Ksepka DT, Balanoff AM, Walsh S, Revan A, Ho A. 2012. Evolution of the brain and sensory organs in Sphenisciformes: new data from the stem penguin *Paraptenodytes antarcticus*. *Zoological Journal of the Linnean Society* 166(1):202-219.

Milner AC, Walsh SA. 2009. Avian brain evolution: new data from Palaeogene birds (Lower Eocene) from England. *Zoological Journal of the Linnean Society* 155(1):198-219.

Walsh S, Milner A. 2011a. Evolution of the avian brain and senses. In: Dyke G, Kaiser G, editors. *Living Dinosaurs: The Evolutionary History of Modern Birds*. Hoboken: John Wiley and Sons. p 282-305.

Walsh S, Milner A. 2011b. *Halcyornis toliapicus* (Aves: Lower Eocene, England) indicates advanced neuromorphology in Mesozoic Neornithes. *Journal of Systematic Palaeontology* 9(1):173-181.

Wylie DR, Gutiérrez-Ibáñez C, Iwaniuk AN. 2015. Integrating brain, behavior, and phylogeny to understand the evolution of sensory systems in birds. *Frontiers in Neuroscience* 9:281.

Budget

Budget items covered by this proposal			
Item	**Source**	**Cost**	**Total**
Roundtrip flight from Columbus, OH to Buenos Aires, Argentina	Google Flights	$1,276	$1,276.00
		Total requested from GSS	**$750.00**
		Remainder to be covered by personal funds	$526.00
Budget items covered by other funding sources			
Item	**Source**	**Cost**	**Total**
Renting car in Buenos Aires, Argentina	https://www.hertz.com.ar/	$70.82 for 2 days	$70.82
Gas for round trip from MACN to YPF Tecnología	US General Services Administration	$0.535/mile for 79.66 miles	$42.62
Per diem in Buenos Aires, Argentina	US State Department	$129.00/day for 7 days	$903.00
Housing in Buenos Aires, Argentina	US State Department	$267.00/night for 6 nights	$1,602.00
		Total from other funding sources	$2,618.44
		Project Total	$3,894.44

Budget Justification

All costs listed in the budget are associated with travel to and from Buenos Aires, Argentina to study MACN-N-11, a specimen of *Patagopteryx deferrariisi* at the Museo Argentina de Ciencias Naturales. The rental car will only be needed for the day of CT scanning at YPF Tecnología in La Plata, Buenos Aires province, Argentina, so I will pick it up on that day and return it the following day.

I plan to apply for a grant through the Jurassic Foundation to help with costs not covered by the Original Work Grant, but if I am unsuccessful in that application, I will cover the remaining costs out of pocket.

Curriculum Vitae Removed to Maintain Applicant Privacy

Glossary

computed tomography (CT) scanning: an X-ray-based imaging technique that takes multiple X-ray images from 360°, allowing the user to see inside an object without cutting it open and, with proper data processing, to generate three-dimensional models from two-dimensional slice data.

Cretaceous Period: one of the subdivisions of the Mesozoic Era spanning 145-66 million years ago.

deep time: the concept of geologic time, which includes all the time in Earth's history, not just the portion during which humans have been present.

endocast: the internal cast of a hollow object; in the context of this proposal, it always refers to the space in the skull once occupied by the brain.

extant: still in existence or surviving; not extinct.

Mesozoic Era: the interval of geological time spanning about 252-66 million years ago comprising the Triassic, Jurassic, and Cretaceous Periods; non-avian dinosaurs lived and went extinct during this Era.

morphology: the subdiscipline of biology focused on the study of the form, structure, and anatomy of organisms.

neural: relating to the nervous system.

neuroanatomy: the anatomy of the nervous system.

neurons: the specialized cells that make up brains and nerves.

non-avian dinosaurs: the dinosaurs, or members of the group Dinosauria, that are not birds; this is an important distinction, as simply saying "dinosaurs" would refer to all members of Dinosauria, i.e. non-avian dinosaurs and birds.

optic tectum: a cellular structure within the midbrain, involved in a major visual pathway in birds, which makes up the outer surface of the optic lobe, a prominent endocast structure.

secondarily: describing a change in a trait from the ancestral state of that trait; often refers to reversions, so for example, flightless birds known as ratites (ostriches, kiwi, etc.) are secondarily flightless because they are descended from flighted birds, but the flighted birds from which they descended would have evolved from flightless ancestors.

