Evolution and Integration of Avian Caudal Skeletal Morphology

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This dissertation titled
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ABSTRACT

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Evolution and Integration of Avian Caudal Skeletal Morphology

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The great diversity of avian species relates to their prodigious capacity for locomotor variation. The derived structure and function of the avian tail represents one evolutionary innovation that contributes to this variation. The tail supplements the role of the wings in flight, increasing lift and maneuverability while decreasing drag. These functions are made possible through specialized caudal anatomy, consisting of an articulated fan of feathers (rectrices) supported by a truncated series of caudal vertebrae and a unique terminal element, the pygostyle. The morphology of the caudal skeleton is variable across birds, but despite its evolutionary significance, little is known about the causes of this variation. This dissertation explores how ecomorphological patterns and trait interactions shape caudal skeletal variation in a phylogenetic comparative context. First, the relationship between flight behavior and caudal skeletal morphology is examined. Pygostyle shape is found to be a good predictor of foraging style, suggesting that the tail skeleton evolves in response to functional demands. Next, the association between the rectrices and the underlying skeleton is tested. Indeed, a strong correlation between these two tissues is detected, supporting the hypothesis that the rectrices and pygostyle coevolve. In addition to its functional/locomotor role, the tail fan is used for intraspecific display in many species, with sexual selection acting to generate sexually dimorphic ornamental rectrices. The evolution of these ornaments is predicted to
necessitate changes in the caudal skeleton to support and maneuver such structures. However, no sexual dimorphism in caudal skeletal morphology is detected in species with dimorphic tail fans. In conjunction with the previous finding that caudal bones and feathers coevolve, these results illustrate the complexity of trait interactions within the tail and suggest that such covariation patterns are malleable. Finally, integration between the subregions of the tail skeleton is evaluated. The pygostyle and free caudal vertebrae exhibit significant intracolumnar covariation, suggesting the tail skeleton evolves as a coordinated whole. Together, these results offer insight into how form-function relationships and trait interactions have influenced the evolution of the unique avian caudal skeleton and provide a framework for exploring macroevolutionary patterns and ontogeny in this system.
DEDICATION

For my family
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CHAPTER 1: INTRODUCTION

The remarkable diversity and species richness of birds is at least partially attributable to the acquisition of key morphological innovations related to flight (Benson and Choiniere, 2013a). Like bats, pterosaurs, and non-avian flying theropods, crown birds use the forelimb wing to produce lift and thrust in flight and to facilitate active control over maneuverability (e.g., Dyke et al., 2006; Swartz and Middleton, 2008; Caple et al., 2010; Bell et al., 2011). Likewise, all of these groups use the hind limb for a variety of functions, including terrestrial locomotion, perching and prey handling (e.g., Gatesy, 1991; Habib and Ruff, 2008; Hutchinson and Allen, 2009). But the locomotor system of crown birds is unique in the presence of a third locomotor module: the tail (Gatesy and Dial, 1996a). The evolution of this locomotor role for the tail is thought to have played a major role in the ecological and morphological diversification of birds (Gatesy and Dial, 1996a; Benson and Choiniere, 2013a). This dissertation investigates the evolution of the tail skeleton in an effort to understand how ecology and trait interactions have shaped this novel structure.

The importance of the avian tail stems from its use as part of the flight system, complementing the role of the wings. The tail generates lift and reduces drag, increasing flight efficiency (Maybury et al., 2001; Evans et al., 2002). The tail also acts as a control surface for steering, increasing maneuverability and agility (Thomas and Balmford, 1995). It is used to increase static stability, the ability to resist perturbations in flight (Thomas and Taylor, 2001; Taylor and Thomas, 2002). The tail fanning apparatus enhances flight performance with its capability to spread (abduct) and fold (adduct) the
feathers (Fig. 1A). The ability of the tail to perform any of these aerodynamic roles is dependent on the shape of the tail fan (Thomas and Balmford, 1995; Thomas, 1996). Crown birds with an articulated tail fan can thus actively modify the aerodynamic properties of the tail to suit specific flight behaviors completely independently from the actions of the wing (Gatesy and Dial, 1996b). For example, folding the tail decreases drag and makes straight-line flight more efficient whereas a fully spread tail is used to generate high lift during landing/takeoff and maneuvering (Thomas and Balmford, 1995). This means a crown bird that possesses this derived tail structure and function is capable of utilizing a wider repertoire of flight behaviors than a stem bird or bird-like nonavian paravian theropod dinosaur with a long bony tail (e.g., Microraptor, Jeholornis) or one with a short tail lacking a fan of feathers (e.g., Sapeornis, Confuciusornis; Gatesy and Dial, 1996b). This wider breadth of possible flight behaviors was no doubt instrumental in allowing birds to explore new ecological niches and eventually to diversify into the range of taxa seen today.

The acquisition of the aerodynamic function of the tail required the tail to become dissociated with the hind limb. Long-tailed paravians and non-paravian theropods used the hind limb and tail as an integrated unit for terrestrial locomotion (Gatesy and Dial, 1996a; Benson and Choiniere, 2013a). In these clades, the caudofemoralis muscle attached to the fourth trochanter of the femur and the proximal caudal vertebrae and served as the primary hip retractor (Gatesy, 1990; Gatesy and Dial, 1996a; Hutchinson and Gatesy, 2000). The evolution of both the tail and hind limb was constrained by this
Figure 1. Digital 3D model of caudal skeleton and rectrices.
Dorsal view of a generalized avian tail apparatus (A) with rectrices folded on the left side and spread on the right side. Isolated caudal skeleton in left lateral (B) and dorsal (C) views. fcv, free caudal vertebra; pyg, pygostyle; syn, synsacrum. Specimen OUVC 10322, *Zenaida macroura.*
functional association (Benson and Choiniere, 2013a). With the shortening of the tail in pygostylian birds this functional association was broken, allowing the hind limb and tail to evolve independently of one another. In addition to facilitating morphological diversification of the tail, this also spurred increased rates of hind limb evolution and speciation (Benson and Choiniere, 2013a). Thus, tail evolution and the reorganization of the locomotor system into three modules was a major driver of avian diversification.

The locomotor functions of the tail are made possible through the derived structure of the caudal apparatus. The aerodynamic part of the tail consists of a fan of tail feathers (rectrices) that can be spread or folded (Fig. 1A). The roots (calami) of the rectrices insert into the paired fibroadipose structures, the rectricial bulbs. The rectricial apparatus is supported by a specialized, shortened tail skeleton that is divided into two distinct subregions (Fig. 1). Proximally, caudal mobility is facilitated by several (five to nine) free caudal vertebrae (Baumel, 1988). Distally, the terminal three to seven caudal vertebrae are co-ossified to form the pygostyle (Fig. 1B,C). The pygostyle is a laterally compressed, plowshare-shaped bone that serves to yoke together the two rectricial bulbs, thereby anchoring the fan of tail feathers (Baumel, 1988; Gatesy and Dial, 1996b). The pygostyle also serves as the origin and insertion of the bulbi rectricium muscle, which spreads the tail fan (Gatesy and Dial, 1996b). Together, these bones, feathers, muscles and other soft tissues work as a coordinated system to allow for the complex functions of the tail.

The importance of the tail apparatus in locomotion and diversification has led to a wealth of research on the evolution of tail structure and function across birds. The
evolution of rectricial morphology is well understood, both from the perspective of
natural selection (e.g., Thomas and Balmford, 1995; Moreno and Moller, 1996;
Fitzpatrick, 1997; 1999) and sexual selection/mate choice (Andersson and Andersson,
1994; Regosin and Pruett-Jones, 2001a; Evans, 2004; Pryke and Andersson, 2005; Clark,
2010). Numerous other studies have characterized the function of this system as an
aerodynamic structure (Balmford et al., 1993; Thomas, 1993; 1996; Park et al., 2000;
Sachs, 2007; Evangelista et al., 2014). Taken together, these studies have generated a
clear picture of the evolution of the tail fan.

One aspect of tail evolution, however, has been largely neglected: the variation in
the caudal skeleton. Caudal skeletal morphology is highly diverse among birds (e.g., Fig.
2). Although this variation has been recognized for well over a century (Van Oort, 1904),
relatively few formal investigations of the causes and consequences of this variation have
been carried out. The work that has been done in this area focuses on the shortening of
the tail along the theropod lineage in deep time (Pittman et al., 2013). Other researchers
have characterized caudal skeletal variation within modern taxa with highly specialized
tail structure and function, such as Falconidae and Picinae (Richardson, 1942; 1972;
Manegold and Töpfer, 2012). Finally, some limited work has been done to understand the
developmental origin of the pygostyle itself (Rashid et al., 2014).

This dissertation seeks to characterize morphological variation in the avian caudal
skeleton in a broad, comparative context by focusing on two related lines of
investigation. First, the relationship between form and function in the tail skeleton is
explored in the context of **ecomorphology**. Because of the aerodynamic function of the tail fan, flight style is strongly linked with tail fan shape. Birds that use different flight modes (e.g., flapping, gliding) have evolved tail fan morphology that is capable of producing the aerodynamic forces that increase the efficiency of those behaviors. (Thomas and Balmford, 1995). Because of the topological and functional association between the tail feathers and the underlying tail skeleton, Chapter 2 addresses whether a similar form-function relationship is detectable in the caudal vertebrae and pygostyle. Birds that utilize different flight or foraging techniques might require changes in the
caudal skeleton in the same way that forelimb bones are shaped by flight behavior (Nudds et al., 2007; Simons, 2010; Bell and Chiappe, 2011).

In addition to this ecomorphological work, subsequent chapters each address hypotheses regarding trait covariation within the caudal apparatus. In addition to being functionally associated, there is evidence from the fossil record that the tail fan apparatus and the pygostyle have coevolved, with coordinated changes in each structure occurring in a stepwise manner in the ancestors of modern birds (Clarke et al., 2006). Chapter 3 investigates patterns of skeletal and rectricial covariation using comparative methods in order to understand how these structures coevolve in modern birds. The statistical relationship between feathers and bones in the tail is then applied to reconstruct tail fan shape in a fossil specimen in which no integument is preserved, paving the way for future studies of integumentary evolution in extinct birds.

Whereas the primary function of the avian tail is as a locomotor module, many taxa also use the tail as a display structure. Often ornamental tails are sexually dimorphic, with males exhibiting elongate rectrices compared to females (Brown and Gutierrez, 1980; Cuervo and Moller, 2001; Park et al., 2001). Long, elaborate tail fans deviate from the aerodynamic optimum, reducing lift and increasing drag, thereby increasing the energetic costs of flight (Evans and Thomas, 1992; Balmford et al., 1993; Norberg, 1995; Evans, 1998; Park et al., 2000; Clark and Dudley, 2009). In order to support and maneuver elaborate, energetically costly tail fans, the underlying caudal skeleton may require morphological modifications in males of sexually dimorphic species, for example to allow for the attachment of larger caudal elevator and depressor muscles. Chapter 4
compares patterns of sexual dimorphism in the caudal skeleton in order to test this hypothesis and to explore how feather-bone interactions are affected by sexual selection.

Finally, in Chapter 5, trait covariation is evaluated among the individual elements of the caudal skeleton. The tail skeleton is subdivided into two morphologically and functionally distinct subregions, the pygostyle and the free caudal vertebrae. Despite their morphological divergence, these two regions share a common developmental origin, with the pygostyle developing from the coossification of the distal vertebrae (Catala et al., 2000). To what extent is each of these subregions independent from the other?

Quantifying the degree of integration and modularity allows for a consideration of the structure of variation within a system, as well as constraint and evolvability (e.g., Cheverud, 1996; Goswami and Polly, 2010; Adams and Felice, 2014). Moreover, the results of this work are used to generate testable hypotheses regarding the functional, genetic and developmental underpinnings of integration patterns in the tail.

Taken together, these individual studies explore morphological evolution through two complementary lenses: ecomorphology and trait covariation. Throughout, phylogenetic comparative methods are employed to maintain an explicit evolutionary perspective. This dissertation expands our understanding of the evolution of the tail skeleton in birds, an important morphological novelty. At the same time, the insights gained here provide a foundation for future paleontological, genetic, and developmental work that will explore how variation is generated in this system. Finally, the methodology used and conclusions drawn here are broadly applicable to other biological...
systems, as they address the way that ecology shapes the skeleton, how integument and bones interact, and how intracolumnar variation is maintained in the axial skeleton.
CHAPTER 2: ECOLOGY AND CAUDAL SKELETAL MORPHOLOGY IN BIRDS:
THE CONVERGENT EVOLUTION OF PYGOSTYLE SHAPE IN UNDERWATER FORAGING TAXA

Introduction

Understanding the processes that generate phenotypic diversity is an important goal in evolutionary biology (Adams and Nistri, 2010; Mallarino and Abzhanov, 2012). The evolutionary diversification of phenotypes can be influenced by many factors, including natural selection, sexual selection, biomechanical constraints, developmental processes, and trait interactions (Schluter, 1996; Owens et al., 1999; Ricklefs, 2004; Berner et al., 2008; Ward and Mehta, 2010; Sanger et al., 2011; Mallarino and Abzhanov, 2012). By testing hypotheses regarding the patterns and causes of morphological diversity in highly variable structures, we may better characterize the role that such variation has played in the diversification of clades (Fitzpatrick, 1985; Schluter and McPhail, 1992; Hunter, 1998; Wainwright, 2007; Bleiweiss, 2009; Monteiro and Nogueira, 2010; Close and Rayfield, 2012).

The avian tail is one such highly variable structure, with modern birds using the tail as an integral component of the flight apparatus (Baumel, 1988; Gatesy and Dial, 1996a; 1996b). The role of the tail in flight is to supplement the lift produced by the wings during slow flight, reduce whole-body drag, and both stabilize and maneuver the bird during flight (Thomas and Balmford, 1995; Thomas, 1996; Maybury et al., 2001; Sachs, 2007). Bird tail morphology is specialized for its function as part of the locomotor apparatus and consists of an articulated fan of tail feathers, separate muscular systems for
Tail movements and tail fanning, and a modified, shortened tail skeleton (Baumel, 1988). The avian caudal skeleton consists of several (five to nine) free caudal vertebrae (Fig. 1). The terminal element of the caudal skeleton is the pygostyle, represented by a single, coossified unit consisting of the fused caudal-most vertebrae, ranging from three to seven in number (Baumel, 1988; Baumel and Witmer, 1993). This serves as an attachment site for caudal musculature, tail feathers, and as an anchor for the tail fanning mechanism itself (Baumel, 1988; Gatesy and Dial, 1996b).

The drivers of tail feather (rectrix: plural, rectrices) diversity are somewhat well understood. Tail fan shape determines the functional and aerodynamic properties of the tail (Balmford et al., 1993; Thomas, 1993). Not surprisingly then, tail-fan shape diversity reflects differences in ecology. As examples, birds that live in dense woodland environments benefit from the increased maneuverability granted by a long tail fan (Thomas and Balmford, 1995), whereas those that capture their prey in the air generally exhibit a deeply forked tail that increases agility (Thomas and Balmford, 1995). High-speed fliers often have a shortened tail fan that reduces drag and thus increases flight efficiency, similar to the situation observed in long distance migrants (Thomas and Balmford, 1995; Fitzpatrick, 1999).

Tail-fan shape also serves non-aerodynamic functions. In some species, males exhibit an elaborate tail fan that deviates from the “optimal” shape predicted from aerodynamic models (Brown and Gutierrez, 1980; Bancroft, 1984; Evans and Thomas, 1992; Balmford et al., 1994; Thomas, 1997). For example, male red-collared widowbirds in breeding plumage have a tail five times longer than that of females (Pryke and
Andersson, 2005). Sexually dimorphic rectrices like these are honest indicators of male quality and have been shown to evolve as a result of female preference (Andersson, 1992; Pryke and Andersson, 2005). Such ornaments have evolved in numerous clades despite being energetically and aerodynamically costly (Thomas, 1997; Pryke and Andersson, 2005). Thus, tail-fan phenotypic diversity is shaped not only by natural selection for increased flight performance, but also by sexual selection (Thomas, 1997).

In contrast to rectricial diversity, drivers of caudal skeletal diversity are poorly understood. The degree of morphological variation of the free caudal vertebrae and the pygostyle has long been recognized (Van Oort, 1904; Burt, 1930; Fisher, 1946; Owre, 1967; Raikow, 1970; Richardson, 1972). There is substantial interspecific variation in the number and form of the free caudal vertebrae in addition to the shape of the pygostyle (Fig. 2). However, little comparative consideration of caudal skeletal structure and function has been undertaken, with the exception of a few clades with highly specialized tails. For example, falconids and some hummingbirds have paired accessory pygostyle elements just ventral to the pygostyle. Accessory pygostyle bones are associated with the depressor caudae musculature (Richardson, 1972). These structures are hypothesized to have evolved to accommodate stresses on the tail during rapid maneuvering and braking in these highly aerial clades (Richardson, 1972). Woodpeckers (Picidae) are also noted for their derived caudal skeletal structure and function. Extremely arboreal woodpeckers use the tail as a prop for support during vertical climbing. The pygostyle of woodpeckers has a laterally expanded ventral surface (discus pygostyli) that increases the surface area for the attachment of both the rectrices and caudal musculature. The discus pygostyli is
more expanded in species that use the tail as a prop more frequently (e.g., species that spend a considerable proportion of time utilizing the vertical or near-vertical components of the arboreal environment) and, as such, this derived caudal skeletal morphology has been interpreted as an adaptation for the unique function of the tail in woodpeckers (Burt, 1930; Richardson, 1942; Manegold and Töpfer, 2012).

The specialized caudal skeletal morphology in Falconidae and Picidae suggests that variation in caudal skeletal anatomy, like rectricial anatomy, likely evolves in response to variation in tail function. To date, there has been no broad comparative investigation of structure-function relationships in the avian caudal skeleton. Variation in forelimb (wing) skeletal morphology is strongly linked to flight style (Simons, 2010; Simons et al., 2011). For example, in Pelecaniformes, species that utilize different flight styles (e.g., flap, flap-glide, dynamic soar, static soar) are characterized by different forelimb skeletal anatomy. The length and diameter of the carpometacarpus (the forelimb element that supports the primary flight feathers of the wing) vary among functional groups, reflecting the different biomechanical demands of each flight style (Simons, 2010). Likewise, hind limb morphology reflects aspects of ecology and locomotor behavior. For example, foot-propelled diving birds exhibit an enlarged area of attachment (i.e., the cnemial crest) for knee extensor musculature that functions to both stabilize the knee joint and produce powerful knee extension during swimming (Hinić-Frlog and Motani, 2010). More generally, hind limb proportions can be used to discriminate among habitat types (e.g., arboreal, wading, swimming, terrestrial) with some confidence, suggesting that pelvic limb variation is influenced by differences in the locomotor
demands of each substrate type (Zeffer et al., 2003). The present study investigates the
degree to which the avian caudal skeleton, like components of the appendicular skeleton,
reflects differences in locomotor behavior.

Given that forelimb and hind limb skeletal anatomy differs among functional
groups within birds, does caudal skeletal anatomy also exhibit clear structure-function
relationships? As a framing statement then, we predict that birds that utilize different
foraging strategies (e.g., aerial, terrestrial, pursuit diving) or flight styles (e.g., flap, soar,
flap-glide) are characterized by variable caudal skeletal morphology that reflects this
function. We examine this working hypothesis in a phylogenetic comparative context
using morphometric data derived from both the free caudal vertebrae and the pygostyle
and assess their relationships with both foraging and locomotor characteristics.

Materials and Methods

Taxon Sampling

Morphometric data were collected from 158 specimens representing a total of 51
species (35 genera, see Appendix 1). Taxa were sampled primarily from the diverse
waterbird assemblage, often referred to as the “Aequornithes” (Hackett et al., 2008;
Mayr, 2011). Waterbirds include Ciconiiformes (storks), Gaviiformes (loons),
Pelecaniformes (pelicans, cormorants, and allies), Procellariiformes (albatrosses and
petrels), and Sphenisciformes (penguins) (Hackett et al., 2008; Mayr, 2011). Although
the monophyly of this clade has been contested (Ericson et al., 2006; Jetz et al., 2012), it
was chosen as a focal group for several reasons. First, Aequornithes is among the most
diverse avian groups in terms of morphology and body size range (Mayr, 2011; Smith,
Second, diversity in flight behavior and foraging behavior within this clade is well categorized (Pennycuick, 1982; Anderson, 1991; Frederick and Bildstein, 1992; Le Corre, 1997; Pennycuick, 1997; Spear and Ainley, 1997; Brewer and Hertel, 2007). Finally, taxa were sampled primarily from the waterbird assemblage because unlike many other neornithine clades, there is minimal-to-no sexual dimorphism of the tail feathers (rectrices) within the group (Coulson, 2002). Even taxa with elaborate rectrices, such as tropicbirds, are sexually monomorphic for this trait (Veit and Jones, 2003). As such, differences in caudal skeletal morphology between males and females are not expected to influence the analyses conducted herein. In addition, six of the 51 taxa are not waterbirds but members of the somewhat distantly related Charadriiformes (shorebirds: Larus argentatus, Stercorarius parasiticus, Fratercula cirrhata, Uria aalge, Cephus columba, Ibidorhyncha struthersii). These taxa are ecologically convergent with some waterbirds (e.g., alcids and penguins are both marine wing-propelled divers) and thus represent a useful comparison to waterbirds for understanding the correlated evolution of form and function.

In order to explore the relationship between caudal skeletal morphology and flight behavior, each taxon was assigned to both a flight style group and a foraging style group. These categorizations are based on published observations and other comparative ecomorphology studies (Pennycuick, 1982; Kahl, 1987; Pennycuick, 1987; del Hoyo et al., 1992; Hobson and Welch, 1992; McMahon and Evans, 1992; Barr, 1996; Kelly et al., 2003; Brewer and Hertel, 2007; Simons, 2010; Close and Rayfield, 2012). The flight style categories were chosen as Flap, Flap-Glide, Dynamic Soar, Static Soar, Wing-
Propelled Flightless and Foot-Propelled Flightless. Taxa were placed in one of five foraging style groups: Aerial, Terrestrial, Plunge Dive, Foot-Propelled Pursuit Dive, and Wing-Propelled Pursuit Dive. The aerial foraging group contains any taxon that habitually utilizes airborne foraging techniques including hawking, dipping, pattering, and kleptoparasitism. See Appendix 1 for both flight-style and foraging-style assignments.

Skeletal Morphology and Analytical Approaches

In order to fully characterize caudal skeletal morphology, two datasets were collected. First, free caudal vertebral morphology was quantified using linear measurements. The following metrics were collected: centrum craniocaudal length, centrum width, centrum height, transverse process craniocaudal length, transverse process width, spinous process craniocaudal length, spinous process width, spinous process height, ventral process craniocaudal length, ventral process width, ventral process height (Fig. 3). These metrics were collected at three serial positions within the caudal vertebral series. The first (i.e. post-synacral) free caudal vertebra, the vertebra halfway along the length of the caudal series, and the last (i.e. propygostylar) free caudal vertebra. For individuals with an even number of free caudal vertebrae, the two middle vertebrae were measured and averaged. In order to take into account the effect of body size, the geometric mean of five additional measurements was used as a proxy for body size: sternal length, sternal width, height of sternal keel, synsacral length, and femur length (Mosimann, 1970; Mosimann and James, 1979; Simons, 2010). Specimens and their institutional identification numbers are listed in Appendix 1. Linear measurements of free
Figure 3. Free caudal vertebra: skeletal metrics
Free caudal vertebra in dorsal (A), ventral (B), left lateral (C), and anterior (D) views. Skeletal metrics collected: Centrum length (CL), centrum width (CW), centrum height (CH), transverse process length (TPL), transverse process width (TPW), spinous process length (SPL), spinous process width (SPW), spinous process.

Caudal vertebrae and body size proxies are provided in Appendix 2, averaged by species. A phylogenetic least-squares regression was conducted to correct raw measurements for body size, with the species’ means of the residuals used as variables for subsequent analyses (Revell, 2009; 2011). All linear measurements were obtained using digital calipers (Fowler digital calipers, Fred V. Fowler Company, Inc., Auburndale, MA).

The second dataset characterizes the morphology of the pygostyle using geometric morphometrics. Given that the pygostyle is irregularly shaped (Fig. 1, 2), laterally compressed, and lacks explicitly defined homologous landmarks, Elliptical Fourier Analysis (EFA) was used to quantify morphological variation in this structure. EFA is an outline analysis method commonly used on landmark-poor outline shapes (Rohlf and Archie, 1984; Ferson et al., 1985; Crampton, 1995; Sheets et al., 2006; Van Bocxlaer and Schultheiss, 2010).
Fourier analysis utilizes a digitized outline of a shape consisting of a series of x and y coordinates for each pixel around the contour of a given shape. Separate Fourier decompositions are carried out for the change in the sequences of x- and y-coordinates around the perimeter. The result is a set of harmonically related (sine and cosine) equations, with each one referred to as a harmonic. For each harmonic, the sine and cosine equations describe the shape of an ellipse (Ferson et al., 1985; Crampton, 1995). Taken together, many harmonics may be used to describe more and more complex shapes (Fig. 4). The total number of variables (Fourier descriptors) is $4n$, where $n$ is the number of harmonics (Crampton, 1995). As with traditional, landmark based geometric morphometrics, the effects of size, position, and rotation must be removed such that only shape information remains. This is accomplished by standardizing Elliptical Fourier Descriptors by the first harmonic of each specimen. The resulting shape variables are referred to as Normalized Elliptical Fourier (NEF) descriptors (Ferson et al., 1985; Crampton, 1995; Claude, 2008). This normalization process also reduces the number of variables to $4n-3$. NEF coefficients can then be used as variables in multivariate statistical analyses.

In order to conduct the EFA, each specimen (Appendix 1) was photographed in lateral view (Fig. 2). Pygostyle outlines were digitized, Fourier transformed, and normalized using SHAPE v. 1.3 (Iwata and Ukai, 2002). In order to remove superfluous variables from the dataset, the number of harmonics to retain was determined using the Fourier power method (Crampton, 1995; Claude, 2008). For a given harmonic, $n$, Fourier power is calculated as
The number of harmonics retained is determined by the number required to obtain 99% of the cumulative power (Crampton, 1995). For the 160 pygostyle specimens photographed, eight harmonics comprise 99% of the cumulative power (Momocs R package; Bonhomme et al., 2013). For each species, harmonic descriptors were averaged, resulting in 51 observations (species) and 37 variables (NEF descriptors).

**Figure 4. Outline reconstruction using elliptical Fourier descriptors.**
Black contours represent the original outline shape of the pygostyle of *Phoebastria immutabilis*. Red contours represented the reconstructed shape using the corresponding number of harmonics. Increasing the number of harmonics increases the detail of the reconstructed shape and the accuracy with which it approximates the true shape.

**Phylogenetic Signal**

Taxa in interspecific comparative studies cannot be treated as independent data points in statistical analyses because the phylogenetic relatedness of organisms introduces a degree of non-independence (Felsenstein, 1985; Garland et al., 2005). The effect of phylogeny on caudal morphology was first quantified and then formally taken into account as part of each statistical approach.
For both the free caudal vertebrae and pygostyle datasets, phylogenetic signal was quantified using Pagel’s $\lambda$ (Pagel, 1999). Pagel’s $\lambda$ is a tree transformation parameter that measures the degree to which evolutionary relationships predict the observed patterns of variation/similarity in the data. This parameter varies between $\lambda = 0$ and $\lambda = 1$. If $\lambda = 0$, phylogenetic relatedness has no influence on the data and the tree can be transformed into a star phylogeny (equivalent to using ahistorical comparative methods). If $\lambda = 1$ the data fit a Brownian motion model of evolution given the original untransformed branch lengths. The optimal lambda for each dataset was calculated using the phytools R package (Freckleton et al., 2002; Revell, 2011).

For the EFA dataset, an additional metric of phylogenetic signal was used. Calculating an optimal $\lambda$ for a given dataset assumes that the data are multivariate. Shape data are in fact a single multidimensional character, and as such, is better served by calculating phylogenetic signal using the alternative ‘consistency index’ (Klingenberg and Gidaszewski, 2010). This metric varies from 0 to 1, where 0 = high homoplasy (low phylogenetic signal) and 1 = low homoplasy (high phylogenetic signal). The index is calculated using a permutation test. First, the amount of morphological change along the branches of the tree is calculated. Next, the shape data are shuffled among the tips of the tree and the amount of shape change is recalculated and compared to the observed value. If phylogeny has little effect, swapping the data among the tips will be equally likely to increase or decrease the amount of total tree length, and thus, on average not impart a noticeable effect. Conversely, if the effect of phylogeny is high, shuffling tip data are predicted to increase amount of change along the tree (Klingenberg and Gidaszewski,
The consistency index for pygostyle shape was calculated using the geomorph package in R (Adams and Otárola-Castillo, 2013).

The higher-level phylogenetic relationships among the members of the “waterbird” and shorebird clades are somewhat contested (Ericson et al., 2006; Hackett et al., 2008; Smith, 2010; Mayr, 2011; Jetz et al., 2012). In order to take into account this phylogenetic uncertainty, each analysis was conducted using two alternative topologies, one using a “backbone” based on Hackett et al. (Hackett et al., 2008) and the other using a “backbone” from Ericson et al. (Ericson et al., 2006). The former topology resolves Aequornithes as a monophyletic group, whereas the latter does not. The two phylogenetic hypotheses also differ in their placement of Phaethontidae. For each backbone topology, a sample of 5000 trees was obtained from the posterior distribution of trees on http://www.birdtree.org (Jetz et al., 2012). A Maximum Clade-Credibility (MCC) tree for each topology was produced using TreeAnnotator v1.6.2 (Drummond et al., 2012). The two MCC trees were used for all comparative analyses.

**Comparative Analyses**

The two primary goals of the analyses conducted herein are to determine whether birds belonging to different ecological groups are characterized by different caudal skeletal morphology, and if so, identify which components of caudal skeletal morphology best explain differences among the groups. Phylogenetic MANOVAs (geiger R package; ) were used to test for significant differences in morphology among functional groups. For the free caudal vertebrae dataset, separate tests were conducted for the first caudal vertebra, mid-caudal vertebra, and propygostylar vertebra. For the pygostyle shape
dataset, the dimensionality of the data was first reduced by conducting a phylogenetic principal components analysis on an evolutionary variance-covariance matrix of the normalized Fourier descriptors (Revell, 2009). Custom R scripts for computing and plotting phylogenetic PCA of elliptical Fourier data are provided in Appendix 3. The significant principal components (those that explain 5% or more of the total observed variance) were used as the dependent variables in the MANOVAs. MANOVAs were repeated using flight style and foraging style as the grouping factor and with both the Hackett et al. (Hackett et al., 2008) backbone tree and Ericson et al. (Ericson et al., 2006) backbone tree.

In order to determine which aspects of morphological variation best explain the differences among functional (flight or foraging) groups, we used a Phylogenetic Flexible Discriminant Analysis (pFDA), a multigroup classification tool related to Linear Discriminant Analysis and Canonical Correlation Analysis (Hastie et al., 1994; Motani and Schmitz, 2011; Schmitz and Motani, 2011). This method involves using a phylogenetic generalized least squares regression to construct a model estimating the relationship between the dependent variables (morphology) and group identity. The model is then used to predict group identity for each taxon given the data (Motani and Schmitz, 2011; Schmitz and Motani, 2011). The accuracy of the model—the degree to which group identity can be predicted by its morphology—can be evaluated by its misclassification rate. The misclassification rate equals the proportion of species that were improperly assigned to their respective class using the model (lower misclassification rate means higher accuracy of the model). Finally, the pFDA model can
be used to generate an ordination plot to assist in the interpretation of the characters that differentiate each group. As with the MANOVAs, pFDA was repeated using both topologies and both eco-functional classification schemes.

Results

*Phylogenetic Signal Results*

Phylogenetic relationships influence both free caudal vertebral anatomy and pygostyle shape. Pagel’s $\lambda$ was slightly different for the first, middle, and last vertebra, but ranged between 0.418 and 0.723 (Table 1), thus the phylogenetic signal in free caudal vertebra can be characterized as moderate to high. Pagel’s $\lambda$ was also calculated using the NEF descriptors for pygostyle shape and found to be 0.42, indicating a moderate degree of signal (Table 1). Using the consistency index, a more appropriate measure of phylogenetic signal for geometric morphometric data, phylogenetic signal for pygostyle shape was found to be approximately 0.45 ($p < 0.001$), confirming a moderate level of phylogenetic influence on morphology. The results of the tests of phylogenetic signal were not substantially different when either of the two topologies were used, nor were the results of any of the subsequent analyses. As such, results are presented for the Hackett topology only (Hackett et al., 2008). These results justify the use of the phylogenetic comparative methods used below.

### Table 1. Phylogenetic signal

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Pagel's $\lambda$</th>
<th>Log Likelihood</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Vertebra</td>
<td>0.6786913</td>
<td>-432.4101</td>
</tr>
<tr>
<td>Middle Vertebra</td>
<td>0.53254101</td>
<td>-552.9049</td>
</tr>
<tr>
<td>Last Vertebra</td>
<td>0.7236591</td>
<td>-574.1383</td>
</tr>
<tr>
<td>Pygostyle Shape</td>
<td>0.4181343</td>
<td>5239.667</td>
</tr>
</tbody>
</table>
Phylogenetic MANOVA Results

The first, middle, and last free caudal vertebrae were analyzed using phylogenetic MANOVA for both topologies and for both eco-functional classification schemes (flight style and foraging style). In nearly all cases we found a significant difference in caudal vertebral anatomy among the groups (Table 2). Birds that utilize different flight styles differ in the dimensions of their first, middle, and last free caudal vertebrae (p < 0.05), regardless of the choice of phylogenetic tree. Taxa that utilize different foraging styles have significantly different post-synsacral and pre-pygostylar vertebrae (p < 0.05). Middle caudal vertebrae did not exhibit significant differences (p > 0.1).

Phylogenetic MANOVAs were also used to examine whether different flight or foraging groups differ in pygostyle shape. The PC scores from a phylogenetic PCA were used as the dependent variables in the MANOVA. The PCA indicates that the first six PC axes combined explain > 85% of the cumulative variance (> 5% per axis), and these six axes were retained for the MANOVAs. Pygostyle shape does not differ significantly among flight style groups (p > 0.1, Table 3). Among foraging groups, however, pygostyle shape is nearly significantly different (p = 0.0519). If the non-aquatic foraging groups (i.e., terrestrial and aerial) are combined, such that the groups are Plunge Dive, Foot-propelled Pursuit Dive, Wing-propelled Pursuit Dive, and Non-diving, the results of the phylogenetic MANOVA are significant at p < 0.01 (Table 3).
### Table 2. Phylogenetic MANOVA results: free caudal vertebrae.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Grouping</th>
<th>Degrees of Freedom</th>
<th>Pillai-Bartlett Trace</th>
<th>Approximate F Number</th>
<th>Ahistorical p-Value</th>
<th>Phylogenetic p-Value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Vertebra</td>
<td>Flight Style</td>
<td>5</td>
<td>1.9958</td>
<td>3.4877</td>
<td>2.42E-09</td>
<td>0.002997</td>
<td>*</td>
</tr>
<tr>
<td>Middle Vertebra</td>
<td>Flight Style</td>
<td>5</td>
<td>2.136</td>
<td>2.6443</td>
<td>4.85E-07</td>
<td>0.02198</td>
<td>*</td>
</tr>
<tr>
<td>Last Vertebra</td>
<td>Flight Style</td>
<td>5</td>
<td>2.1851</td>
<td>2.7521</td>
<td>1.61E-07</td>
<td>0.01998</td>
<td>*</td>
</tr>
<tr>
<td>First Vertebra</td>
<td>Foraging Style</td>
<td>4</td>
<td>1.632</td>
<td>3.6183</td>
<td>3.11E-08</td>
<td>0.03696</td>
<td>*</td>
</tr>
<tr>
<td>Middle Vertebra</td>
<td>Foraging Style</td>
<td>4</td>
<td>1.8754</td>
<td>3.1297</td>
<td>1.04E-07</td>
<td>0.1179</td>
<td></td>
</tr>
<tr>
<td>Last Vertebra</td>
<td>Foraging Style</td>
<td>4</td>
<td>2.3282</td>
<td>4.9376</td>
<td>6.45E-14</td>
<td>0.000999</td>
<td>*</td>
</tr>
</tbody>
</table>

### Table 3. Phylogenetic MANOVA results: principal components of pygostyle shape.

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Degrees of Freedom</th>
<th>Pillai-Bartlett Trace</th>
<th>Approximate F Number</th>
<th>Ahistorical p-Value</th>
<th>Phylogenetic p-Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flight Style</td>
<td>5</td>
<td>1.0598</td>
<td>1.9724</td>
<td>3.01E-03</td>
<td>0.3766</td>
<td></td>
</tr>
<tr>
<td>Foraging Style</td>
<td>4</td>
<td>1.2827</td>
<td>3.4615</td>
<td>1.04E-06</td>
<td>0.05195</td>
<td></td>
</tr>
<tr>
<td>Diving Type</td>
<td>3</td>
<td>1.1795</td>
<td>4.751</td>
<td>5.05E-08</td>
<td>0.008991</td>
<td>*</td>
</tr>
</tbody>
</table>

**Phylogenetic FDA (pFDA) Results**

To assist with interpreting which specific variables best explain differences among groups, we used pFDA ordinations. Using flight style as the grouping factor, pFDA of each of the three free caudal vertebrae generated a misclassification rate of 37-41% (Fig. 5). The majority of misclassifications occurred between flapping and flapping-gliding taxa, in addition to commonly misclassifying both static and dynamic soaring taxa as flappers. In general, only wing-propelled flightless birds (*Pygoscelis papua* and *Pygoscelis adeliae*) and one foot-propelled flightless bird (*Phalacrocorax harrisi*)
consistently occupy distinct regions of pFDA morphospace. *Pygoscelis* is characterized by a dorsoventrally restricted, laterally wide centrum and spinous process and a laterally restricted transverse process. *Phalacrocorax harrisi* exhibits a large spinous process and a small vertebral centrum. The remaining 48 taxa, representing the flap, flap-glide, static soar, and dynamic soar groups are clustered together in pFDA morphospace and lack any strong discriminating characteristics among the groups.

When foraging style is used as the grouping factor, the misclassification rate is 23-39% (Fig. 6). The highest misclassification rates for foraging style occur in the first and middle caudal vertebra datasets (39% and 31% respectively). In these datasets, aerial foragers and plunge-diving foragers were most commonly misclassified. Several plunge divers were misclassified as aerial or terrestrial foragers. Aerial foragers were most commonly misclassified as terrestrial, but were occasionally placed among the pursuit-diving or plunge-diving groups. The results (Figs. 6a-b) of these two pFDA analyses illustrate that terrestrial, foot-propelled diving, and wing propelled diving birds occupy somewhat distinct regions of morphospace, whereas aerial and plunge-diving birds occupy a common region of morphospace that overlaps with the other groups.

A misclassification rate of 24% for the propygostylar vertebra dataset is the least severe among the examined free caudal elements (Fig. 6c). The patterns observed here are somewhat different than for vertebrae positioned more cranially along the series. Aerial foragers are again the most frequently misclassified, sometimes being placed among the terrestrial or pursuit-diving foragers. There is considerably less classification error for the other foraging groups. When errors do occur, taxa are most often placed
Figure 5. Flight style pFDA plot: free caudal vertebrae. (A) Flight style pFDA plot: first caudal vertebra. Misclassification rate = 41.18%. (B) Flight style pFDA plot: middle free caudal vertebra. Misclassification rate = 37.25%. (C) Flight style pFDA plot: last free caudal vertebrae. Misclassification rate = 37.25%.

among the aerial foragers. Terrestrial, plunge-diving, and wing-propelled pursuit-diving foragers each group in distinct regions of the pFDA plots (Fig. 6c). Plunge-diving and wing-propelled pursuit-diving foragers are high on Discriminant Axis 1, indicating both groups have an craniocaudally restricted centrum and a craniocaudally restricted, yet wide ventral process. These groups are distinct from one another in that plunge divers score low on Discriminant Axis 2 (dorsoventrally expanded, narrow centrum, large spinous process, and small transverse process) but wing-propelled divers are high on Axis 2 (dorsoventrally restricted, wide centrum, small spinous process, and large transverse process). In contrast, terrestrial foragers are low on Axis 1 but high on Axis 2. This position in morphospace corresponds to a dorsoventrally restricted, elongate and wide centrum, large transverse process, and small spinous process. Aerial foragers and foot-propelled pursuit divers occupy an overlapping region of morphospace roughly centered on the origin.

The misclassification rate for pFDAs of pygostyle shape data is much lower than for free caudal vertebrae. When flight style is used as the grouping factor, only seven out of 51 taxa are misclassified (13.7%). All seven of these cases involve ambiguous placements among flap, flap-glide, and static soaring taxa. As with free caudal vertebrae, wing-propelled flightless species (penguins) and foot-propelled flightless species (Phalacrocorax harrisi and Rollandia microptera) each occupy distinct regions of pFDA morphospace (Fig. 7). Foot-propelled flightless species score extremely low on Discriminant Axis 1 and moderately high on Axis 2, whereas wing-propelled flightless species score very low on Axis 2. Foot-propelled flightless birds have a pygostyle with a
strong dorsal deflection and a pointed caudal margin. Wing-propelled flightless birds (penguins) have an extremely elongate, straight pygostyle. The remaining flight style groups (flap, flap-glide, static soar, dynamic soar) are somewhat restricted to a smaller region of morphospace, but overlap among these groups is minimal. Static soaring and flapping taxa have quite similar pygostyle shape, with a slight dorsal deflection and a slight taper. Static soarers have a slightly more pointed caudal margin whereas the typical flapping bird pygostyle is slightly rounded. Dynamic soaring birds have a rounded pygostyle with a deep articulation for the propygostylar vertebra. Flap-gliding taxa have a somewhat elongate, blunt pygostyle with a shallow articular surface.

The pFDA of pygostyle shape using foraging style as the grouping factor produces the most accurate discrimination, with just 2 of 51 taxa being misclassified (3.9%). The only misclassified taxa are the aerial foragers *Phoebastria immutabilis* and *Fregata magnificens*, both misclassified as terrestrial foragers. Their respective congeners, *Phoebastria nigripes* and *Fregata minor*, were correctly classified. A plot of the first two discriminant axes (Fig. 8) reveals that each foraging group is characterized by a distinct pygostyle shape. Plunge divers and wing-propelled pursuit divers both have a very elongate pygostyle; the plunge-diver pygostyle is tapered whereas wing-propelled pursuit divers are not. Foot-propelled pursuit divers are situated in pFDA phylomorphospace in between the wing-propelled divers and terrestrial foragers and exhibit a dorsoventrally expanded pygostyle that tapers to a point caudally. Terrestrial taxa have a pygostyle that is expanded dorsoventrally and is dorsocaudally directed.
Finally, the average pygostyle of the aerial foraging group is similar to the terrestrial foraging condition, but exhibits a distinct narrowing midway along its length, giving an hourglass-like shape.

Figure 7. Flight style pFDA plot: pygostyle shape. Misclassification rate = 13.73%.
Figure 8. Foraging style pFDA plot: pygostyle shape.
Misclassification rate = 3.92%.

Discussion

Does Free Caudal Vertebral Morphology Differ Among Ecological Groups?

The two analyses, MANOVA and pFDA, give ostensibly conflicting results regarding the association between free caudal vertebral morphology and flight behavior (foraging and flight styles). Phylogenetic MANOVAs identified a significant difference
in free caudal vertebral morphology among flight style groups. In contrast, the pFDAs of each free caudal vertebra using flight style as a grouping factor produced high misclassification rates. These conflicting results indicate that free caudal vertebral morphology is not useful for discriminating among flight style groups. Similar results were found using foraging style as a grouping factor — the MANOVAs for the first and last free caudal vertebrae return a significant difference among groups, yet pFDAs of these data result in a high misclassification rate.