Appendix

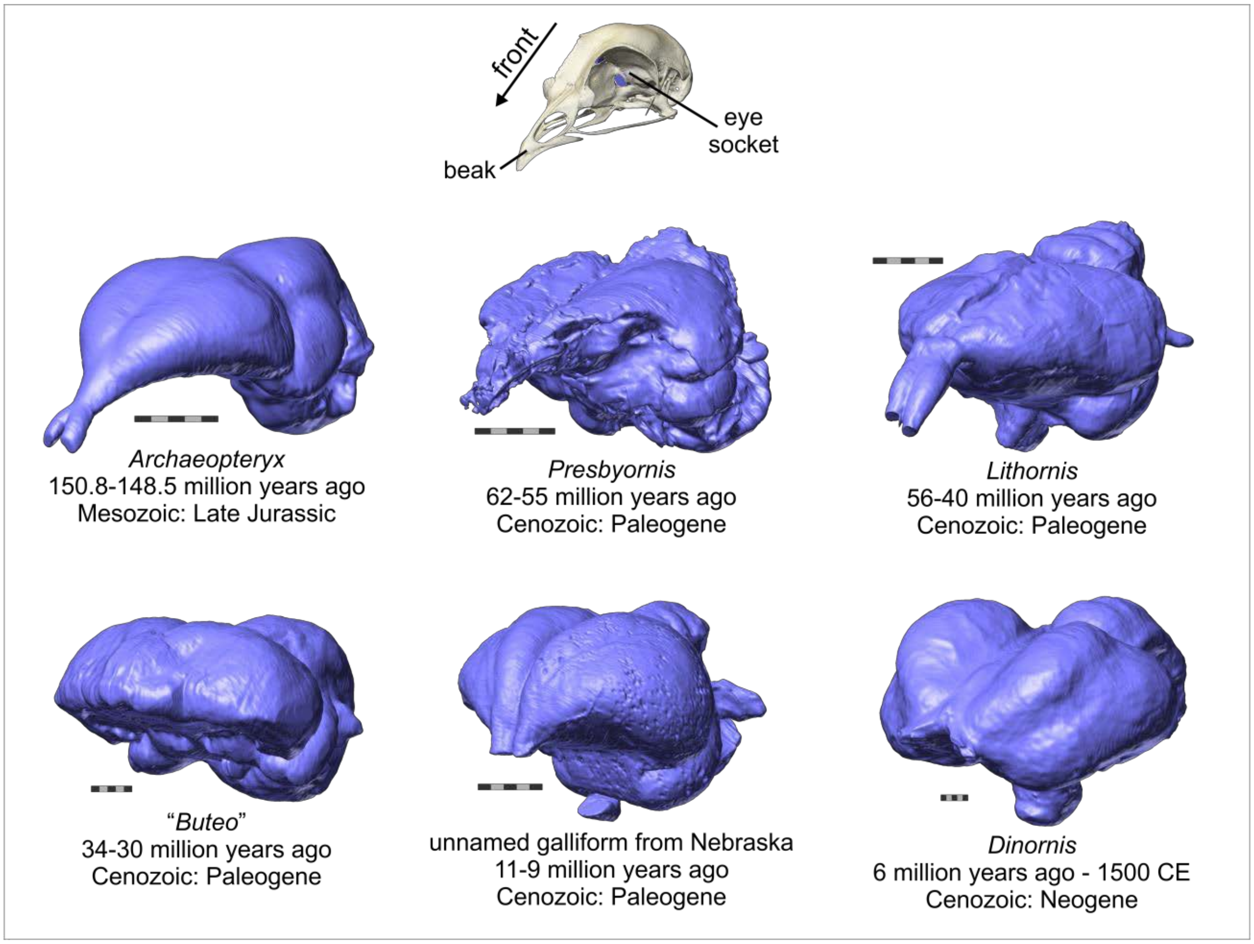

Figure 1: Endocasts of the extinct birds included in my dissertation sample so far. The turkey skull at the top provides orientation. The endocasts in the top row were generated and contributed by my advisor, Dr. Lawrence Witmer, and his lab technician, Ryan Ridgely. The endocasts in the bottom row were generated by me from CT scan data collected by various collaborators.

Appendices Removed to Maintain Applicant Privacy