The discordance between these results of the MANOVA and pFDA analyses is most likely attributed to the limitations of each analysis and the structure of the dataset. Whereas MANOVA is parametric, pFDA is not (Garland et al., 1993; Hastie et al., 1994; Schmitz and Motani, 2011). Thus, pFDA is more robust to deviations from multivariate normality and homoscedasticity than phylogenetic MANOVA. None of the free caudal vertebral datasets meet the criterion of multivariate normality. In order to mitigate the effects of the structure of the data, Pillai-Bartlett’s Trace was used as the test statistic because it is more robust to deviations from the assumptions of the MANOVA than the more common Wilk’s lambda test statistic (Hand and Taylor, 1987). The non-significant results of the pFDA suggest that the significant results of the phylogenetic MANOVA are spurious and that the structure of the free caudal vertebrae dataset is not well suited for such an analysis. The results of the pFDAs using these data can be interpreted with greater confidence.

Birds cannot be reliably assigned to flight style groups on the basis of free caudal vertebral morphology. The pFDA plots show that most taxa cluster together, with only
the flightless birds *Pygoscelis* spp. and *Phalacrocorax harrisi* characterized by distinct caudal vertebral morphology compared to their close relatives. Although *Pygoscelis* and *Phalacrocorax harrisi* do not have similar free caudal morphology, the fact that these flightless swimming birds are distinct from all other sampled taxa suggests that this morphological divergence may be related to their specialized locomotor styles. The penguins possess a reduced transverse process and a wide, dorsoventrally compressed centrum. In contrast, *P. harrisi* is characterized by a large spinous process with a small centrum, creating a dorsoventrally expanded, laterally compressed vertebra. The different vertebral anatomy in these clades of flightless taxa could be related to functional differences in tail use among wing-propelled flightless (*Pygoscelis*), foot-propelled (*Phalacrocorax*) flightless, and volant taxa. For example, the range of motion of the tail in elevation is limited by the “knocking together” of the spinous processes (Baumel, 1988). The tall spinous process of *P. harrisi* may thus restrict the extent to which it can elevate the tail. Given that tail elevation is observed primarily during takeoff (Baumel, 1988), it is possible that the unique caudal vertebral morphology of this taxon represents a relaxation of constraints maintaining the function of this structure as part of the flight apparatus.

Among the volant groups, most misclassification errors pertain to the flapping group. This manifests as either ambiguity between flapping and flap-gliding taxa or with dynamic- and static-soaring birds being classified in the flapping group. This suggests that flapping birds may have greater disparity in vertebral morphology than other flight style groups, and thereby occupy a greater region of pFDA morphospace. This is
consistent with the hypothesis that birds with powerful wings for flight should display increased variance in tail form as constraints on the tail as a component of the aerial locomotor apparatus are relaxed (Bleiweiss, 2009).

When foraging style is used as the grouping factor for pFDA ordinations, the results are similar: high misclassification rates for first and middle caudal vertebrae and a moderate misclassification rate for the last caudal vertebrae. Misclassification error decreases and foraging groups separate better in phylomorphospace moving from cranial to caudal through the free vertebral series. The propygostylar vertebra has moderate predictive power with only 24% misclassification. The differences in predictive power among the three positions along the caudal vertebral column could be related to the association between more distal caudal vertebrae. The distal caudal vertebrae are variably ankylosed as part of the pygostyle and the distal-most free caudal vertebra articulates with the pygostyle. Baumel (1988) noted that in rock dove (*Columba livia*) the propygostylar vertebra is reduced in size and hypothesized that this was an adaptation for increased freedom of movement at the propygostylar joint. Given that pygostyle shape seems to be influenced by flight behavior (see below) and that the propygostylar vertebra is functionally linked with pygostyle, it is possible that the same evolutionary forces drive pygostyle and propygostylar vertebral morphological variation but do not influence more cranial regions of the caudal series.

*Does Pygostyle Shape Differ Among Ecological Groups?*

In contrast to free caudal vertebral morphology, pygostyle shape is an excellent predictor of foraging style in waterbirds. The results of the phylogenetic MANOVAs and
pFDAs are more congruent with one another using NEF descriptors of pygostyle shape. Each foraging group is characterized by a significantly different pygostyle shape (Fig. 8). Aerial foragers exhibit a vertically-deflected pygostyle with a blunt caudal margin and dorsoventral constriction midway along its length, resulting in a distinctive hourglass shape. Terrestrial foragers have a generally similar pygostyle shape when compared to aerial foragers, but lack the dorsoventral constriction. Foot-propelled pursuit divers have a pygostyle that is dorsoventrally expanded at the cranial end but that tapers to a point caudally. Wing-propelled pursuit divers exhibit an exceptionally long pygostyle that does not taper. Plunge divers have a generally similar pygostyle, but one that tapers gradually. In general, underwater foragers (plunge dive and pursuit dive) have straight, elongate pygostyles, whereas birds that do not forage underwater (aerial and terrestrial) have craniocaudally restricted, dorsally-oriented pygostyles. The only misclassifications occurred between non-aquatic groups, supporting a dichotomy between aquatic and non-aquatic foragers.

Underwater foraging birds exhibit convergence in pygostyle morphology (Fig. 9). The significance of a straight, elongate pygostyle is likely related to the mechanical demands on the tail when moving through water as opposed to air. Observational data on captive Great Cormorants (*Phalacrocorax carbo*) and several species of penguins indicate that the tail is used as a steering structure, controlling pitch and yaw for high speed underwater turns (Ross, 1976; Clark and Bemis, 1979; Hui, 1985). Stifftail ducks (Oxyurinae), a group of foot-propelled diving specialists, also use the tail as a rudder (Raikow, 1970; McCracken et al., 1999). Quantitative data on the use of the tail in
underwater locomotion in other birds is not available. Nonetheless, the ubiquitous use of the tail as a control surface in aerial locomotion and the observed use of the tail during swimming in certain clades suggest that the tail is no doubt an important part of the swimming locomotor apparatus in diving birds. An elongate pygostyle may confer some advantage when moving the tail through water.

Underwater locomotion imposes certain unique challenges. During flight, the wings and tail produce lift, resisting the downward force of gravity. Conversely, while diving underwater a bird must counteract its own buoyancy, an upward force pulling it toward the surface. Accordingly, wing-propelling diving birds use different power strokes for flying and swimming— the flight stroke produces a downward force whereas the swim stroke produces an upward force (Lovvorn et al., 1991; Wilson et al., 1992; Lovvorn and Liggins, 2002; Hamilton, 2006). Birds that are capable of flight and underwater diving thus experience both dorsally and ventrally directed forces acting on the tail, whereas birds that do not dive experience primarily dorsally directed force (lift). This may influence the difference in pygostyle morphology between underwater foragers and aerial/terrestrial foragers: underwater foragers exhibit a dorsoventrally symmetrical pygostyle whereas aerial and terrestrial taxa possess an asymmetrical, dorsally deflected pygostyle (Fig. 8). Perhaps birds that utilize underwater locomotion require more symmetrical attachments for dorsiflexor and ventroflexor musculature, resulting in a more symmetrical pygostyle. Alternatively, an elongate, straight pygostyle may be related to resisting biomechanical forces rather than supplying muscle attachments. Diving birds such as alcids and penguins exhibit specialized limb bone geometry being
Figure 9. Foraging style and pygostyle shape mapped onto phylogenetic topology. Phylogeny based on Hacket (Hackett et al., 2008; Jetz et al., 2012). Branch colors represent foraging style; internal branches are colored gray to indicate that ancestral foraging style is uncertain. Node 1, Aequornithes (waterbirds); Node 2, Charadriiformes (shorebirds).
better suited to resisting the high bending and torsional forces associated with the denser medium of water (Habib and Ruff, 2008). The geometry of the pygostyle of aquatic birds may similarly be able to resist such forces. These hypotheses require comparative surveys of caudal muscle anatomy (e.g., cross sectional area, pennation, fiber type) and pygostyle mechanical properties.

Another possible consequence of the evolution of a long, straight pygostyle is the orientation of the rectrices. Baumel (Baumel, 1988) noted variability in the degree of concavity of the tail fan. Pigeons and some other taxa have medial rectrices that are positioned dorsally within the rectricial bulb relative to lateral rectrices, such that the array of tail feathers forms a “vaulted” or “tented” arrangement (Baumel, 1988). Other taxa, such as Anser, Ardea, Chaetura, and Quiscalis have a flat arrangement of the rectrices, with the rachises of each tail feather lying roughly on the same plane (Baumel, 1988). A dorsally-oriented pygostyle may facilitate the dorsoventral stacking of rectrices in birds with a tented tail whereas a straight pygostyle may be indicative of flat tail fan. Conformation of a link between pygostyle shape and rectricial configuration will require an extensive survey of soft tissue morphology. Additionally, the functional consequences of a tented tail are not currently known, as aerodynamic models of the avian tail assume a flat tail fan (Thomas, 1993).

Convergent caudal morphology in diving birds is not surprising given the numerous morphological specializations observed in these forms. Diving waterbirds and anseriforms have a reduced level of skeletal pneumaticity relative to their non-diving relatives (O'Connor, 2004; Smith, 2012). Foot-propelled diving birds have pelvic girdle
and hind limb morphology that increases mechanical advantage for paddling (McCracken et al., 1999). Diving pelecaniform birds (anhingas, cormorants), have distinct forelimb cross-sectional geometry with high levels of cortical bone, likely related to buoyancy modulation (Simons et al., 2011). Similarly, the humerus of wing-propelled divers such as penguins and alcids exhibits thick cortical bone, making this element resistant to bending and torsion under the high mechanical loads involved with flapping underwater (Habib and Ruff, 2008). Foot-propelled divers typically exhibit a suite of traits related to increasing swimming performance such as a long, narrow pelvis, a large, stable knee articulation, and a posteriorly placed hip joint (Hinić-Frlog and Motani, 2010). Taken together with the results of this study, it is clear that the evolution of diving behavior in birds results in a wide range of morphological adaptations to cope with the unique demands of underwater locomotion.

Finally, the high predictive power of the pFDA of pygostyle shape suggests that pygostyle morphology may be useful for interpreting the ecology of extinct pygostylian birds. Past studies have used forelimb, hind limb, and furcula morphology to predict ecology (flight style and/or foraging style) in extinct birds with some success (Hinić-Frlog and Motani, 2010; Bell and Chiappe, 2011; Wang et al., 2011; Close and Rayfield, 2012). Incorporating information from the tail with data from the other two avian locomotor modules (wings and legs) could improve inferences of foraging behavior from the fossil record. For example, the Cretaceous diving bird Baptornis is characterized by an elongate pygostyle (Martin and Tate, 1976).
Conclusions

Pygostyle shape is an excellent predictor of foraging style in waterbirds. Underwater foraging birds, such as cormorants, penguins, puffins, gannets, and tropicbirds, exhibit convergent evolution toward a strait, elongate pygostyle (Fig. 9). Moreover, each underwater foraging group (foot propelled, wing propelled, and plunge diving) has a distinctive pygostyle shape (Fig. 8). Free caudal vertebral morphology, in contrast, is a less informative predictor of flight style or foraging style groups. These results contribute to the body of knowledge on how the acquisition of underwater locomotor behaviors influences avian morphology. The tail skeleton, much like the forelimbs, hind limbs, and skeletal pneumaticity, is modified in swimming birds.

The disassociation of the tail module from the hind limb module in basal birds is thought to have been an important innovation that allowed for ecological diversification (Gatesy and Dial, 1996a; Dial, 2006; Benson and Choiniere, 2013a). Each of the three locomotor modules (wings, legs, and tail) can evolve semi-independently and have been emphasized to varying degrees, increasing the diversity of locomotor repertoires available to birds (Gatesy and Dial, 1996a; 1996b; Dial, 2006; Benson and Choiniere, 2013b). The use of the tail for locomotion is predicted to be emphasized in birds that are capable of complex flight behavior with small bodies and elevated nests (Dial, 2006). The importance of the tail module in diving waterbirds with colonial nesting and large bodies is previously unrecognized. The diversification of diving birds may have been facilitated by evolution of caudal structure and function for underwater locomotion in the
same way that diversification into other niches is thought to be related to correlated evolution of the wings and tail for aerial locomotion.
CHAPTER 3: COEVOLUTION OF CAUDAL SKELETON AND TAIL FEATHERS IN BIRDS

Introduction

Variation in tail feathers (rectrices) is one of the most conspicuous signs of avian morphological diversity. Tail shape is extremely variable, from the deeply forked, V-shaped tail of the Magnificent Frigatebird to the delicate streamers of the Red-tailed Tropicbird. The tail in all its forms is a key component of the aerial locomotor apparatus. The wings produce flight by generating lift, thrust, and turning moments (e.g., Pennycuick, 1975; Hedenström, 2002). The tail supplements the role of the wings by generating lift, reducing whole-body drag, contributing to static stability, and serving as a rudder for maneuvering (Thomas and Balmford, 1995; Thomas, 1996; Maybury et al., 2001; Sachs, 2007). The aerodynamic properties of the tail, and thus the potential for the tail to perform these functions, is determined by the shape of the fan of rectrices that make up the tail (Thomas and Balmford, 1995). This tight form-function relationship between tail shape and flight performance means that hypotheses grounded in aerodynamic principles have been useful for understanding the evolution of tail feather diversity (e.g., Fitzpatrick, 1999; Park et al., 2000; Clark, 2010). Whereas the caudal skeleton similarly exhibits morphological disparity, the evolution of this variation is less well understood (Van Oort, 1904). Previous work has shown that caudal skeletal morphology is related to foraging behavior. For example, birds that forage underwater convergently evolve a characteristic pygostyle morphology, consisting of an elongate, straight shape (Felice and O’Connor, 2014). This study explores a potential alternative
source of variation in the caudal skeleton: its association with the caudal feathers it supports. Herein I test the covariance between integument and bone in this important locomotor module. Furthermore, I evaluate the utility of caudal skeletal morphology for predicting tail fan shape in fossil birds that do not preserve feathers.

The morphology of the rectrices and the caudal skeleton are predicted to covary for several reasons. First, the rectrices exhibit close topological and functional association with the underlying skeleton (Fig. 1). The calami of these rectrices insert within a fibroadipose structure called the rectricial bulb, which is in turn supported by the caudal skeleton. Specifically, the rectricial bulb is affixed to the pygostyle, the terminal caudal element (Baumel, 1988; Gatesy and Dial, 1996b). The pygostyle is a laterally compressed, plowshare-shaped bone formed by the co-ossification of the terminal few (five to nine) caudal vertebrae (Baumel, 1988). It not only acts as an attachment for the rectricial bulbs (and in turn the rectrices), but also as an attachment for the muscle that facilitates tail fanning (m. bulbi rectricium) and several of those that produce dorsoventral and lateral movements of the tail (e.g., m. depressor caudae, m. lateralis caudae, Baumel, 1988; Gatesy and Dial, 1996b). Together, the muscles, skeleton, and integument function as an integrated whole.

In addition to these functional associations, the early evolution of the avian tail suggests that there is correlated evolution of skeleton between rectrices and pygostyle. The earliest examples of pygostyles are found in stem-group Neornithes such as Confuciusornithidae and Enantiornithidae (e.g., Gatesy and Dial, 1996a; Gatesy, 2001; 2002; Zhou and Zhang, 2006). These taxa exhibit an elongate, rod-like pygostyle that
consists of as many as 12 fused caudal vertebrae, typically longer than the combined length of the free caudal vertebrae, and at most two elongate, streamer-like rectrices (Clarke et al., 2006). The evolution of an articulated tail fan capable of spreading and folding is coincident with the first occurrence of a modern plowshare-shaped pygostyle in the stem ornithurine *Yixianornis* (Clarke et al., 2006). For this reason, it is thought that the evolution of the tail apparatus of birds is characterized by coordinated evolution of the caudal integument and the pygostyle.

Finally, woodpeckers (Picinae) exhibit perhaps the most specialized tail of any bird, an adaptation that is expressed through coevolution of bones and feathers. Arboreal specialist members of this clade utilize the tail as a prop to support the body during vertical climbing (Burt, 1930; Richardson, 1942). These taxa are characterized by a wedge-shaped tail fan with thickened rachises and stiffened vanes on all but the outer two rectrices (Manegold and Töpfer, 2012). This derived feather morphology is accompanied by a pygostyle with an enlarged lateral surface (lamina pygostyli), providing increased area for attachment of the rectrices, and an “enormously enlarged,” concave ventral surface (discus pygostyli), increasing the area of attachment for enlarged tail depressor muscles (Manegold and Töpfer, 2012). These derived caudal feather and skeletal traits evolved in concert and in a stepwise manner. More stem-ward members of Picinae have only the medial few pairs of rectrices stiffened and lack a enlarged discus pygostyli, but do exhibit the expanded lamina pygostyli. This amounts to a stepwise evolution of the tail apparatus for its derived function as part of an arboreal locomotor apparatus, with correlated changes in the tail skeleton and rectrices (Manegold and Töpfer, 2012). The
trunk-foraging Brown Creeper (*Certhia Americana*, a member of passeriformes) also uses the tail as a prop during vertical climbing. This taxon similarly exhibits an expanded pygoystyle and stiffened medial rectrices, also thought to be adaptations for arboreal locomotion (Richardson, 1942). These examples are one line of evidence supporting the hypothesis that the components of the avian tail co-evolve.

Thus, co-evolution of the skeleton and integument of the tail is in evidence from the gross morphology of the system, its early evolutionary history, and from a specific example of adaptation in trunk-climbing birds. It is therefore reasonable to ask whether this pattern of covariation between rectrices and caudal skeleton can be observed in a broad comparative sample of extant birds.

**Materials and Methods**

Skeletal and rectricial morphology was quantified in 48 taxa, sampled from the waterbird (Aequornithes) and shorebird (Charadriiformes) groups (Appendix 1). These clades were chosen for study as they exhibit morphological, body size, and ecological disparity (Smith, 2012). Among the members of these clades are soaring (e.g., albatrosses), flapping (e.g., loons, gulls), and swimming (e.g., penguins, auks) taxa (e.g., Pennycuick, 1982; Spear and Ainley, 1997b; Shealer, 2002). Body sizes vary greatly among the sampled taxa, from Wilson’s storm petrel (*Oceanites oceanicus*, 32 g) to the great albatross (*Diomedia epomorpha*, 8200 g; Dunning, 1993). Caudal skeletal diversity in these clades is also well documented (Felice and O’Connor, 2014). The diversity represented in these two clades makes this taxonomic sample a good focal group for characterizing evolutionary patterns in caudal morphology. Importantly, sexual
dimorphism in rectricial morphology is not present in the taxonomic sample chosen (Coulson, 2002). Even groups such as frigatebirds and tropicbirds that are characterized by elaborate tail feathers are sexually monomorphic for rectricial morphology (Coulson, 2002; Veit and Jones, 2003). Specific taxon sampling within these clades was designed to meet several criteria. First, sampling within Aequornithes was designed to include representatives of all the major groups within the clade and was modeled after related studies of evolutionary morphology in waterbirds (e.g., Simons, 2010; Smith, 2012). Second, selected members of Charadriiformes serve as an outgroup to Aequornithes, with individual taxa exhibiting convergent skeletal morphology and ecology between the two clades (Felice and O'Connor, 2014). Finally, species were selected for analysis if multiple skeletal and study-skin specimens were available in major museum collections.

Skeletal morphology was quantified using two methods (described previously, Felice and O'Connor, 2014). The morphology of the free caudal vertebrae was characterized using linear measurements collected using digital calipers (Mitutoyo Model 573-731, Plymouth, MI). In order to fully capture the extent of morphological variation of the free caudal vertebrae, the following measurements where used: centrum length, centrum width, centrum height, transverse process length, transverse process width, spinous process length, spinous process width, spinous process height, ventral process length, ventral process width, ventral process height (Fig. 3). Additional skeletal elements (sternal length, sternal width, height of sternal keel, synsacral length, and femur length) were measured and used to calculate a geometric mean to act as a body size proxy for each specimen: (Mosimann, 1970; Mosimann and James, 1979; Simons, 2010). A linear
regression reveals a significant relationship ($R^2 = .84, p < 0.001$) between body size proxy values and published body mass values (Dunning, 1993). This indicates that the proxies calculated from skeletal data suitably approximate the actual body masses of these taxa. Body mass proxies were then used to conduct a phylogenetic least-squares regression using the phylogenetic topology, described below (Fig. 10), under a Brownian Motion model, to correct raw measurements for body size. The species’ means of the residuals were used as variables for subsequent analyses (Revell, 2009; 2011).

Second, pygostyle morphology was quantified using Elliptical Fourier Analysis (EFA). EFA is a geometric morphometric method that is useful for describing shape variation in two-dimensional forms, like the pygostyle, that have few clearly defined homologous landmarks (Rohlf and Archie, 1984; Crampton, 1995). Using this method, the outline of a given shape is summarized as a series of harmonically related sine and cosine equations. Taken together, these sets of equations, termed harmonics, may be used to describe an increasing degree a complexity of the original outline shape. The result is a multidimensional data set containing $4n$ Fourier descriptors for each specimen, where $n$ is the number of harmonics used. This dataset is then corrected for the effects of size, rotation, and position, and subsequently can be used in multivariate statistical analyses in the same way that landmark-based morphometric data sets are used (Rohlf and Archie, 1984; Crampton, 1995; Claude, 2008). Each pygostyle specimen was photographed in left lateral perspective. Outline shapes were digitized and EFA was applied using the SHAPE
Figure 10. Phylogenetic tree of 47 extant waterbird and shorebird taxa. Based on (Hackett et al., 2008; Jetz et al., 2012). Extinct taxon Limnofregata azygosternon indicated with dotted line.
software suite (Iwata and Ukai, 2002). Using the Fourier power equation (Crampton, 1995; Claude, 2008) it was determined that eight harmonics are required to reconstruct 95% of the detail of the digitized outline. Following convention, the first 8 harmonics were thus used for subsequent analysis of shape variation.

Finally, tail fan shape was quantified using linear measurements of the rectrices. The length of the outermost and innermost tail feathers were measured from 223 study skin specimens (Collected from the following institutions: AMNH, American Museum of Natural History, New York, New York; CM, Carnegie Museum of Natural History, Pittsburgh, PA; FMNH, Field Museum of Natural History, Chicago, IL; NMNH, National Museum of Natural History, Washington, DC; OUVC, Ohio University Vertebrate Collection, Athens, OH. Complete list of specimens in Appendix 1). Feather length was measured from the point that the calamus emerges from the skin to the distal extent of the feather. When the tail is folded, the rectrices are stacked dorsoventrally. In order to minimize the risk of damage to museum specimens, only the more dorsal of the two innermost rectrices and the more ventral of the two outermost rectrices where measured. Tail fan shape is summarized as the logarithm of the length of the outer rectrix divided by the logarithm of the length of the inner rectrix (Bleiweiss, 2009). Thus, a high tail ratio signifies a deeply forked tail fan and a low tail ratio indicates a graduated tail fan. A tail ratio close to 1.0 indicates that inner and outer tail feathers are nearly the same length. This tail shape is termed ‘square’ as it appears somewhat rectangular when folded, although the fully spread tail fan appears semicircular (Bleiweiss, 2009).
When considering a broad taxonomic dataset such as that assembled herein, it is important to acknowledge the shared evolutionary history of the study species involved and to explicitly address the nonindependence of the data in any statistical approach (Felsenstein, 1985). In order to take into account the effects of shared ancestry, I tested for phylogenetic signal utilizing a phylogenetic topology (Fig. 10) based on Hackett and colleagues recent work (Hackett et al., 2008; Jetz et al., 2012). A posterior distribution of 5000 trees was obtained from www.birdtree.org, and a maximum clade-credibility (MCC) tree was constructed from this sample using TreeAnnotator v1.6.2 (Drummond et al., 2012). Using this MCC tree, the strength of phylogenetic signal was tested using two methods. First, the optimal value of the tree transformation parameter lambda ($\lambda$) was estimated, a variable that quantifies the extent to which phylogenetic patterns predict variation in the phenotypic data (Pagel, 1999; Freckleton et al., 2002). Optimal lambda values were calculated separately for the two skeletal morphology datasets (pygostyle and free caudal vertebrae). For the geometric morphometric dataset summarizing pygostyle shape, phylogenetic signal was also estimating using an alternative approach referred to as the consistency index (Klingenberg and Gidaszewski, 2010). This method specifically formulated for the purpose of quantifying the effect of phylogeny on multi-dimensional data (such as geometric morphometric data) and is thus a more appropriate test in this case. Both methods find significant levels of phylogenetic signal, justifying the use of phylogenetic comparative methods in subsequent analyses. The optimal value of lambda is 0.28 for pygostyle shape and 0.27 for the free caudal vertebra data. The
consistency index confirms a moderate level of phylogenetic signal (signal = 0.53, p-value = 0.001, iterations = 999).

The relationship between skeletal morphology and tail fan shape was then evaluated using several statistical approaches. First, a phylogenetic generalized least squares regression (PGLS) was used to test whether skeletal morphology could be used to predict tail fan ratio. Two regressions were calculated: one with the pygostyle shape data as the independent variables and one with free caudal vertebrae data as the independent variables. In both analyses, the dependent variable was tail fan shape and the phylogenetic tree described above was used as the comparative framework.

I also utilized a more general, categorical approach in contrast to the continuous data approach of the PGLS. In this case, each taxon was assigned into one of three tail fan shape categories: the quartile with the highest tail fan ratio was defined as forked-tailed, the quartile with the lowest ratio was defined as graduated-tailed, and the median 50% was defined as square-tailed (Fig. 10). Using this classification scheme, a phylogenetic MANOVA was used to test whether each tail fan group exhibits significantly different caudal skeletal morphology (Garland et al., 1993). Again, separate analyses were carried out for the pygostyle and free caudal vertebrae data sets. A Phylogenetic Flexible Discriminant Analysis (PFDA) was also used in order to determine if skeletal morphology can be used to consistently predict gross tail fan shape. PFDA is a multi-group classification method related to linear discriminant analysis that is used to predict group identity (in this case tail fan group) using multivariate continuous data (in this case skeletal morphology) (Motani and Schmitz, 2011; Schmitz and Motani, 2011).
PFDA was also used to generate ordination plots in order to better visualize and interpret differences in skeletal morphology among tail fan groups.

Finally, if there is significant covariation between skeletal morphology and rectrices, skeletal morphology should be able to be used to predict tail fan shape in fossil birds that do not preserve integument. I test this assertion using an exemplar specimen of the extinct bird *Limnofregata azygosternon* (Olson, 1977). *Limnofregata* represents the sister taxon to modern frigatebirds (Smith, 2010). Frigatebirds are pelagic marine waterbirds that are seemingly adapted for efficient aerial foraging in the open ocean, where food resources are patchy and unpredictable (Weimerskirch et al., 2004). Putative adaptations for this foraging strategy include long, narrow wings, feet specialized for perching rather than paddling, and a long tail that is deeply forked (Weimerskirch et al., 2004; Olson and Matsuoka, 2005).

In contrast to the oceanic modern frigatebirds, the middle to late Eocene *Limnofregata* is found in lacustrine sediments (Olson, 1977; Olson and Matsuoka, 2005). Its hind limb morphology indicates that it exhibited more substantial toe webbing than *Fregata* (the genus of extant frigatebirds), suggesting it was more capable of alighting on water than *Fregata*. This morphological and paleoenvironmental evidence has led paleontologists to interpret *Limnofregata* as exhibiting an ecology more similar to *Larus* (gull) than *Fregata*. That is, *Limnofregata* is thought to be more of an opportunistic predator and scavenger like modern *Larus* than a specialized highly aerial predator like *Fregata* (Olson and Matsuoka, 2005). Indeed, it has been hypothesized that the deeply forked tail of modern frigate birds only evolved in an oceanic context as an adaptation for
soaring flight (Olson and Matsuoka, 2005). An alternative hypothesis, supported by aerodynamic models of tail function, predicts that agile aerial foragers benefit from forked tails, as this configuration increases lift to drag ratio and moment-to-drag ratio (efficiency of producing turns; Thomas and Balmford, 1995). Thus, if *Limnofregata* was a generalist/opportunistic forager like a gull, it may be expected to have a “square” tail shape like a gull. If it was an aerial forager like *Fregata*, it would be expected to have a forked tail.

To test whether *Limnofregata* possesses the distinct deep forked tail like *Fregata*, I quantified the shape of the pygostyle of *Limnofregata azygosternon* (specimen FMNH PA 723) using EFA and subjected it to the phylogenetic FDA analysis described above to predict tail fan shape in this specimen. For this iteration of the PFDA, I constructed an informal phylogenetic tree by starting with the topology described above and grafted *Limnofregata* as the sister to *Fregata* (Fig. 10, dashed line), as recovered by a recent morphology-based phylogenetic analysis (Smith, 2010). This produces an ultrametric tree, meaning that the extinct taxon is represented as being contemporaneous with the extant taxa. I acknowledge that this is not a completely accurate representation of the true evolutionary history of the taxonomic sample. However, this informal tree is an appropriate method to incorporate the extinct taxon into the PFDA analysis given our taxonomic sample and our current knowledge regarding the evolutionary relationships among extinct and modern waterbirds (e.g., Smith, 2012; Zanno and Makovicky, 2013). The alternative, constructing a formal phylogenetic hypothesis using a total evidence
method (morphological and molecular data) in order to include this single extinct taxon is outside of the scope of this study.

Results

Each of the two regions of the caudal skeleton exhibits a different relationship with rectricial morphology. First, there is no covariation between morphology of the free caudal vertebrae and tail fan shape. The results of the PLGS regression using free caudal vertebrae data to predict tail fan ratio are not significant (Table 4). Additionally, when tail fan shape is treated as categorical, a phylogenetic MANOVA shows that there is not a significant difference in free caudal vertebral morphology among forked-, square-, and graduated-tailed birds (Table 5).

Pygostyle shape, however, is more closely related to tail fan morphology. Although PGLS regression finds that the relationship between pygostyle fan shape and tail ratio is not significant (Table 4), the statistical approaches classifying tail shape more generally as a categorical variable do recover a strong relationship between caudal skeleton and integument. Forked-, square-, and graduated-tailed birds indeed exhibit significantly different pygostyle shape (Table 5).

### Table 4. Phylogenetic generalized least squares regression results

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Table 5. Phylogenetic MANOVA results

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In order to visualize the differences in pygostyle shape among these groups, I calculated average pygostyle shape for each group and constructed outlines for each using inverse Elliptical Fourier Analysis (Claude, 2008; Bonhomme et al., 2013). Taxa with graduated tails (e.g., gannets and cormorants) exhibit a pygostyle that is craniocaudally elongate and that tapers to a point caudally (Fig. 11a). In contrast, the pygostyle of forked-tailed birds (e.g., storm petrels, frigatebirds) is rounded caudally and deflected caudodorsally (Fig. 11c) compared to the straight configuration observed in the graduated-tail group. The pygostyle of this group also has well-defined ventral processes and exhibits distinct craniodorsal and caudoventral concavities at midlength, generating the hourglass shape in lateral view (Fig. 11c). Finally, the pygostyle shape of square-tailed birds is somewhat intermediate between that of the forked and graduated groups (Fig. 11b). It shows the pronounced ventral process of the forked group. The hourglass shape is also present in the square-tailed group, but it is less strongly defined. The pygostyle in the square-tailed group is less dorsally deflected than that of the forked-tail group but not as straight as in the graduated-tail group. The pygostyle shape of forked-tailed and graduated-tailed birds therefore represent two extremes, with the square-tailed taxa exhibiting an intermediate condition.
Given that pygostyle shape differs among these groups, I subjected these data to a PFDA in order to assess whether pygostyle shape can be used to predict the tail fan category to which a species belongs. The results indicate that pygostyle shape is an excellent predictor of tail fan shape with just 2.1% misclassification error (Fig. 12). The first discriminant axis explains 55% of the between-group variance. This axis describes the change in height of the pygostyle along its length. Taxa that score high on axis one (forked tailed taxa, e.g., *Nesofregetta fuliginosa, Oceanodroma furcata*) exhibit a pygostyle with a defined hourglass shape, with a narrowing midway along its length followed by an expansion at the caudal extent (e.g., Fig. 11c). Those that score low on this axis (square tailed taxa, e.g., *Ardea herodias, Cochlearius cochlearius*) exhibit a pygostyle this is taller at mid-length and tapers slightly at the caudal margin, thus lacking the hourglass shape. The second discriminant axis explains the remaining 45% of the between group variance. This axis separates the graduated-tailed taxa from the other groups and describes the extent to which the pygostyle is deflected dorsally. Graduated-tailed taxa (e.g., *Phaethon rubricauda, Pygoscelis papua*) score very high on axis two.
Figure 12. Results of phylogenetic flexible discriminant analysis. Circles: graduated-tailed taxa, triangles: squared-tailed taxa, crosses: forked-tailed taxa. Misclassification rate = 2.1%.

exhibit a straight pygostyle (e.g., Fig. 11a). Forked- and square-tailed taxa score low on axis two and exhibit a dorsally deflected pygostyle (e.g., Fig. 11b,c).

Of the 48 taxa measured, only *Eudocimus albus* (American White Ibis) was not accurately classified by the PFDA model: it belongs to the square tail fan group, but was predicted to have a forked tail fan. Taken together, these results suggest that there is a
relationship between pygostyle shape and caudal feather configuration. This relationship is not strong enough to predict precise tail fan ratio (as in PGLS) but can be used to predict general tail fan shape (as in PFDA).

When the fossil bird *Limnofregata* was subjected to the PFDA, it was predicted to have exhibited a forked tail fan, a phenotype often found in agile aerial foragers. A qualitative examination of the pygostyle morphology of *Fregata* and *Limnofregata* reveals some similarities (Fig. 13). Both are characterized by the distinctive dorsally deflected orientation associated with a forked tail fan. Additionally, both have a well-
defined ventral process, although it is more expanded in Fregata. Both show slight craniodorsal and caudoventral concavities, characteristic of forked-tailed birds. The caudoventral concavity is positioned more proximally in Limnofregata than in Fregata. The dorsal margin is craniocaudally expanded in Fregata, but not in Limnofregata. Although pygostyle shape of these two genera are distinct from one another, they both exhibit the characteristic pygostyle morphology indicative of a forked tail fan.

Discussion

The caudal apparatus of birds is an important part of the aerial locomotor apparatus, yet the evolution and diversification of its component parts has until now been understudied. Evidence from the fossil record (Clarke et al., 2006) and from birds with highly specialized tail structure and function (Manegold and Töpfer, 2012) suggests that the fan of tail feathers coevolves with the caudal skeleton that supports it. The results of this phylogenetic comparative analysis of caudal morphology in waterbirds and shorebirds support the hypothesis of coevolution of caudal feathers and the pygostyle. Birds with different tail fan shapes (forked, graduated, or square) have significantly different pygostyle shapes. Moreover, pygostyle shape can be used to accurately predict tail fan shape using PFDA. In several instances, common pygostyle shape and tail fan shape evolve in distantly related taxa. For example, the various fork-tailed taxa (e.g., African sacred ibis, Threskiornis aethiopicus; frigatebirds, Fregata; great blue heron, Ardea Herodias; hydrobatids, Nesofregetta, Oceanites, Oceanodroma; tufted puffin, Fratercula cirrhata) all exhibit a characteristic dorsally deflected, hourglass-shaped pygostyle. These taxa are somewhat dispersed across the phylogeny (Fig. 10), suggesting
that feathers and skeleton independently co-evolved in each of these lineages. Likewise, distantly related graduated-tailed taxa (e.g., cormorants, *Phalacrocorax*; penguins, *Pygoscelis*; tropicbirds, *Phaethon*) exhibit a common long, straight pygostyle shape. This convergent evolution of both tail fan shape and pygostyle shape supports the hypothesis that these components of the caudal apparatus co-evolve. Interestingly, many of the graduated-tailed taxa are underwater foraging birds (e.g., Shealer, 2002). The straight, tapered pygostyle shape that is correlated with this tail fan shape is also characteristic of underwater foraging taxa (Felice and O'Connor, 2014) This suggests that that an interplay of functional demands and covariation among traits serves to generate the diversity of caudal morphology observed among birds.

Another notable finding is the discordance between the results of continuous (PGLS) and categorical (MANOVA and PFDA) approaches. Whereas no significant relationship was found between tail feathers and pygostyle using PGLS, a correlation was recovered using MANOVA and PFDA. The disparity in results could be due to the structure of the data. The geometric morphometric dataset describing pygostyle shape does not meet one of the assumptions of MANOVA and PGLS. Mardia’s tests of multinormality (Mardia, 1974; Korkmaz et al., 2014) were used to determine that this dataset exhibits significant kurtosis, deviating from multivariate normality. Conversely, the assumption of homoscedasticity is met (Anderson, 2006; Oksanen et al., n.d.). MANOVA using the Pillai-Bartlett trace test statistic has been shown to be somewhat robust to such a departure from normality (Olson, 1974). PFDA, as a nonparametric analysis, is also robust to departures from normality. The non-significant results of the
PGLS analysis could represent Type II error resulting from the failure of the data to meet the assumptions of the method. Alternatively, the difference in the results of the various analyses could be related to the resolution of each test. Whereas the MANOVA and PFDA analyses evaluate the relationship between pygostyle shape and general tail fan shape (forked, square, graduated), the PGLS analysis tests the relationship between pygostyle shape and the exact tail fan ratio of each taxon. It is possible that the phenotypic covariation between the pygostyle and the rectrices is simply strong enough to allow for a prediction of gross tail fan shape but not the exact dimensions of the fan.

Using the categorical approach, the sole missclassified taxon was the white ibis (*Eudocimus albus*), a square-tailed species which was predicted to have a forked tail fan. The white ibis scores higher on discriminant axis one than any other square tailed taxon and also lower on axis one than any forked-tailed taxon (Fig. 12). This intermediate position indicates that the white ibis lacks the distinguishing features of either the forked or graduated groups (i.e., it lacks both the “hourglass” shape and a caudal tapering). This serves to illustrate that whereas feather morphology and pygostyle morphology are closely linked, other factors influence the morphology of each of these tissues, complicating the relationship. For example, the correlation between foraging behavior and caudal skeletal morphology is an additional source of skeletal variation (Felice and O’Connor, 2014). White ibis forages while standing in deep water (Frederick and Bildstein, 1992). As such, it exhibits pygostyle morphology characteristic of terrestrial foraging birds (i.e., hind limb based stalking and standing, Felice and O’Connor, 2014).
This ecological signal could be overwhelming the feather-bone variation signal and causing the misclassification error.

The predictive power of the PFDA was used to determine that the extinct frigatebird *Limnofregata* probably exhibited a forked tail similar to that of its extant relatives. It has been proposed that *Limnofregata*, found in lacustrine deposits, was a generalist akin to *Larus*, and that the frigatebird lineage only evolved specializations for aerial foraging in the context of a marine habitat (Olson and Matsuoka, 2005). These results, however, suggest that *Limnofregata* had already acquired one characteristic of aerial foragers, a forked tail fan. Therefore, it is possible that *Limnofregata* and *Fregata* share this foraging style in common. Importantly, this example illustrates that this method for reconstructing rectricial morphology on the basis of pygostyle shape has potential for use with other fossil birds (e.g., *Baptornis, Gansus*) with known pygostyle morphology (Martin and Tate, 1976; You et al., 2006). The accuracy of soft tissue reconstructions in stem Neornithes will depend on a more comprehensive taxonomic sampling than what is presented here.

In contrast to the relationship between pygostyle shape and tail fan shape, no relationship is found between the morphology of the free caudal vertebrae and the tail feathers. Variation in free caudal vertebrae is also not associated with locomotor behavior (Felice and O'Connor, 2014). Additional work is needed to determine the drivers of free caudal vertebral evolution. One possibility is that the proportions of free caudal vertebrae are less influenced by their association with the pygostyle and tail fanning apparatus than by other factors, such as whole-body trends in axial skeletal morphology.
Whereas these findings indicate that there is a relationship between the pygostyle and the tail fan, it is still unclear exactly what mechanistic or functional linkage underlies this relationship. Different gross tail fan shapes may exhibit different configurations of the calami relative to the pygostyle. For example, birds with long medial rectrices (graduated tail fans) may have longer calami on the medial rectrices, necessitating an elongate pygostyle. As the pygostyle is also the site of attachment for many of the muscles associated with tail fanning and mobility, differences in pygostyle shape may be attributed to different muscular demands among the tail fan groups. For example, the orientation or size of the tail fanning muscle (m. bulbi rectricium) may vary with tail fan shape, in turn influencing pygostyle shape. These hypotheses require an in-depth investigation of soft tissue anatomy across a variety of taxa.

The results presented here provide evidence that the morphology of the pygostyle and the rectrices are related to one another and may indeed coevolve. Additionally, both tail fan shape (Thomas and Balmford, 1995) and pygostyle shape (Felice and O'Connor, 2014) have been shown to be correlated with locomotor behavior. Taken together, these findings reinforce the tail as a complex, interconnected system that plays an important role in avian locomotion.
CHAPTER 4: ASSESSING CAUDAL SKELETAL DIMORPHISM IN SEXUALLY DIMORPHIC PASSERIFORME BIRDS

Introduction

Evolution in tail morphology has been a major component of the diversification of birds. The tail represents one of three locomotor modules, along with the forelimb (wings) and hind limb (Gatesy and Dial, 1996a). The tail serves to supplement the role of the wings in flight by producing lift, reducing drag, and contributing to agility and maneuverability (Thomas, 1996). As an aerodynamic structure, the tail consists of a fan of tail feathers (rectrices) that can be spread or folded (Fig. 1). Much like the wings or tail of an airplane, the shape of the tail fan in birds determines its aerodynamic properties, and thus a bird’s aerial capabilities (Thomas, 1993). As such, different flight behaviors are associated with characteristic tail fan shapes that provide advantageous aerodynamic properties for that behavior. For example, birds that catch their prey on the wing typically exhibit a forked tail, a shape that is hypothesized to maximize agility (Thomas and Balmford, 1995).

In addition to natural selection, sexual selection has undoubtedly played an important role in shaping variation in tail feathers among birds. Indeed, observation of ornamental plumage of sexually dimorphic birds such as the peacock, rock-thrush, and bird of paradise led Darwin to the conclusion that competition for mates may also serve as a selective pressure, providing the basis for the term “sexual selection” (Darwin, 1859; 1871). Elaborate tail feathers are observed in the males of numerous lineages, including species of quail (Brown and Gutierrez, 1980), swallow (Park et al., 2001), duck, grouse,
pheasant, parakeet, hummingbird, nightjars, kingfisher, and numerous passerines (Cuervo and Moller, 2001). Various mechanisms have been proposed for the evolution and maintenance of these display structures, including the handicap principle, Fisherian “runaway” selection, and sensory exploitation (Andersson, 1994).

From a locomotor standpoint, elongate tail feathers are of special interest given the function of the tail. Aerodynamic theory predicts that natural selection should act to optimize the shape of the tail fan to meet aerodynamic demands (Norberg, 1995; Thomas and Balmford, 1995). Extremely long tails deviate from the aerodynamically “optimum” shape, imposing a cost. This cost is manifested in several ways. Long tails increase drag and therefore result in a decrease flight performance, both in flight speed and energetic cost of flight (Evans and Thomas, 1992; Balmford et al., 1993; Norberg, 1995). In turn, maneuverability, foraging rates, and predator escape capability can all be affected negatively by the presence of a long tail (Evans, 1998; Park et al., 2000; Rowe et al., 2001; Clark and Dudley, 2009).

Whereas female mate choice has resulted in the evolution of elongate tail feathers in males, sexual selection may lead to the evolution of further morphological differences as males evolve ways to mitigate the performance costs of elongate tails. As one example, in species with sexually dimorphic tails, males often exhibit longer wings as well (Evans and Thomas, 1992; Andersson and Andersson, 1994; Balmford et al., 1994). Longer wings produce more lift, compensating for the increased drag produced by an elongate tail (Evans and Thomas, 1992). Given the costs of locomotion associated with long tail feathers, it is reasonable to predict that other morphological features may evolve
in junction with tail feather length (Balmford et al., 1994). This study tests whether the evolution of sexually dimorphic tail feathers is correlated with dimorphism in the underlying caudal skeleton that supports the tail fan.

Several lines of evidence suggest that caudal feather and caudal skeletal evolution may be linked. First, caudal feathers and bones are topologically and functionally closely associated. The fan of tail feathers is anchored in bilateral fibroadipose structures known as rectricial bulbs. Rectricial bulbs flank the terminal element of the axial skeleton, the pygostyle (Fig. 1). The fanning (abduction) action of the tail feathers is facilitated by the bulbi rectricium muscle, which has its proximal and distal attachments on the pygostyle (Gatesy and Dial, 1996b). Other movements of the tail used in locomotion and display (e.g., elevation, depression, lateral deviation, rotation) are achieved through the action of muscles (e.g., levator caudae, depressor caudae, lateralis caudae, pubocaudalis) that attach to the pygostyle and free caudal vertebrae (Baumel, 1988). Long tails may require specialized musculoskeletal morphology to allow for sufficient mobility during display and locomotion.

Second, in monomorphic species, pygostyle shape is correlated with tail fan shape (Felice, 2014). Each tail fan shape (e.g., forked, graduated, square) is associated with characteristic pygostyle morphology. For example, taxa with graduated tails exhibit a long, tapered pygostyle, whereas those with forked tails exhibit a dorsally deflected, blunt-tipped pygostyle. This relationship is strong enough that tail fan shape can be accurately predicted on the basis of pygostyle shape (Felice, 2014). Such a correlation
between skeletal and integumentary morphology suggests that the evolution in tail fan shape and caudal skeletal morphology are linked.

Finally, in clades with highly specialized tails, rectricial and caudal skeletal morphology coevolve. Highly arboreal birds such as woodpeckers (Picinae) and the Brown Creeper (*Certhia americana*) utilize the tail as a prop during vertical climbing. These taxa are characterized by stiffened medial rectrices that are specialized for this function (Richardson, 1942; Manegold and Töpfer, 2012). The acquisition of reinforced tail feathers was accompanied by derived pygostyle morphology, including a larger area of surface attachment for the rectricial bulbs and for depressor muscles of the tail (Burt, 1930; Clark and Dudley, 2009; Manegold and Töpfer, 2012). These skeletal and integumentary features are thought to be adaptations that make the tail more durable and more useful as a support structure for climbing (Richardson, 1942). Importantly, these traits were acquired in a stepwise manner though the woodpecker lineage. The medial rectrices become reinforced and stiffened in the outgroup to Picinae *sensu stricto* with an associated change in the size of the lamina pygostyli. In derived members of Picinae, those taxa most highly specialized for vertical trunk climbing, additional rectrices are modified to increase strength and the discus pygostyli is increased in size. These coordinated modifications of both the rectrices and pygostyle morphology have been interpreted as evidence of coevolution of the feathers and bones of the tail (Manegold and Töpfer, 2012).

Taken together, these lines of evidence suggest that skeletal and feather morphology co-evolve in the tail. If this general pattern is also present in taxa with
sexually dimorphic tail feathers, then it is reasonable to predict that caudal skeletal morphology may also exhibit a sexually dimorphic signal. Herein, I test whether males and females of dimorphic species exhibit distinct caudal skeletal morphology. Several aspects of caudal vertebral morphology will be evaluated. First, the shape of the free caudal vertebrae is expected to change in conjunction with tail fan shape, facilitating movements of heavy, drag-inducing display feathers. Second, because pygostyle shape is correlated with differences in tail fan shape among monomorphic taxa, males and females of dimorphic taxa may also exhibit dimorphism in pygostyle shape. Finally, the evolution of larger tail feathers in males may require larger pygostyle surface area for the attachment of the medial rectrices, as observed in trunk foraging birds. The degree of sexual dimorphism in each of these skeletal features was assessed in a variety of passeriform taxa to investigate whether skeletal morphology evolves in a coordinated manner with sexually selected rectrices.

Materials and Methods

Caudal skeletal and integumentary morphology was quantified in four species that exhibit sexually dimorphic tail feathers (Bancroft, 1984; Cuervo and Moller, 2000; 2001; Regosin and Pruett-Jones, 2001b). These taxa include the Boat-Tailed Grackle (Quiscalus major), Pin-Tailed Whydah (Vidua macroura), Scissor-Tailed Flycatcher, (Tyrannus forficatus), and White-Rumped Shama (Copsychus malabaricus). As a basis for comparison, caudal morphology was also quantified in a monomorphic congener for each of the four dimorphic species (Fig. 14). These are the Common Grackle (Q. quiscula), Village Indigobird (V. chalybeata), Gray Kingbird (T. dominicensis), and Oriental
Magpie-Robin (*C. saularis*), respectively. The latter four taxa are predicted to be sexually monomorphic in caudal skeletal morphology. Each monomorphic-dimorphic species pair is closely related, having diverged approximately three to twelve million years before present (Fig. 14, (Jetz et al., 2012). A total of 329 skeletal specimens (191 male, 139 female, Table 6) were measured representing these eight taxa. These data were collected from specimens housed in the following museum collections: AMNH, American Museum of Natural History, New York, New York; CM, Carnegie Museum of Natural History, Pittsburgh, PA; FMNH, Field Museum of Natural History, Chicago, IL; KU, University of Kansas Museum of Natural History, Lawrence, KS; LACM, Natural History Museum of Los Angeles County, Los Angeles, CA; LSUMZ, LSU Museum of Natural Science, Baton Rouge, LA; NMNH, National Museum of Natural History, Washington, DC; OUVC, Ohio University Vertebrate Collection, Athens, OH; UMMZ, University of Michigan Museum of Zoology, Ann Arbor, MI; YPM, Yale Peabody Museum, New Haven, CT.

**Table 6. Taxonomic sampling.**

*Shaded rows exhibit sexually dimorphic tail feathers.*

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of Males</th>
<th>Number of Females</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Copsychus malabaricus</em></td>
<td>28</td>
<td>18</td>
</tr>
<tr>
<td><em>Copsychus saularis</em></td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td><em>Quiscalus major</em></td>
<td>38</td>
<td>33</td>
</tr>
<tr>
<td><em>Quiscalus quiscula</em></td>
<td>47</td>
<td>19</td>
</tr>
<tr>
<td><em>Tyrannus dominicensis</em></td>
<td>20</td>
<td>28</td>
</tr>
<tr>
<td><em>Tyrannus forficatus</em></td>
<td>24</td>
<td>15</td>
</tr>
<tr>
<td><em>Vidua chalybeata</em></td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td><em>Vidua macroura</em></td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>191</strong></td>
<td><strong>138</strong></td>
</tr>
</tbody>
</table>
Figure 14. Phylogenetic relationships of focal passeriform taxa. Red tips are taxa with sexually dimorphic rectrices, blue tips are those with sexually monomorphic rectrices. Rectricial morphology of each dimorphic taxon is illustrated to the right of its name. Topology and divergence dates derived from Jetz et al. (2012)

These focal taxa represent an ideal sample for studying patterns of caudal dimorphism as they exhibit a wide range of rectricial phenotypes, breeding behaviors, and body sizes. *Vidua macroura* are polygynous with highly territorial males in which the medial two rectrices are extremely elongate (Shaw, 1984). In contrast, *V. chalybeata* exhibits sexually monomorphic tail feathers and utilizes a “dispersed lek” courtship behavior (Shaw, 1984). Both *V. macroura* and *V. chalybeata* are brood parasites that utilize the nests of waxbills (Savalli, 1995). Patterns of sexual dimorphism are particularly well studied in the monogamous *Tyrannus forficatus*. Males of this species
have elongate outermost rectrices and the length of the tail feathers in both sexes is correlated with fitness traits such as increased clutch size (Regosin and Pruett-Jones, 2001b). Similar to *T. forficatus*, the dimorphic *Copsychus malabaricus* and monomorphic *C. saularis* are considered monogamous (Aguon and Conant, 1994; Siddique, 2014). Unlike *T. forficatus*, the sexually dimorphic ornament found in *C. malabaricus* consists of an elongate graduated (diamond shaped) tail fan, with medial rectrices longer than lateral rectrices (Balmford et al., 1994). Finally, species within *Quiscalus* are polygynous to variable degrees. The level of polygyny is positively correlated with the magnitude of sexual dimorphism, with highly polygynous species (e.g., *Q. major*) exhibiting dimorphism in both body size and tail length, and less polygynous species (e.g., *Q. quiscula*) exhibiting low-to-absent dimorphism (Bjorklund, 1991). Moreover, these taxa exhibit variation in foraging behavior. *Quiscalus* spp. are omnivorous ground foragers. *Copsychus malabaricus* is similarly a ground forager, but specializes on insects (Fan et al., 2015). Its congener, *C. saularis* is also insectivorous, foraging primarily in the lower branches of trees (ZannDarjono, 1992). *Vidua* spp. are granivorous (Savalli, 1995). Both *Tyrannus forficuatus* and *T. dominicensis* are insectivorous air-salliers (i.e., launching from a perch to pursue aerial prey), but *T. dominicensis* occasionally feeds on fruits (De Graaf et al., 1985). Because the focal taxa are variable in ecology, rectricial phenotype and in the mating system under which the ornament evolved, if sexually dimorphic tail feathers are associated with sexually dimorphic skeletal morphology then this pattern should be detected across the entire sample.
The morphology of the free caudal vertebrae was quantified using methods described previously (Felice and O'Connor, 2014). Briefly, digital calipers (Mitutoyo Model 573–731, Plymouth, MI) were used to collect the following metrics: centrum length (craniocaudal), centrum width, centrum height, spinous process length, spinous process width, spinous process height, transverse process length, transverse process width, ventral process length, ventral process width, ventral process height (Fig. 3). Because the number of free caudal vertebrae varies among individuals and taxa, a basis for making homologous comparisons of morphology among individuals is required. As such, metrics were assessed for both the first (postsynsacral) and last (propygostylar) free caudal vertebrae, as well as the vertebra halfway along the free caudal series. In individuals with an even number of free caudal vertebrae, the morphology of the middle two vertebrae was quantified and the average of each measurement was used. Additionally, a body size proxy was calculated for each skeletal specimen. The body size proxy used herein consists of the geometric mean of several skeletal dimensions: femur length, sternal length, sternal width, sternal keel height, and synsacral length (Mosimann and James, 1979; Simons, 2010; Felice and O'Connor, 2014). To mitigate the effects of body size on free caudal vertebral dimensions, the logarithm of each vertebral metric was divided by the logarithm of the body size proxy for that individual (Jungers et al., 1995). The resulting values were used as the free caudal vertebral dataset for the subsequent analyses. The complete dataset of all free vertebral measurements and body size proxy measurements is available as an online digital supplement to this dissertation.
Some skeletal specimens had incomplete caudal series. In these cases, pygostyle shape (see below) was quantified but not free caudal vertebral morphology. In most taxa, this decreased the sample size for free caudal vertebral morphology by 6.5-19%.

However, for *V. chalybeata* and *V. macroura*, incomplete specimens were more common (20-62% incomplete), decreasing the sample size to a level whereby it was not feasible to incorporate either taxon into MANOVA approaches. Thus, this analysis was omitted for both species of *Vidua*.

Pygostyle shape was quantified using Elliptical Fourier Analysis (EFA), a geometric morphometric technique that is suitable for data with few clearly defined homologous landmarks (Rohlf and Archie, 1984; Crampton, 1995). With EFA, the outline of a shape is subjected to a Fourier decomposition, which summarizes the shape as a series of sine and cosine equations, termed harmonics. The coefficients of these harmonics describe a portion of the detail of the original shape. The harmonics are normalized to remove the effects of size, position, and rotation (Crampton, 1995; Claude, 2008). Harmonic coefficients can then be used as multidimensional data in statistical analyses, analogous to the Procrustes-aligned coordinates used in landmark based geometric morphometrics (e.g., Bonhomme et al., 2013).

Each pygostyle specimen was photographed from the left lateral perspective. The outline of each specimen was digitized, Fourier decomposition was applied, and normalization was carried out using the SHAPE software package (Iwata and Ukai, 2002). The Fourier power equation was used to determine that 95% of the total power to reconstruct the outline shapes in the sample is described by the first five harmonics.
Thus, five harmonics were retained for all subsequent analyses of pygostyle shape. Finally, in order to evaluate differences in pygostyle size between sexes, the lateral surface area of each pygostyle was measured using ImageJ (Abràmoff et al., 2004). Pygostyle size then was corrected for body size using the same method used for free caudal vertebral metrics.

The presence of sexual dimorphism in caudal skeletal morphology was evaluated using two different analytical approaches. First, for the free caudal vertebral morphology and pygostyle shape data sets, a permutational MANOVA (multivariate analysis of variance) was used (Anderson, 2006). This analytical approach was chosen to accommodate significance testing utilizing the geometric morphometric (GMM) data describing pygostyle shape, which contains a high number of trait dimensions (variables) relative to the number of observations. In such cases, it is preferable to use distance-based (Q-mode) statistics such as permutational MANOVA rather than traditional parametric (R-mode) tests of significance, such as Wilk’s lambda and Pillai-Bartlett trace (Adams, 2014). Permutational MANOVAs were carried out using the vegan package in R (Oksanen et al., n.d.). Because some of the Fourier coefficients are negative values, Kulczynski distance was selected as the most suitable distance metric (Legendre and Legendre, 2012), and significance was tested using 1000 permutations. Second, a one-tailed t-test was used to test whether males of species with dimorphic tail feathers exhibit a larger pygostyle than females.
Results

Sexual dimorphism was assessed for three aspects of the caudal vertebral morphology in each of the eight taxa examined: free caudal vertebral morphology, pygostyle shape, and pygostyle size. An examination of free caudal vertebrae reveals no significant difference in morphology between males and females in either the taxa with monomorphic rectrices or those with dimorphic rectrices (Table 7), with the exception of *Quiscalus major*. Likewise, permutational MANOVA reveals that pygostyle shape is not significantly different between males and females in any of the eight taxa (Table 8). However, a difference in pygostyle size between the sexes was detected in *Quiscalus quiscula, Q. major*, and *Vidua macroura* (Table 9). In each case, males exhibit a larger pygostyle than females. All other taxa were found to be monomorphic for this trait.

Discussion

An assessment of skeletal shape and size variation in a selection of sexually dimorphic species and closely related sexually monomorphic taxa within Passeri and Tyranni reveals very little evidence of correlations of bony morphology with variation in feather morphology. Specifically, no differences were detected between males and females in pygostyle shape. Similarly, each comparison reveals that free caudal vertebral morphology is also monomorphic between sexes, with the exception of *Quiscalus major*. Five of the eight taxa are monomorphic in pygostyle size. The three taxa with sex-specific differences in pygostyle size are *Quiscalus quiscula, Q. major* and *Vidua macroura*. These findings are surprising in light of recent evidence that pygostyle shape and tail fan shape are correlated across a wide range of Aequornithes and
Table 7. Results of permutational MANOVA, free caudal vertebral morphology sexual dimorphism.
Shaded rows exhibit sexually dimorphic tail feathers. Note that negative sum of squares and F numbers are allowable in pt-MANOVA when using non-Euclidean distance metrics (Gower, 1985; Chapman and Underwood, 1999).

<table>
<thead>
<tr>
<th>Species</th>
<th>Sum of Squares</th>
<th>F Number</th>
<th>R²</th>
<th>p-Value</th>
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<tr>
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Table 8. Results of permutational MANOVA, pygostyle shape sexual dimorphism.
Shaded rows exhibit sexually dimorphic tail feathers.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sum of Squares</th>
<th>F Number</th>
<th>R²</th>
<th>p-Value</th>
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<td>Tyrannus dominicensis</td>
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<td>1.06</td>
<td>0.02</td>
<td>0.37</td>
</tr>
<tr>
<td>Tyrannus forficatus</td>
<td>0.03</td>
<td>0.53</td>
<td>0.01</td>
<td>0.81</td>
</tr>
<tr>
<td>Vidua chalybeata</td>
<td>0.05</td>
<td>0.75</td>
<td>0.05</td>
<td>0.62</td>
</tr>
<tr>
<td>Vidua macroura</td>
<td>0.14</td>
<td>1.83</td>
<td>0.08</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Table 9. Results of t-test, pygostyle size dimorphism
Shaded rows exhibit sexually dimorphic tail feathers.

<table>
<thead>
<tr>
<th>Species</th>
<th>T-Statistic</th>
<th>Degrees of Freedom</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copsychus malabaricus</td>
<td>0.82</td>
<td>26.36</td>
<td>0.21</td>
</tr>
<tr>
<td>Copsychus saularis</td>
<td>0.61</td>
<td>17.93</td>
<td>0.27</td>
</tr>
<tr>
<td>Quiscalus major</td>
<td>4.47</td>
<td>59.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Quiscalus quiscula</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>Tyrannus dominicensis</td>
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<td>43.69</td>
<td>0.34</td>
</tr>
<tr>
<td>Tyrannus forficatus</td>
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<td>.66</td>
</tr>
<tr>
<td>Vidua chalybeata</td>
<td>1.01</td>
<td>4.79</td>
<td>0.16</td>
</tr>
<tr>
<td>Vidua macroura</td>
<td>2.78</td>
<td>21.11</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Charadriiformes (Felice, 2014). Comparing patterns of caudal evolution and use among these clades suggests that functional variation, rather than skeleton-integumentary interactions, is the primary cause of caudal skeletal evolution.
Clades that have been shown to exhibit pygostyle-tail fan covariation also have highly variable locomotor behavior and ecology (Felice, 2014; Felice and O'Connor, 2014). Members of Aequornithes and Charadriiformes typically utilize the tail module as a significant component of the locomotor system, whether in flight and swimming (e.g., Hui, 1985; Dial, 2003). This locomotor function of the tail is thought to be a major force driving morphological evolution in the caudal skeleton in these clades (Felice and O'Connor, 2014). In contrast, passeriforms, such as those represented in this analysis, reduce the importance of the tail in locomotion (Dial, 2003; Bleiweiss, 2009). Birds that habitually utilize different locomotor regimes emphasize the use of the three locomotor modules (tail, wings, and hind limb) to different degrees. Investment in one locomotor module can result in a reduction of selective pressure to main structure and function in the other modules (Dial, 2003; 2006). Highly aerial taxa (e.g., Passeriformes, Hirundinidae, Apodiformes) possess a powerful pectoral girdle capable of serving a primary role in lift and thrust production for flight, as well as advanced aerial control (e.g., stability, maneuverability, see Dial, 2003). As such, the role of the tail in flight is diminished, thereby reducing selective pressures on maintaining the structure and function of the tail to serve as an aerial rudder (Bleiweiss, 2009). In this way, the tail apparatus may be free to evolve alternative functions (e.g., intersexual display) and structures (e.g., elongate streamers). This tradeoff is supported from evidence within hummingbirds (Trochilidae): elongate tail feathers are only found in the most highly aerial lineages with extremely powerful pectoral girdle musculature, and thus, forelimb dominant locomotion (Bleiweiss, 2009). This suggests that (A) natural selection on tail
function is a primary driver of caudal skeletal evolution and (B) the magnitude and rate of skeletal evolution in this system may be low in clades that deemphasize the tail’s locomotor function.

These ideas are supported by an examination of the natural history and skeletal dimorphism patterns in *Vidua*, the lone genus in this analysis that supports the hypothesis that males and females with different tail fan shapes also exhibit different caudal skeletal morphologies (Table 9). *Vidua macroura* is unique within this study in that there is a critical difference in tail function between males and females. Males display to potential mates by hovering and performing rapid upward flicking of the elongate tail (Shaw, 1984). Its monomorphic congener, *V. chalybeata*, also hovers during courtship displays, but does not utilize tail flicking (Payne, 1973). This difference in tail function between the two species could explain why this is the only one of the examined pairs that exhibits the predicted pattern of skeletal dimorphism. In this case, it seems that in addition to tail ornament size, tail function and behavior is shaped by sexual selection in *V. macroura*. For this reason, and because of previous evidence between pygostyle morphology and tail function (Richardson, 1972; Manegold and Töpfer, 2012; Felice and O'Connor, 2014), it is likely that the evolution of caudal skeletal diversity is shaped more by functional variation than by any strict association between the caudal skeleton and the integument.

*Quiscalus quiscula* and *Q. major* were also found to have sex differences in pygostyle size (but not shape). In *Quiscalus*, males have larger pygostyles in both the species with monomorphic rectrices (*Q quiscula*) and the species with dimorphic rectrices (*Q. major*). This is contrary to the prediction that sexual dimorphism in the
caudal skeleton would reflect dimorphism in the rectrices. *Quiscalus* is alone among the genera examined here in that sexual dimorphism is expressed as a size difference in the entire tail fan, compared to the other taxa in which ornaments are only one or two pairs of rectrices. For this reason, it is possible that among the species examined here that only the grackles exhibit a significant difference in tail mass between males and females (although rectricial mass was not quantified here). A large difference in rectricial mass could necessitate a larger pygostyle surface area to allow for a larger bulbi rectricium and thus deeper “rooting” of the rectricial calimi. Such a function for an increase in lamina pygo styli size has been previously suggested in Picinae (Manegold and Töpfer, 2012).

Perhaps more interesting is that both species of *Quiscalus* exhibit dimorphism in pygostyle size, although this pattern was only predicted to be present in *Q. major*. This pattern may be attributed to the degree of monomorphism present in *Q. quiscula*. Although *Q. quiscula* was considered monomorphic for the purposes of this study, it may show some dimorphic features. Dimorphism in body size and tail length is present to variable degrees throughout *Quiscalus*, with *Q. major* being among the most dimorphic species and *Q. quiscula* among the least (Bjorklund, 1991). Pygostyle dimorphism may reflect an overall pattern of dimorphism occurring throughout this genus (Bjorklund, 1991). *Q. major* (but not *Q. quiscula*) is also the only species with a significant difference in free caudal vertebral morphology between males and females (Table 7). However, the goodness-of-fit is very low ($R^2 = 0.09$), which indicates that whereas the relationship between sex and free caudal vertebral morphology is statistically significant in this taxon,
sex differences explain very little of the total variation in free caudal vertebrae compared to other factors.

It is important to consider the evolutionary history of the clade examined in this analysis to understand the lack of morphological divergence in conjunction with the evolution of dimorphic rectrices. In each of the four species pairs in this study, the monomorphic and dimorphic taxa diverged relatively recently, ranging between 2.9 mya (Quiscalus spp.) to 11.5 mya (Copsychus spp., Jetz et al., 2012). Assuming that each dimorphic species evolved from monomorphic ancestor, then the males of these species have experienced only 2.9-11.5 million years in which to accumulate any morphological changes in the caudal skeleton and integument. In contrast, much more time has elapsed since the divergence of lineages with different caudal skeletal and integumentary morphology within waterbirds and shorebirds. For example, frigatebirds (Fregata) have a deeply forked tail fan and a characteristic pygostyle shape that is associated with that integumentary morphology. The closely related group comprised of gannets, cormorants, and anhingas exhibits a graduated tail and a divergent pygostyle phenotype (Felice, 2014). These two clades diverged from one another around 59 mya (Jetz et al., 2012). This divergence date is typical of groups previously identified as having significant differences in caudal skeletal morphology and is approximately six times greater than the greatest temporal distance between species pairs in this study. Skeletal morphology could be evolving at a slower rate than integumentary morphology. If so, the lack of caudal skeletal differences between males and females in dimorphic taxa could be a consequence of insufficient time under the influence of sexual selection to result in significant
differences in the axial skeleton. Different evolutionary rates among individual structures represents a testable hypothesis: skeletal and integumentary morphology can be quantified across a wide phylogenetic sample, then rates of morphological evolution can be quantified under various evolutionary models such as Brownian motion and Ornstein-Uhlenbeck (Revell et al., 2012; Higham et al., 2015). Such an analysis would also allow for the detection of differential evolutionary rates among clades (e.g., between Passeriformes and Aequornithes).

From another perspective, caudal skeletal morphology may not be modified to mitigate the aerodynamic costs of the tail because the wings are instead modified to compensate for a costly tail. Across a variety of clades, males of dimorphic species with longer tails also have longer wings (Balmford et al., 1994). Increasing wing length increases lift production, potentially offsetting the increased drag (aerodynamic cost) produced by long tails. For this reason, it is believed that birds “compensate” for the costs of an elongate tail by modifying the wings (Balmford et al., 1993). In this case, there may simply be no need to modify the axial skeleton in conjunction with the evolution of elongate rectrices.

Ultimately, no overarching pattern of dimorphism in caudal skeletal morphology was observed in passeriform taxa with sexually dimorphic tail feathers. Previous research has emphasized that caudal skeletal morphology is shaped primarily by tail function (Richardson, 1972; Manegold and Töpfer, 2012; Felice and O'Connor, 2014). None of the examined taxa exhibited significant sex differences in free caudal vertebral morphology or pygostyle shape. Only a small minority of the taxa examined exhibit
pygostyle size dimorphism, most notably *V. macroura*. This species exhibits sex differences in tail function as well as tail feather length and pygostyle size. Taken together, these results suggest that caudal skeletal morphology, including pygostyle shape and size, evolves in concert with tail function and is largely unaffected by sexual selection for large tail feathers.
CHAPTER 5: INTEGRATION PATTERNS BETWEEN SUBREGIONS OF THE AVIAN TAIL SKELETON

Introduction

Birds are the most diverse tetrapod group, probably due in no small part to their derived locomotor system. The evolution of flight allowed birds to increase dispersal capability and an opportunity to exploit a stunning variety of ecological niches. High performance flight and diverse flight behaviors are possible in large part due to evolutionary innovations in the structure and function of the tail. The tail serves a critical role in flight, assisting the wings in producing lift, reducing drag, and facilitating maneuverability/agility (Thomas, 1996; Gatesy and Dial, 1996a; 1996b). The tail is also hypothesized to be a critical part of steering in underwater locomotion (Hui, 1985; Felice and O'Connor, 2014). These functions are possible due to the derived morphology of the tail apparatus. This system consists of a fan-like array of tail feathers (rectrices), which serve as the aerodynamic component, muscles and soft tissues that facilitate both the folding/spreading of the rectrices and movement of the entire tail, and a unique caudal skeleton (Baumel, 1988; Gatesy and Dial, 1993). In neornithines, the tail skeleton consists of an abbreviated vertebral series divided into two subregions (Fig. 1). The more proximal of the two regions contains five to nine mobile “free caudal vertebrae,” with the distal unit terminating as the pygostyle (Baumel, 1988). The pygostyle consists of the three to seven caudal-most vertebrae, fused together to form a single unit. The pygostyle functions primarily as an anchor for the soft tissues in which the rectrices are rooted and as an attachment for some of the muscles of the tail (e.g. depressor caudae, Baumel,
1988). Although a high degree of variation in caudal skeletal morphology has been recognized for over a century (Van Oort, 1904; Steiner, 1938; Richardson, 1942; 1972), it is only recently that the functional, developmental, and evolutionary implications of this diversity have been examined. For example, underwater foraging birds have convergently evolved a common caudal skeletal phenotype that consists of a long, narrow, tapered pygostyle, suggesting that locomotor behavior shapes aspects of pygostyle morphology (Felice and O'Connor, 2014). Moreover, there is a strong correlation between tail fan shape and pygostyle shape, suggesting that the caudal integument and skeleton co-evolve (Felice, 2014). Even in light of these recent efforts, many aspects of morphological variation in this key skeletal structure remain unexplained.

One major unexplored factor in the evolution of avian caudal skeletal variation is the extent to which modularity influences the sub-regions of the caudal skeleton. Modularity describes the degree to which subunits of organisms represent semi-independent units. Highly modular structures are composed of individual component parts that may evolve more-or-less independently with minimal developmental interaction. Conversely, highly integrated structures, those that exhibit high covariation, are expected to evolve in concert. Such patterns of integration and modularity are influenced by genetic effects (such as pleiotropy), developmental effects, and functional constraints (Cheverud, 1996). Therefore, it has become increasingly apparent that modularity among anatomical subunits is a major factor to be considered when determining potential constraints on the evolution of morphological variation (Callebaut, 2005; Young and Hallgrimsson, 2005; Goswami and Polly, 2010; Marugán-Lobón, 2010;
Young et al., 2010; Klingenberg et al., 2011; Marroig et al., 2012; Parsons et al., 2012; Clune et al., 2013).

Because of the unique regionalized morphology of the avian caudal skeleton (i.e., pygostyle vs. free caudal vertebrae), it is reasonable to consider the degree to which the two sub-regions are semi-independent of one another. Both units have distinct morphology and functions, yet they are necessarily coordinated to form a complete functional unit (Baumel, 1988; Gatesy and Dial, 1996b). The pygostyle serves primarily to support the tail-fan and the muscles that abduct the rectrices, whereas the free caudal vertebrae serve as attachments for the muscles that move and stabilize the tail (Baumel, 1988; Gatesy and Dial, 1996b). Despite these morphological and functional differences, the pygostyle and free caudal vertebrae represent serial homologs that share a common developmental origin (Van Oort, 1904; Steiner, 1938; Catala et al., 2000; Christ et al., 2000; Rashid et al., 2014). Indeed, the pygostyle first develops as a series of distinct caudal vertebrae that fuse later in ontogeny (Van Oort, 1904; Steiner, 1938; Catala et al., 2000). Moreover, the coordinated movements of the tail intervertebral joints and tail fan are performed by the intrinsic muscles of the tail, which share a common innervation from the caudal nervous plexus that is derived from the distal 6-7 segments of the spinal cord (Baumel, 1988). Thus, in some ways the avian caudal skeleton is parceled into discrete morphological and functional unit, but, in other ways it is a coordinated whole with a common underlying developmental architecture. This study seeks to quantify the extent to which the morphologies of these subregions are correlated with one another in order to evaluate whether the caudal skeleton evolves as an integrated unit or as distinct
modular subunits. Because of the common developmental origin and serial homology of the free caudal vertebrae and the pygostyle (Catala et al., 1995; Catala, 2002), I hypothesize that the two subregions will show significant integration. This contrasts with the alternative hypothesis of total modularity between pygostyle and free caudal vertebrae (e.g., very low covariation between blocks).

Materials and Methods

Caudal skeletal modularity was evaluated using a data set consisting of 267 skeletal specimens (Table 6) representing eight species of passeriforms: Boat-Tailed Grackle (Quiscalus major), Common Grackle (Q. quiscula), Pin-Tailed Whydah (Vidua macroura), Village Indigobird (V. chalybeata), Scissor-Tailed Flycatcher, (Tyrannus forficatus), Gray Kingbird (T. dominicensis), White-Rumped Shama (Copsychus malabaricus), and Oriental Magpie-Robin (C. saularis). This sample exhibits both intraspecific and interspecific variation in the caudal skeleton (this volume, Chapter 4), in addition to having a sufficient number of observations to evaluate modularity. Moreover, as passeriforms, these eight taxa utilize somewhat similar locomotor strategies (e.g., Viscor and Fuster, 1987). As such, confounding effects related to variation in caudal skeletal morphology attributable to functional variation (i.e., Felice and O'Connor, 2014) should be minimized, allowing for more accurate evaluation of modularity.

In order to test for patterns of modularity and integration, caudal skeletal morphology must first be quantified. Two different methods were used to describe the morphology of each of the two caudal subregions. First, free caudal vertebral morphology was quantified using linear morphometrics of centrum length (craniocaudal), centrum
width, centrum height, spinous process length, spinous process width, spinous process height, transverse process length, transverse process width, ventral process length, ventral process width, ventral process height (Fig. 3). All metrics were collected using digital calipers (Mitutoyo Model 573–731, Plymouth, MI). Because the number of free caudal vertebrae is variable among individuals, a basis for homologous comparisons among observations was required. For this reason, metrics were quantified from each individual at three positions along the caudal vertebral column: the cranial-most (post-synsacral) caudal vertebra, the caudal-most (propygostylar) free caudal vertebra, and the vertebra midway along the caudal series. For individuals with an even number of free caudal vertebrae, the average measurements of the middle two caudal vertebrae were used to represent the central caudal element.

To minimize the influence of scaling on free caudal vertebral morphology and evaluate primarily shape variation, a body size correction was carried out on this dataset. First, a body size proxy was calculated for each specimen by taking the geometric mean of several skeletal dimensions: femur length, sternal length, sternal width, sternal keel height, and synsacral length (Mosimann and James, 1979; Simons, 2010; Felice and O'Connor, 2014). The logarithm of each vertebral measurement was divided by the logarithm of the body size proxy for that individual, generating a “size-corrected” dataset (Jungers et al., 1995).

The irregular shape of the pygostyle was quantified using Elliptical Fourier Analysis (EFA), a geometric morphometric technique well suited for analyzing variation in outline shapes (Rohlf and Archie, 1984; Crampton, 1995; Carlo et al., 2011). This
technique allows for complex outlines to be summarized as a series of equations termed “harmonics” and does not require the selection of homologous landmarks (Rohlf and Archie, 1984; Crampton, 1995). For each specimen, the pygostyle was photographed in left lateral perspective. Outlines were digitized, Fourier transformed, and normalized (to remove the effects of size, rotation, and orientation) using the SHAPE software package (Iwata and Ukai, 2002). The Fourier power was calculated to determine that five harmonics were required to describe 95% of the detail in the outlines comprising this sample (Crampton, 1995).

Modularity between pygostyle shape and free caudal vertebral morphology was evaluated using two-block partial least squares (Rohlf and Corti, 2000; Adams and Felice, 2014). Two-block partial least squares (PLS), sometimes referred to as singular warps analysis, is an analytical tool used to assess trait covariation or integration between two sets of variables (i.e., “blocks”). These block of data may represent two regions of an anatomical structure as described by geometric morphometrics (e.g., Bookstein et al., 2003; Kulemeyer et al., 2009), or an anatomical structure and another set of continuous variables, such as environmental/trophic traits or fitness variables (e.g., Rüber and Adams, 2001; Arnqvist and Rowe, 2002; Kuchta and Svensson, 2014). PLS evaluates covariation between subsets of traits using the variance-covariance matrix of the entire data set, $S$. The subset of $S$ describing covariation between the two blocks of data is subjected to a singular value decomposition, producing the linear combinations of variables that maximize covariation between the blocks. The original data are projected onto these vectors of the linear combinations of variables, producing PLS scores. The
strength of integration is then evaluated by calculating the correlation between the vectors of PLS scores. Finally, permutation procedures can be used to assess the statistical significance of this correlation (Rohlf and Corti, 2000; Adams and Felice, 2014). This method is related to least squares regression, but is more suitable for studies of integration in that it does not assume one data set to be dependent on the other.

Given that the data analyzed here are sampled across multiple species, the phylogenetic structure of the data must be taken into account (Felsenstein, 1985). To this end, a phylogenetically-informed implementation of PLS was utilized (Adams and Felice, 2014). In this application, the trait variance-covariance matrix $S$ is substituted with the evolutionary covariance matrix $R$, which describes the extent to which continuous characters co-evolve under a Brownian motion model of evolution with a given phylogenetic topology (Felsenstein, 1988; Revell and Harmon, 2008; Adams and Felice, 2014). Thus, a phylogenetic hypothesis of the evolutionary relationships of the focal taxa was required. This was generated by downloading a sample of 5000 trees from the www.birdtree.org database. From this sample, a maximum clade credibility MCC tree (Fig. 14) was generated using TreeAnnotator (Drummond et al., 2012). In order to take into account intraspecific variation, a star phylogeny was constructed for each species with the number of tips equal to the number of individuals of that species and branch lengths equal to 0.1. The polytomy for each taxon was then grafted to the corresponding tip on the MCC tree, resulting in a final tree with 267 tips and 15 internal nodes. This final tree was used for all subsequent analyses.
It bears consideration that autocorrelation among the variables describing each of the free caudal vertebrae within an individual may affect the results of the PLS analysis. Thus, two PLS analyses were carried out. The first evaluates integration between the shape of the pygostyle and the three (cranial-most, central and propygostylar) free caudal vertebrae. The second analysis evaluates integration between the pygostyle and the propygostylar vertebra only. This vertebra is most likely to show covariation with the pygostyle given that these two elements are topologically most closely associated and that they interact mechanically (e.g., they form a joint). In both analyses, significance was assessed using permutations with 1000 iterations.

Those aspects of vertebral morphology that account for the covariation between the subregions were interpreted by examining the shape change in both blocks of data across PLS axes 1 and 2. Using an inverse Fourier transformation, pygostyle shapes were generated to illustrate shape change described across PLS Axis 1 (Fig. 15). This shape change is also illustrated through two example pygostyle specimens, one that scores very low on Axis 1 (Fig. 16A) and one that scores very high on Axis 1. The shape change in the free caudal vertebrae was interpreted from the loadings of the individual variables on PLS Axis 2, and illustrated using photomanipulation of a 3D digital model of an isolated free caudal vertebra (Fig. 16D).

Results

Both PLS analyses resulted in significant correlation between the free caudal vertebrae and the shape of the pygostyle. The strength and significance of integration was similar whether free caudal vertebral morphology was represented by three vertebrae
Figure 15. Plot of PLS scores for pygostyle shape vs. free caudal vertebral morphology
Images in bottom left and top right corners represent the extreme forms along each axis.
Figure 16: Shape change visualizations.
The change in pygostyle shape across PLS Axis 1 (A) and PC Axis 1 (B) as visualized by Inverse Fourier method. (C) Representative pygostyles of specimens near the minimum (left image, Tyrannus forficatus, KU 19669) and maximum (right image, Copsychus malabaricus, FMNH 347527) of PLS Axis 1. Grey arrows indicate articular surface. (D) Digital 3D models illustrating the shape change in free caudal vertebral shape across PLS Axis 2. These models were created by photom manipulation of a single specimen, OUVC 10322.
or with the propygostylar vertebra alone (Table 10). Therefore, results are described for the whole dataset analysis below. The first singular value of the PLS analysis explains 55.6% of the total covariation between the pygostyle and free caudal vertebrae. The vectors of PLS scores computed from this singular value are the only pair of PLS axes to exhibit significant correlation (PLS correlation = 0.55, p-value = 0.001). This indicates that some aspects of caudal skeletal morphology are significantly integrated, but that features of each module are free to vary independently.

Table 10. Results of two-block partial least squares analysis

<table>
<thead>
<tr>
<th>Data Blocks</th>
<th>PLS Correlation</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Free Caudal Vertebrae vs. Pygostyle Shape</td>
<td>0.55</td>
<td>0.001</td>
</tr>
<tr>
<td>Propygostylar Vertebra vs. Pygostyle Shape</td>
<td>0.54</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Examination of the shape change along both axes reveals several general patterns (Fig. 16). Individuals that score low on both axes (i.e., bottom left quadrant of Fig. 15) have short spinous and ventral processes and long narrow centra. This free caudal vertebral morphology is correlated with a pygostyle shape that has a rounded craniodorsal margin. The lamina pygostyli is craniocaudally elongate at the level of the articular region (Fig. 16A). In contrast, individuals that score high on both axes (i.e., top right quadrant of Fig. 15) exhibit free caudal vertebrae with shorter, wider centra and taller spinous and ventral processes (Fig. 16D). This phenotype is correlated with a pygostyle that has a sharply angled craniodorsal margin and ancranially projecting ventral process. The area caudal to the articular region and above the ventral process is craniocaudally short. Taken together, these patterns suggest that the shape of the craniodorsal margin and the ventral process of the pygostyle are correlated with the morphology of the
spinous process and ventral process, respectively, of the free caudal vertebrae. As spinous process height increases, the craniodorsal margin of the pygostyle becomes higher and less rounded. Likewise, as the height of the ventral process of the free caudal vertebrae increases the ventral process of the pygostyle becomes longer, narrower, and more cranially projecting. A similar relationship is exhibited by the vertebral centrum and the shape of the pygostyle at about mid-height: the pygostyle is craniocaudally constricted at this level in individuals with small centra and expanded in those with large centra.

To better understand the aspects of pygostyle morphology that vary independently of the free caudal vertebrae, a phylogenetic principal components (PC) analysis was carried out on the pygostyle shape data (Felice and O'Connor, 2014). The first five PC axes explain a significant portion of the total variation in pygostyle shape (greater than 5% of the total variation each). The first PC axis, accounting for 30.4% of the total variation, describes similar patterns of variation to the first PLS axis, including the shape of the craniodorsal margin and the length and anterior extent of the ventral process (Fig. 16B). The variation described by the first PC axis and first PLS axis are highly correlated, as confirmed with a linear regression of the first eigenvectors of each analysis ($R^2 = 0.72, p < 0.001$). The similarities in the patterns observed along PLS Axis 1 and PC Axis 1 indicate that the covariation between modules described in the PLS analysis is indeed describing the majority of the variance in pygostyle shape in the sample. The next four PC axes explain progressively smaller proportions of the total variation, but illustrate the variable properties of the pygostyle that are not linked to free caudal vertebral morphology (Fig. 17). These include craniocaudal length (PC2, 18.2% of
Figure 17. Variation in pygostyle shape described by each principal component axis. Center column represents the mean pygostyle shape. Left and right columns represent shapes at the extreme ends of each PC axis, -2 standard deviations from the mean and +2 standard deviations from the mean, respectively.
total variation) and curvature (PC3, 15.5%) of the ventral process, the depth of the articular region (PC4, 9.8%), and the angle at which the ventral process projects anteriorly (PC5, 6.6%).

Discussion

The observed patterns of covariation between the free caudal vertebrae and the pygostyle indicate that these two vertebral regions are moderately integrated. The general shape of the pygostyle, including the size and shape of the ventral process, the height and shape of the craniodorsal margin, and the length of the center of the pygostyle are correlated with the proportions of the vertebral centra and the height of the dorsal and ventral processes of the preceding vertebrae. This significant integration pattern indeed suggests that the tail skeleton evolves as a coordinated whole but that some features may be able to vary and evolve independently.

Two complementary hypotheses can be formulated to explain the specific covariation patterns observed. First, the morphological evolution of both subregions may be responding to common selective pressures on tail function. The muscles that facilitate dorsiflexion/elevation (levator caudae) and ventroflexion/depression (depressor caudae) of the tail apparatus have attachments spanning both the free caudal vertebrae and the pygostyle/rectrices (Baumel, 1988; Gatesy and Dial, 1993). The link between spinous process height and the shape of the craniodorsal margin of the pygostyle could reflect an increased area for attachment of a large levator caudae muscle. Likewise, the correlation between the height of the ventral processes of the free caudal vertebrae and the pygostyle may be a result of selection for a larger depressor caudae muscle and thus larger
attachment sites for this muscle. The evolution of strong tail depressors and an expanded ventral process of the pygostyle are associated with aerial braking (i.e., deceleration) in highly aerial predators (Richardson, 1972). More generally, coordinated evolution of the dorsal and ventral regions of the entire caudal skeleton would be consistent with previous studies that have shown a link between structure and function in the pygostyle (Richardson, 1942; 1972; Manegold and Töpfer, 2012; Felice and O'Connor, 2014). Very little is currently known about the extent of intraspecific variation in caudal musculature (although see Moreno and Moller, 1996). In-depth analysis of variation in caudal muscle anatomy and how this variation is associated with skeletal and functional diversity is necessary to evaluate how these factors interact to generate the integration patterns described above.

An alternative hypothesis can be derived from the developmental origin of the pygostyle. The pygostyle develops from the fusion of several distal caudal vertebrae (Van Oort, 1904; Steiner, 1938). In chicks, this process begins around embryonic day 10, after the individual chondrified caudal vertebrae have formed from the sclerotome compartment of the segmented somites (Catala et al., 2000). If the distal vertebrae that fuse to form the pygostyle exhibit similar morphology to the more proximal caudal vertebrae that do not fuse, the shape of the pygostyle may be reflecting the general caudal vertebral phenotype as a sort of developmental palimpsest (Hallgrimsson et al., 2009). Additionally, the genetic and developmental mechanisms governing morphogenesis of the two caudal regions no doubt have some overlap. The covariation between the spinous processes of the caudal vertebrae and the dorsal margin of the pygostyle could be related
to a common developmental signal governing the expansion of dorsally oriented structures, with the same being true in the ventral regions of the free caudal vertebrae and pygostyle. This leads to the prediction that the dorsal part of the pygostyle is derived from the same embryonic structures as the spinous processes of the free caudal vertebrae (dorsal sclerotome) and the ventral processes of both caudal regions arise from the same embryonic structures (ventral sclerotome: Christ et al., 2000). However, little is known about the morphogenesis of the pygostyle itself (Rashid et al., 2014). Further research into the developmental pathways regulating the fusion and growth of the pygostyle is needed to understand how integration is mediated on a genetic/molecular level.

One notable observation of the components of free caudal vertebral morphology that covary significantly with pygostyle shape is that they are primarily characteristics describing height and length, but not width. Many of the quantified traits that capture variation in the lateral dimension, including spinous process width, ventral process width, and all transverse process proportions, were not found to be integrated with pygostyle shape. This is likely an effect of the procedure used to quantify pygostyle morphology. Because the pygostyle is a laterally compressed structure, shape variation was captured in two dimensions using EFA. This method thus obscures any pygostyle variation in the mediolateral dimension that might be correlated with the lateral features of the free caudal vertebrae. Across Neornithes, pygostyle shape variation appears to occur primarily in the lamina pygostyli (i.e., the lateral surface quantified here), but some variation is apparent in the shape and size of the discus pygostyli (i.e., the ventral surface of the ventral process: Van Oort, 1904; Manegold and Töpfer, 2012). This omission may also
explain the moderate strength of integration seen in this study. With increased information about the pygostyle in three dimensions, this value may increase. An analysis incorporating the variation in the shape of the discus pygostyles and width of the pygostyle is necessary to reveal the ways in which the lateral features of the free caudal vertebrae contribute to integration within the tail.

In addition to evaluating the influence of muscular variation on caudal skeletal integration as described above, the development of a complete picture of caudal skeletal evolution in neornithines requires that these studies be synthesized with information regarding the relationship between the tail skeleton and the rectrices. The degree to which caudal skeletal morphology and integumentary morphology are correlated is variable among different lineages (this volume, Chapter 4), but there is some evidence that pygostyle shape and tail fan shape coevolve (Felice, 2014). Whether the tail fan-pygostyle relationship influences intracolumnar integration is still unclear. One testable prediction is that the functional unit comprised of the pygostyle-rectrices-rectrical fanning mechanism represents a more strongly integrated module than pygostyle-free caudal vertebrae. Moreover, there are potential tradeoffs among these trait interactions, such that taxa that emphasize intracolumnar integration have increased skeletal-integumentary modularity. This type of pattern might be expected in lineages for which the biomechanical properties of the tail skeleton are particularly important, such as penguins that use the tail as an underwater rudder (Hui, 1985). Conversely, lineages such as woodpeckers (Picinae) with extremely derived tail functions can be predicted to exhibit strong integration among all components of the tail apparatus. Hence, a
biomechanical milieu whereby tails must withstand variably extreme forces during turning and/or arboreal support may have a stronger influence on such relationships. The methods used here to evaluate morphological integration are theoretically generalizable to accommodate three or more blocks of data (Bookstein et al., 2003), which would allow for covariation patterns to be quantified among the tail fan, pygostyle, and free caudal vertebrae.

This study also provides a foundation for investigating patterns of intracolumnar integration and modularity in a broader evolutionary context and how these patterns are shaped by natural selection and interactions with additional traits. The passeriform taxa included in this study are closely related (Jetz et al., 2012), utilize similar habitats and locomotor regimes (e.g., Viscor and Fuster, 1987), and exhibit a narrow range of body sizes (Dunning, 1993). Thus, an important next step involves evaluating how free caudal-pygostyle integration has changed across a wider taxonomic sample that is variable in these parameters. As an example, within Aequornithes and Charadriiformes, shifts in foraging behavior (e.g., diving, aerial, terrestrial) are associated with shifts in pygostyle shape (Felice and O'Connor, 2014). Do these changes in pygostyle morphology require changes in the free caudal vertebrae as well, due to the integration between these two subregions, or does the degree of integration decrease? Recent advances in analytical techniques allow for trait integration to be quantified in a phylogenetic framework, such that evolutionary shifts in these patterns can be detected and analyzed (Adams and Felice, 2014; Goswami et al., 2014). Moreover, it will be important to evaluate whether specific lineages with highly specialized tail structure and function feature divergent patterns of
integration within the caudal skeleton. For example, aerial predators such as Falconiformes and scansorial specialists such as Picinae exhibit derived pygostyle shapes (Richardson, 1942; 1972; Manegold and Töpfer, 2012). Similarly, lineages that reduce the size and importance of the tail module, such as grebes (Podicipediformes), might also experience evolution in caudal integration patterns. Comparative analysis of the intracolumnar integration can be used to determine whether the evolution of these novel phenotypes involved an increase in modularity and evolvability.

The two subregions of the avian caudal skeleton, the free caudal vertebrae and pygostyle, exhibit strongly divergent phenotypes. This reflects the derived function of the pygostyle as a structural support for the rectrices and the muscles of the fanning mechanism. Despite the functional and anatomical differences between these subregions, the entire tail skeleton is significantly integrated. Future studies characterizing the soft-tissue diversity and development of the tail will aid in elucidating how such integration is maintained. Whatever the proximate causes of integration in this system, the caudal skeletal apparatus is thus likely to evolve as a coordinated whole.
CHAPTER 6: CONCLUSIONS

The evolution of the avian tail, including its role in the origin and diversification of birds, its function as a component of the flight apparatus, and its use as an intraspecific display structure, has been of interest to ornithologists, evolutionary biologists and paleontologists alike for over a century (Darwin, 1871; Van Oort, 1904; Steiner, 1938; Andersson and Andersson, 1994; Gatesy and Dial, 1996b). In particular, the relationship between tail function and the shape of the tail fan is well understood (Balmford et al., 1993; Thomas and Balmford, 1995; Thomas, 1996; Maybury and Rayner, 2001; Maybury et al., 2001; Evans et al., 2002; Clark, 2010). This dissertation expands on this body of work by, for the first time, exploring the evolutionary morphology of the caudal skeleton in a phylogenetic comparative context. Each of the studies contained here characterize patterns of skeletal variation by using one of two research paradigms: (1) ecomorphology, the interface between form, function, and ecology, and (2) trait coevolution, the extent to which individual components of an organism are correlated with or independent from one another. These two complementary approaches allow for a multifaceted consideration of the mechanisms that generate the previously poorly understood breadth of caudal skeletal variation.

Caudal skeletal morphology was found to be closely linked to foraging behavior throughout a diverse sample of waterbirds (Aequornithes) and shorebirds (Charadriiformes), reinforcing the link between form and function in this system. Birds that habitually utilize foraging styles (plunge dive, foot-propelled pursuit dive, wing propelled pursuit dive, terrestrial, aerial) exhibit characteristic pygostyle shapes. Most
Interestingly, multiple independent lineages of diving birds have evolved a common pygostyle phenotype. This finding suggests that the demands of underwater locomotion exert directional selection on caudal structure and function, driving convergent evolution. Whereas the aerial locomotor role of the tail is well understood, it is largely unknown if and how the tail might function as an underwater rudder. However, some aquatic birds have been observed using the tail as a control surface underwater, including penguins, cormorants, and stifftail ducks (Raikow, 1970; Ross, 1976; Clark and Bemis, 1979; Hui, 1985). In light of the repeated evolution of an elongate, pointed pygostyle in diving birds, it is likely that the tail plays an important, previously underappreciated role in swimming as well as flight. Biomechanical and functional analyses are required to evaluate how the particular pygostyle shape of diving birds might enhance swimming performance and to better characterize this convergence.

More generally, this ecomorphological analysis provides evidence that some of the variation in the avian tail skeleton can be attributed to functional differences associated with the mechanical demands of different locomotor regimes, ecologies, and habitats. A similar pattern has been demonstrated for rectricial morphology (Balmford et al., 1993; Thomas and Balmford, 1995; Thomas, 1996; Maybury and Rayner, 2001; Maybury et al., 2001; Evans et al., 2002; Clark, 2010). Taken together, these lines of evidence suggest that locomotor behavior is an important driver of both caudal skeletal and integumentary evolution.

Given that integumentary and skeletal variation are each shaped by locomotor variation, how might these two systems interact as part of a coordinated whole? This
question was first addressed by examining whether caudal skeletal morphology can be used to predict gross tail fan shape (forked, graduated, square). Indeed, pygostyle shape and the shape of the tail fan are strongly associated. Birds with graduated tails have a strait, tapered pygostyle, whereas those with forked tails have a dorsally deflected, hourglass-shaped pygostyle. This not only suggested that these two subregions of the tail coevolve, but also provided the opportunity to reconstruct the shape of the tail fan in a fossil bird, *Limnofregata azygosternon*, that preserves no feathers. This was made possible due to the fact that *Limnofregata* is nested within the study clade, providing a phylogenetic bracket of taxa for which feather and bone morphologie are known. By expanding sampling to include a wider breadth of crown birds, as well as fossil specimens that preserve rectricial morphology, it will be possible to use the methods presented in this work to reconstruct tail fan evolution across Ornithurae.

The relationship between the rectrices and the caudal skeleton was further examined by investigating how this interaction is expressed within the context of sexual dimorphism. Sexually dimorphic, ornamental tail feathers represent one of the most conspicuous examples of caudal variation in birds. Such elaborate rectrices are often hypothesized to have evolved under the handicap mechanism of sexual selection because they are metabolically costly to grow and maintain, and are also aerodynamically detrimental (Andersson, 1994). Long tails fans, especially streamer tails, deviate from the aerodynamic optimum, reducing the lift-to-drag ratio and making flight behaviors less efficient (Evans and Thomas, 1992; Balmford et al., 1993; Norberg, 1995; Evans, 1998; Park et al., 2000; Rowe et al., 2001; Clark and Dudley, 2009). Given the previously
demonstrated covariation between pygostyle shape and tail fan shape within Aequornithes and Charadriiformes, it was predicted that the evolution of sexual dimorphism in rectricial morphology would similarly be correlated with dimorphism in the tail skeleton.

Surprisingly, males and females of species with sexually dimorphic rectrices do not exhibit dimorphism in vertebral morphology. This suggests that sexual selection can act to modify the shape and size of the tail feathers independently of the tail skeleton. These findings appear to contradict the results of the previous study, which found strong covariation between tail fan shape and pygostyle shape. Several testable hypotheses can be posited to explain this discrepancy. First, the covariance structure between rectrices and pygostyle might be different between Aequornithes/Charadriiformes and Passeriformes. A recent comparative study of morphological integration throughout Mammalia has shown that trait covariation patterns can indeed be variable within a clade (Goswami, 2006; Goswami et al., 2014). Such a shift in morphological integration between traits could be related to a relaxation of evolutionary constraints on the tail in passeriforms to maintain its role as an aerial control structure. Evaluating the strength of integration between the pygostyle and rectrices and how this varies throughout neornithines will require a broad scale comparative study incorporating intra-individual calculations of feather and bone covariation.

An additional factor that may contribute to the difference in rectrix-caudal skeleton relationship in these two groups relates to the time since divergence between lineages with disparate caudal skeletal morphology. Among the waterbirds and
shorebirds, lineages with distinct tail fan and pygostyle morphology have divergence
dates much longer than any of the monomorphic/dimorphic passeriform species pairs. For
example, *Quiscalus major* and *Q. quiscula* diverged approximately 2.25 mya whereas the
forked-tailed *Fregata* lineage and the graduated-tailed gannet and cormorant lineage
diverged 59 mya (Jetz et al., 2012). Assuming that sexually dimorphic species evolved
from ancestors with monomorphic rectrices and caudal skeletons, it is possible that there
has not been enough time to accumulate phenotypic change in the caudal skeleton of
recently diverged species pairs. There is some evidence that avian secondary sexual
structures may evolve at higher rates than other characters (Cuervo and Moller, 1999).
One approach to investigate this hypothesis would involve estimating the rate of
phenotypic evolution in the caudal skeleton compared to the rectrices (e.g., Smaers and
Vinicius, 2009). This analytical approach would also be useful for evaluating whether tail
evolution is differentially constrained in individual clades.

Finally, the lack of change in pygostyle morphology accompanying the
acquisition of ornamental tail feathers calls into question whether the caudal skeleton and
integument do indeed coevolve. Given this additional evidence, an alternative hypothesis
to explain the correlation between the pygostyle and tail fan in Aequornithes and
Charadriiformes is that the morphology of the each component (integument, bones) of the
tail is shaped by natural selection but that no mechanistic association exists between two
structures. As an example, birds with graduated tail fans exhibit a pygostyle that is
craniocaudally elongate and that tapers to a point caudally. The birds in this group
include penguins (*Pygocelis*), boobies (*Sula*), tropicbirds (*Phaethon*) and cormorants
(Phalacrocorax), all of which are diving birds. A consistent correlation has been demonstrated between diving behavior and pygostyle shape, with these taxa exhibiting elongate, straight pygostyles. Previous studies have linked tail fan shape to locomotor function (Thomas and Balmford, 1995). Thus the apparent correlation between feathers and bones could be a result of each of these elements being modified for its locomotor role independently and not due to explicit trait covariation.

One way to elucidate the nature of the correlation between the rectrices and pygostyle would be to examine the interactions between these tissues during development. Despite the chick’s status as a model organism for developmental research, relatively little is known about the embryology of the derived tail structures of birds and specifically the rectricial apparatus (e.g., Van Oort, 1904; Steiner, 1938; Catala et al., 2000). A first step will involve characterizing the normal development of the caudal skeleton and associated soft tissue structures and how the timing of origination and growth of these are correlated. Embryonic manipulations will then allow for an experimental consideration of mesenchymal-epithelial interaction between the developing caudal skeleton and rectrices/rectricial bulbs. These could include unilateral or bilateral removal of the insipient rectricial bulb, removal of the caudal somites or vertebrae, or chimeric transplantation of developing caudal structures between chicks and quails. The caudal phenotypes that result from these types of experiments can then be used to evaluate skeletal and rectricial covariation on a fine scale.

Analysis of developmental patterns will also be a key component of elaborating on the study of intracolumnar vertebral covariation. The study of morphological
integration contained herein found that the free caudal vertebrae and pygostyle are significantly integrated. Despite the divergence in form and function of these the regions, the entire tail skeleton is expected to evolve as a coordinated whole, potentially imposing constraints on morphological variation in each region. The mechanisms that mediate this integration remain unknown. Thus, the next step toward understanding how the interrelationships of axial skeletal structures have shaped caudal variation in birds is to investigate the genetic and developmental processes that regulate the morphogenesis and regionalization within the tail. An upcoming study will test the hypothesis that the fusion and morphological differentiation of the pygostyle can be explained by temporal and spatial differences in the expression of key axial patterning genes between the distal somites that form the pygostyle and the more proximal somites that remain unfused. Moreover, integration throughout the caudal skeleton is predicted to be the result of gene expression patterns that are consistent throughout the length of the developing tail. Specifically, in situ hybridization will be used to evaluate the expression patterns of several candidate genes that are likely to be involved in tail morphogenesis, and then targeted embryonic manipulations will be used to experimentally test their function. Chief among these candidate genes are Pax-1/9 and Msx1/2, which are markers of the lateral and dorsal sclerotome, respectively (Christ et al., 2000; Chen et al., 2013). Because the dorsal components of the free caudal vertebrae and the pygostyle were shown to covary, it is likely that the genes that regulate the growth of individual sclerotomal subregions that will be responsible for phenotypic integration. Additionally, intracolumnar variation in the expression of these and other genes are predicted to
regulate pygostyle fusion. For example, downregulation of *Pax-1* in the intervertebral discs results in the fusion of the anterior-most somites, forming the basioccipital (Wilting et al., 1995). A similar mechanism is hypothesized to be responsible for the fusion of distal elements. Testing these hypotheses will allow for a richer understanding of the embryological origins of the derived avian tail skeleton, the proximate causes of caudal variation, and the determinants of morphological integration within this system.

Although the tail has been an historically understudied region of the tetrapod body, recent work such as this has begun to emphasize the importance of the tail as part of the locomotor system not only in birds (Thomas, 1996; Gatesy and Dial, 1996a; Pittman et al., 2013), but also in other clades including non-neornithine birds and dinosaurs (Libby et al., 2012; Evangelista et al., 2014), lizards (Jusufi et al., 2008), and cursorial carnivorans (Wilson et al., 2013). The studies contained within this dissertation provide some of the first in-depth, comparative examinations of caudal skeletal evolution in modern birds. The results of this work provide new insights into the forces that drive morphological variation in the caudal locomotor module that is unique to ornithurine birds. Specifically, caudal variation is strongly correlated with locomotor diversity, suggesting that the link between form and function is a major influence of pygostyle evolution. Additionally, the interrelationships among the components of the caudal apparatus are also important factors in shaping tail evolution. Across a broad comparative sample, the shape of the pygostyle is a good predictor of tail fan shape, supporting the hypothesis that the feathers and bones of the tail co-evolve. However, the subsequent analysis found that the evolution of costly ornamental tail feathers does not have an effect
on the tail skeleton, calling into question the strength and ubiquity of covariation and coevolution between the rectrices and pygostyle. More comparative, and especially, developmental work is needed to uncover the nature and extent of the feather-skeleton link in the tail. Finally, significant morphological integration was detected between the free caudal vertebrae and the pygostyle, suggesting the entire caudal skeleton evolves as a coordinated whole and providing a foundation for understanding the ways in which pygostyle variation may be regulated.

In addition to broadening our understanding of the evolution of the avian tail, a novel and evolutionary important structure, these results demonstrate the complexity of the study of morphological diversification more generally. The various trait interactions and structure-function relationships uncovered herein illustrate the need to investigate structural variation across multiple organizational levels (e.g., within the skeleton, between the skeleton and other tissues, between tissues and the environment/behavior) and multiple evolutionary scales (e.g., intraspecific variation, interspecific variation). Moreover, the use of fossil specimens, phylogenetic comparative methods, and divergence dates estimated from molecular phylogenies point to the importance of temporal and phylogenetic perspective in such integrative analyses. Together, these approaches allow for the characterization of patterns of variation and covariation in an evolutionary context, building the necessary framework for understanding the causal mechanisms by which this variation is generated. These questions can then be addressed using diverse tools such as functional morphology, biomechanics, and developmental biology. Ultimately, this dissertation provides unprecedented breadth and depth of
knowledge of the morphological evolution of the caudal axial skeleton in bird that will be foundational in the study of anatomical and locomotor diversification in flying theropods.
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## APPENDIX 1: CHAPTER 2 TAXON LIST

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<th>Number of Specimens</th>
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<td>Wing-Propelled Pursuit Dive</td>
<td>2</td>
</tr>
<tr>
<td><em>Rollandia microptera</em></td>
<td>Foot-Propelled</td>
<td>Foot-Propelled Pursuit Dive</td>
<td>1</td>
</tr>
<tr>
<td><em>Rollandia rolland</em></td>
<td>Flap</td>
<td>Foot-Propelled Pursuit Dive</td>
<td>3</td>
</tr>
<tr>
<td><em>Scopus umbretta</em></td>
<td>Flap</td>
<td>Terrestrial</td>
<td>3</td>
</tr>
<tr>
<td><em>Stercorarius parasiticus</em></td>
<td>Flap</td>
<td>Aerial</td>
<td>3</td>
</tr>
<tr>
<td><em>Sula dactylatra</em></td>
<td>Flap-Glide</td>
<td>Plunge Dive</td>
<td>3</td>
</tr>
<tr>
<td><em>Sula sula</em></td>
<td>Flap-Glide</td>
<td>Plunge Dive</td>
<td>3</td>
</tr>
<tr>
<td><em>Threskiornis aethiopicus</em></td>
<td>Flap-Glide</td>
<td>Terrestrial</td>
<td>3</td>
</tr>
<tr>
<td><em>Uria aalge</em></td>
<td>Flap</td>
<td>Wing-Propelled Pursuit Dive</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>First Foot Quadrupedal Vertebrae</td>
<td>Mid-Caudal Vertebrae</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td><strong>Length</strong></td>
<td><strong>Growth</strong></td>
<td><strong>Length</strong></td>
<td><strong>Growth</strong></td>
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<td><strong>mm</strong></td>
<td><strong>mm</strong></td>
<td><strong>mm</strong></td>
</tr>
<tr>
<td><strong>Neck</strong></td>
<td>5.35</td>
<td>5.18</td>
<td>5.18</td>
</tr>
<tr>
<td><strong>Upper</strong></td>
<td>4.20</td>
<td>4.45</td>
<td>4.35</td>
</tr>
<tr>
<td><strong>Torso</strong></td>
<td>3.52</td>
<td>3.52</td>
<td>3.52</td>
</tr>
<tr>
<td><strong>Posterior</strong></td>
<td>3.65</td>
<td>3.65</td>
<td>3.65</td>
</tr>
</tbody>
</table>

**APPENDIX 2: CHAPTER 2 SKELETAL DATA - AEQUORNITHES AND CHARADRIIFORMES**
|--------------------|---------------|--------------|---------------|-------------------------|------------------------|-------------------------|-------------------------|----------------------|----------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|-------------------------|-----------------------|-----------------|----------------|----------------|----------------|----------------|

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APPENDIX 3: R SCRIPT FOR CONDUCTING PHYLOGENETIC PCA USING EFA DATA

R Script:

```r
#####R Script: efa.phyl.pca.R

require(Momocs)
require(ape)
require(geiger)
require(phytools)
require(calibrate)

####
#the first two functions, NEF2COE and pca2shp.new, are slightly
#modified versions of functions from the Momocs R package (Bonhomme et al., 2013)
#for compatibility with the SHAPE software package (Iwata and Ukai, 2002)
#and the rest of the efa.phyl.pca.R functions.
####
NEF2COE takes an input NEF file of normalized elliptical Fourier
descriptors (as created with SHAPE) and outputs a COE object for use
with the subsequent functions.
####

NEF2COE <-
function (nef.path)
{
    nef <- readLines(nef.path)
    HARMO.1 <- grep(pattern = "HARMO", nef)
    nb.h <- as.numeric(substring(nef[HARMO.1], 8))
    nef <- nef[-(1:HARMO.1)]
    nb.coo <- length(nef)/(nb.h + 1)
    coo.i <- 1:nb.coo
    coo.beg <- (coo.i - 1) * (nb.h + 1) + 1
    coo.end <- coo.beg + nb.h
    res <- matrix(NA, nrow = nb.coo, ncol = nb.h * 4, dimnames =
                  list(nef[coo.beg], paste(rep(LETTERS[1:4], each = nb.h),
                              l:nb.h, sep = "")))
    for (i in seq(along = coo.i)) {
        nef.i <- nef[(coo.beg[i]+1):coo.end[i]]
        x <- as.numeric(unlist(strsplit(nef.i, " ")))
        x1<-x[!is.na(x)]
        a.i<-x1[seq(1,length(x1),4)]
        b.i<-x1[seq(2,length(x1),4)]
        c.i<-x1[seq(3,length(x1),4)]
        d.i<-x1[seq(4,length(x1),4)]
        res[i, ]<-c(a.i,b.i,c.i,d.i)
    }
}
```
### pca2shp.new is an internal function used elsewhere and is not intended for use by the end user

```r
pca2shp.new <- function (pos, rot, mean.shp, method = c("efourier", "rfourier", "tfourier"), scale = 1, amp = 1, trans = TRUE, nb.pts = 300, rotate.shp)
{
  if (!is.matrix(pos))
    pos <- as.matrix(pos)
  if (ncol(pos) != ncol(rot))
    stop("rot an pos must have the same ncol")
  if (length(mean.shp) != nrow(rot))
    stop("mean.shp length must equals the col number of rot")
  if (missing(method)) {
    warning("Method not provided. efourier is used.")
    p <- 1
    method.i <- efourier.i
  } else {
    p <- pmatch(tolower(method), c("efourier", "rfourier", "tfourier"))
    if (is.na(p)) {
      warning("Unvalid method. efourier is used.")
    } else {
      method.i <- switch(p, efourier.i, rfourier.i, tfourier.i)
    }
  }

  mprod <- function(m, s) {
    res <- m
    for (i in 1:ncol(m)) {
      res[, i] <- m[, i] * s[i]
    }
    return(res)
  }
  nb.h <- length(mean.shp)/ifelse(p == 1, 4, 2)
  n <- nrow(pos)
  res <- array(NA, dim = c(nb.pts, 2, n), dimnames = list(paste0("pt", 1:nb.pts), c("x", "y"), paste0("shp", 1:n)))
  for (i in 1:n) {
    ax.contrib <- mprod(rot, pos[i, ]) * amp
    coe <- mean.shp + apply(ax.contrib, 1, sum)
    if (p == 1) {
      xf <- list(an = coe[1:nb.h + 0 * nb.h], bn = coe[1:nb.h + 1 * nb.h], cn = coe[1:nb.h + 2 * nb.h], dn = coe[1:nb.h + 3 * nb.h])
    }
  }
}
```
else {
    xf <- list(an = coe[1:nb.h + 0 * nb.h], bn = coe[1:nb.h +
       1 * nb.h])
}
coo <- l2m(method.i(xf, nb.h = nb.h, nb.pts = nb.pts))
coo <- coo.template(c oo, size = scale)
if (!missing(rotate.shp)) {
    coo <- coo.rotate(coo, rotate.shp)
}
if (trans) {
    coo <- coo.trans(coo, pos[i, 1], pos[i, 2])
}
res[, , i] <- coo.force2close(coo)
invisible(res)
}

###
# neftrimmer is a function that removes the constants A1, B1, and C1
# from the matrix of normalized fourier coefficients before statistical
# analysis
###

neftrimmer <-
function(nef){
c<-ncol(nef)
nef[,,-c(1,(c/4+1),(c/2+1))]
}

###
# function to calculate phylogenetic PCA of EFA data (Revell, 2009)
###

efa.phyl.pca <-
function (tree, nefmat, nharm, method = "BM", mode = "cov")
{
    if (class(tree) != "phylo")
        stop("tree must be an object of class 'phylo.'")
    if (colnames(nefmat[,c(1,ncol(nefmat)/4+1,ncol(nefmat)/2+1)]) !=
        c("A1","B1","C1"))
        stop("nefmat must be a complete set of NEF descriptors")
    if (length(strsplt(mode, split = "")[[1]]) <= 2) {
        message(paste("mode = ", mode, ", not a valid option; setting mode = 'cov'", sep = "\n"))
        mode = "cov"
    }
    if (all(strsplt(mode, split = "")[[1]] == strsplt("correlation", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "corr"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
    else if (all(strsplt(mode, split = "")[[1]] == strsplt("covariance", split =
        "")[[1]][1:length(strsplt(mode, split = "")[[1]])])
        mode = "cov"
split = """)[1:length(strsplit(mode, split = ""))[1]])))
mode = "cov"
else {
message(paste("mode = ", mode, " not a valid option; setting mode = "cov", sep = ""))
mode = "cov"
}
}
nefmat<-nefmat[,c(1:nharm,(ncol(nefmat)/4+1):(ncol(nefmat)/4+nharm),(ncol(nefmat)/2+1):(ncol(nefmat)/2+nharm),((3*ncol(nefmat)/4)+1):(((3*ncol(nefmat))/4)+nharm))]
p<-ncol(nefmat)
Y<-neftrimmer(nefmat)

n <- nrow(Y)
m <- ncol(Y)
if (n > length(tree$tip))
stop("number of rows in NEF matrix cannot be greater than number of taxa in your tree")
Y <- as.matrix(Y)
if (is.null(rownames(Y))) {
if (nrow(Y) == n) {
print("NEF matrix has no names. function will assume that the row order of NEF matrix matches tree$tip.label")
rownames(Y) <- tree$tip.label
}
else stop("NEF matrix has no names and does not have the same number of rows as tips in tree")
}
else if (length(setdiff(rownames(Y), tree$tip.label)) != 0)
stop("NEF matrix has rownames, but some rownames of Y not found in tree")
C <- vcv.phylo(tree)[rownames(Y), rownames(Y)]
if (method == "BM") {
  temp <- phyl.vcv(Y, C, 1)
  V <- temp$R
  a <- t(temp$alpha)
  C <- temp$C
}
else if (method == "lambda") {
  temp <- optimize(f = likMlambda, interval = c(0, maxLambda(tree)),
                   X = Y, C = C, maximum = TRUE)
  lambda <- temp$maximum
  logL <- as.numeric(temp$objective)
  temp <- phyl.vcv(Y, C, lambda)
  V <- temp$R
  a <- t(temp$alpha)
  C <- temp$C
}
invC <- solve(C)
if (mode == "corr") {
  Y = Y/matrix(rep(sqrt(diag(V)), n), n, m, byrow = T)
V = V/(sqrt(diag(V)) %*% t(sqrt(diag(V))))
a <- matrix(colSums(invC %*% Y)/sum(invC), m, 1)
}
es = eigen(V)
result <- list()
result$mean.shp <- colMeans(nefmat)
result$num.harms <- nharm
result$Eval <- diag(es$values)
result$Evec <- es$vectors
dimnames(result$Eval) <- list(paste("PC", 1:ncol(Y), sep = ""),
                           paste("PC", 1:ncol(Y), sep = ""))
dimnames(result$Evec) <- list(colnames(Y), paste("PC", 1:ncol(Y),
                                       sep = ""))
A <- matrix(rep(a, n), n, m, byrow = T)
result$S <- (Y - A) %*% result$Evec
Ccv <- t(Y - A) %*% invC %*% result$S/(n - 1)
result$L <- matrix(, m, m, dimnames = list(colnames(Y), paste("PC",
                                                       1:ncol(Y),
                                                       sep = "")))
for (i in 1:m) for (j in 1:m) result$L[i, j] <- Ccv[i, j]/sqrt(V[i,
                                                                i] *
result$Eval[j, j])
if (method == "lambda") {
    result$lambda <- lambda
    result$logL.lambda <- logL
}
A1<-rep(0,m)
B1<-rep(0,m)
C1<-rep(0,m)
result$L.full<-rbind(A1,result$L[1:((p/4)-1),],B1,result$L[(p/4):((p/2)-2),],C1,result$L[((p/2)-1):(p-3),])
result$Evec.full<-rbind(A1,result$Evec[1:((p/4)-1),],B1,result$Evec[(p/4):((p/2)-2),],C1,result$Evec[((p/2)-1):(p-3),])
result$var.contrib<=(diag(result$Eval)/sum(result$Eval))*100
result$sig.PCs <- sum((result$var.contrib>5))
result$phy<-tree
return(result)

###
#function to estimate phylogenetic signal using the consistency index.
#Code is modified from geomorph to utilize EFA data (Adams and Otárola-
#Castillo, 2013).
###
efa.phylo.signal <-
function(phy,A,nharm,iter=999)
{
    require(ape)
    require(geiger)
    N <- length(phy$tip.label)
    A <- A[phy$tip.label, ]
    A <- A[, c(1:nharm, (ncol(A)/4 + 1):(ncol(A)/4 + nharm),
                (ncol(A)/2 + 1):((3 * ncol(A)/4) + 1):((3 * ncol(A))/4 + nhar))
}
A <- neftrimmer(A)
SSC.o <- NULL
anc.states <- matrix(NA, nrow = (nrow(A) - 1), ncol = ncol(A))
for (i in 1:ncol(A)) {
  anc.states[, i] <- fastAnc(phy, A[, i])
}
dist.mat <- as.matrix(dist(rbind(as.matrix(A),
as.matrix(anc.states)))^2)
SSC.o <- 0
for (i in 1:nrow(phy$edge)) {
  SSC.o <- SSC.o + dist.mat[phy$edge[i, 1], phy$edge[i, 2]]
}
P.val <- 1
for (i in 1:iter) {
  A.r <- A[sample(nrow(A)), ]
  row.names(A.r) <- row.names(A)
  SSC.r <- NULL
  anc.states <- matrix(NA, nrow = (nrow(A) - 1), ncol = ncol(A))
  for (i in 1:ncol(A.r)) {
    anc.states[, i] <- fastAnc(phy.r[, i])
  }
dist.mat.r <- as.matrix(dist(rbind(as.matrix(A.r),
as.matrix(anc.states)))^2)
  SSC.r <- 0
  for (i in 1:nrow(phy$edge)) {
    SSC.r <- SSC.r + dist.mat.r[phy$edge[i, 1], phy$edge[i, 2]]
  }
P.val <- ifelse(SSC.r <= SSC.o, P.val + 1, P.val)
}
P.val <- P.val/(iter + 1)
return(list(phy.signal = SSC.o, pvalue = P.val))

###
# phyl.efa.morphospace is a simple function for plotting the results of
the phylogenetic PCA of EFA data. It is a modified version of the
morpho.space function of the Momocs packageb (Bonhomme et al., 2013).
###

phyl.efa.morphospace <- function(pca, xax = 1, yax = 2, xlim, ylim, nb.pts = 300, pos.shp = c("li",
"circle", "range")[3], nr.shp = 6, nc.shp = 5, amp.shp = 1,
scale.shp = 1, rotate.shp = 0, circle.nb.shp = 12, circle.r.shp,
plot = TRUE, layer = TRUE, col.shp = "#70809011", border.shp =
"#708090",
pch.pts = 20, col.pts = "grey40", first.point = FALSE)
{
  if (is.data.frame(pos.shp))
    pos.shp <- as.matrix(pos.shp)
  if (is.matrix(pos.shp)) {
    if (ncol(pos.shp) != 2) {
      stop("When passed with a matrix, pos.shp requires a two
columns matrix")
    } else {  # matrix input
      if (is.null(ncol(pos.shp)))
        pos.shp <- cbind(1:nrow(pos.shp), pos.shp)
      if (is.null(nrow(pos.shp)))
        pos.shp <- pos.shp[1, ]
      if (is.null(ncol(pos.shp)))
        pos.shp <- pos.shp[1, ]
      pos.shp <- as.matrix(pos.shp)
      pos.shp <- pos.shp[, 1:2]
    }
  }
  pos.shp <- pos.shp[, c(xax, yax)]
  pos.shp <- pos.shp[, pos.shp[, 1] >= xlim]
  pos.shp <- pos.shp[, pos.shp[, 1] <= xlim]
  pos.shp <- pos.shp[, pos.shp[, 2] >= ylim]
  pos.shp <- pos.shp[, pos.shp[, 2] <= ylim]
  pos.shp <- pos.shp[, pos.shp[, 1] >= xlim]
  pos.shp <- pos.shp[, pos.shp[, 1] <= xlim]
  pos.shp <- pos.shp[, pos.shp[, 2] >= ylim]
  pos.shp <- pos.shp[, pos.shp[, 2] <= ylim]
  pos.shp <- pos.shp[, pos.shp[, 1] >= xlim]
  pos.shp <- pos.shp[, pos.shp[, 1] <= xlim]
  pos.shp <- pos.shp[, pos.shp[, 2] >= ylim]
  pos.shp <- pos.shp[, pos.shp[, 2] <= ylim]
  pos.shp <- pos.shp[, pos.shp[, 1] >= xlim]
  pos.shp <- pos.shp[, pos.shp[, 1] <= xlim]
  pos.shp <- pos.shp[, pos.shp[, 2] >= ylim]
  pos.shp <- pos.shp[, pos.shp[, 2] <= ylim]
  pos.shp <- pos.shp[, pos.shp[, 1] >= xlim]
  pos.shp <- pos.shp[, pos.shp[, 1] <= xlim]
  pos.shp <- pos.shp[, pos.shp[, 2] >= ylim]
  pos.shp <- pos.shp[, pos.shp[, 2] <= ylim]
  pos.shp <- pos.shp[, pos.shp[, 1] >= xlim]
  pos.shp <- pos.shp[, pos.shp[, 1] <= xlim]
  pos.shp <- pos.shp[, pos.shp[, 2] >= ylim]
  pos.shp <- pos.shp[, pos.shp[, 2] <= ylim]
  pos.shp <- pos.shp[, pos.shp[, 1] >= xlim]
  pos.shp <- pos.shp[, pos.shp[, 1] <= xlim]
  pos.shp <- pos.shp[, pos.shp[, 2] >= ylim]
  pos.shp <- pos.shp[, pos.shp[, 2] <= ylim]
  pos.shp <- pos.shp[, pos.shp[, 1] >= xlim]
  pos.shp <- pos.shp[, pos.shp[, 1] <= xlim]
  pos.shp <- pos.shp[, pos.shp[, 2] >= ylim]
  pos.shp <- pos.shp[, pos.shp[, 2] <= ylim]
  pos.shp <- pos.shp[, pos.shp[, 1] >= xlim]
  pos.shp <- pos.shp[, pos.shp[, 1] <= xlim]
  pos.shp <- pos.shp[, pos.shp[, 2] >= ylim]
  pos.shp <- pos.shp[, pos.shp[, 2] <= ylim]
  pos.shp <- pos.shp[, pos.shp[, 1] >= xlim]
  pos.shp <- pos.shp[, pos.shp[, 1] <= xlim]
  pos.shp <- pos.shp[, pos.shp[, 2] >= ylim]
  pos.shp <- pos.shp[, pos.shp[, 2] <= ylim]
  pos.shp <- pos.shp[, pos.shp[, 1] >= xlim]
  pos.shp <- pos.shp[, pos.shp[, 1] <= xlim]
  pos.shp <- pos.shp[, pos.shp[, 2] >= ylim]
  pos.shp <- pos.shp[, pos.shp[, 2] <= ylim]
  pos.shp <- pos.shp[, pos.shp[, 1] >= xlim]
  pos.shp <- pos.shp[, pos.shp[, 1] <= xlim]
  pos.shp <- pos.shp[, pos.shp[, 2] >= ylim]
  pos.shp <- pos.shp[, pos.shp[, 2] <= ylim]
  pos.shp <- pos.shp[, pos.shp[, 1] >= xlim]
  pos.shp <- pos.shp[, pos.shp[, 1] <= xlim]
  pos.shp <- pos.shp[, pos.shp[, 2] >= ylim]
  pos.shp <- pos.shp[, pos.shp[, 2] <= ylim]
  pos.shp <- pos.shp[, pos.shp[, 1] >= xlim]
  pos.shp <- pos.shp[, pos.shp[, 1] <= xlim]
  pos.shp <- pos.shp[, pos.shp[, 2] >= ylim]
  pos.shp <- pos.shp[, pos.shp[, 2] <= ylim]
  pos.shp <- pos.shp[, pos.shp[, 1] >= xlim]
  pos.shp <- pos.shp[, pos.shp[, 1] <= xlim]
  pos.shp <- pos.shp[, pos.shp[, 2] >= ylim]
  pos.shp <- pos.shp[, pos.shp[, 2] <= ylim]
  pos.shp <- pos.shp[, pos.shp[, 1] >= xlim]
  pos.shp <- pos.shp[, pos.shp[, 1] <= xlim]
  pos.shp <- pos.shp[, pos.shp[, 2] >= ylim]
  pos.shp <- pos.shp[, pos.shp[, 2] <= ylim]
  pos.shp <- pos.shp[, pos.shp[, 1] >= xlim]
  pos.shp <- pos.shp[, pos.shp[, 1] <= xlim]
  pos.shp <- pos.shp[, pos.shp[, 2] >= ylim]
  pos.shp <- pos.shp[, pos.shp[, 2] <= ylim]
  pos.shp <- pos.shp[, pos.shp[, 1] >= xlim]
  pos.shp <- pos.shp[, pos.shp[, 1] <= xlim]
  pos.shp <- pos.shp[, pos.shp[, 2] >= ylim]
{
  pos <- pos.shp
}
else if (pos.shp == "li") {
  pos <- pca$S[, c(xax, yax)]
}
else if (pos.shp == "circle") {
  if (missing(circle.r.shp)) {
    li.2 <- apply(pca$S[, c(xax, yax)], 2, function(x) x^2)
    li.len <- apply(li.2, 1, function(x) sqrt(sum(x)))
    circle.r.shp <- mean(li.len)
  }
  t <- seq(0, 2 * pi, len = circle.nb.shp + 1)[-1]
  pos <- cbind(circle.r.shp * cos(t), circle.r.shp * sin(t))
}
else if (pos.shp == "range") {
  pos <- expand.grid(seq(min(pca$S[, xax]), max(pca$S[, xax])), len = nr.shp),
  seq(min(pca$S[, yax]), max(pca$S[, yax]), len = nc.shp)
  pos <- as.matrix(pos)
}
else {
  stop("shp.pos must be passed with values li, circle, range or a
  matrix of coordinates")
}
if (missing(scale.shp)) {
  scale.shp <- min(apply(pca$S[, c(xax, yax)], 2, function(x)
  diff(range(x)))/(c(nr.shp, nc.shp) - 1))
}
shapes <- pca2shp.new(pos, rot = pca$Evec.full[, c(xax, yax)],
  mean.shp = pca$mean.shp,
  method = "efourier", scale = scale.shp, amp = amp.shp,
  rotate.shp = rotate.shp, nb.pts = nb.pts)
if (plot) {
  if (missing(xlim) & missing(ylim)) {
    w <- apply(shapes, 2, range)
  }
  else {
    w <- cbind(xlim, ylim)
  }
  op <- par(no.readonly = TRUE)
  on.exit(par(op))
  par(mar = c(3, 3, 1, 1))
  plot(pca$S[, c(xax, yax)], xlim = w[, 1], ylim = w[, 2],
  asp = 1, las = 1, col = col.pts, pch = pch.pts,
  cex = 1, cex.axis = 0.7, ann = FALSE)
  abline(h = 0, v = 0, lty = 2, col = "grey80")
  box()
}
if (layer) {
  apply(shapes, 3, coo.draw, points = FALSE, border = border.shp,
  col = col.shp, first.point = first.point)
}
invisible(shapes)
phylo.efa.plot is a very powerful function for plotting the results from efa.phyl.pca. It combines the dudi.plot function of Momocs (Bonhomme et al., 2013) and the phylomorphospace function of phytools (Revell, 2011).

phylo.efa.plot <-
function (pca, fac = NULL, groupings = NULL, xax = 1, yax = 2, grid = TRUE, points = TRUE, pch.points = 1, col.points = "black", cex.points = 0.8, labels = FALSE, label = rownames(pca$S), boxes = TRUE, clabel = 0.6, neighbors = FALSE, draw.tree = FALSE, col.nei = "grey90", lwd.nei = 0.5, star = FALSE, col.star = "grey60", cstar = 1, ellipses = FALSE, col.ellipse = "grey30", cellipse = 1, axesell = TRUE, chull = FALSE, col.chull = "grey30", optchull = c(0.5, 1), arrows = FALSE, edge.arrow = FALSE, box.arrow = TRUE, maxnb.arrow = 10, dratio.arrow = 0.2, shapes = TRUE, pos.shp = c("li", "circle", "range", "full")[3], nr.shp = 5, nc.shp = 6, amp.shp = 1, scale.shp = 0.666, first.point.shp = FALSE, rotate.shp = 0, circle.nb.shp = 12, circle.r.shp, col.shp = "#70809011", border.shp = "#708090", rug = TRUE, rug.ticksize = 0.01, rug.col = "#708090", eigen = FALSE, eigen.ratio = 0.2, palette = col.sari, title = substitute(pca), center.orig = FALSE, zoom.plot = 1, control = list(), tree.lab = FALSE)
{
  if (!missing(fac)) {
    if (!is.factor(fac)) {
      if (ncol(groupings) == 0) {
        fac <- factor(rep("", nrow(pca$S)))
      }
      else {
        groupings <- groupings[rownames(pca$S),]
        fac <- groupings[, fac]
      }
    }
    if ((nlevels(fac) > 1)) {
      if (missing(col.star))
        col.star <- paste(palette(nlevels(fac)), "33", sep = "")
      if (missing(col.ellipse))
        col.ellipse <- palette(nlevels(fac))
      if (missing(col.chull))
        col.chull <- palette(nlevels(fac))
    }
  }
  if (center.orig) {
    ##

li.2 <- apply(pca$S[, c(xax, yax)], 2, function(x) x^2)
li.len <- apply(li.2, 1, function(x) sqrt(sum(x)))
w <- max(li.len) * (1/zoom.plot)
s.label(pca$S, xax = xax, yax = yax, xlim = c(-w, w),
        clabel = 0, cpoint = 0, sub = title, grid = grid)
}
else {
    s.label(pca$S, xax = xax, yax = yax, clabel = 0, cpoint = 0,
            sub = title, grid = grid)
}
xaxp <- par("xaxp")
yaxp <- par("yaxp")
d <- min(ax, ay)
op <- par("mar")
par(mar = rep(0.1, 4))
if (rug) {
    rug(pca$S[, xax], side = 1, ticksize = rug.ticksize,
        col = rug.col, lwd = 0.4)
    rug(pca$S[, yax], side = 2, ticksize = rug.ticksize,
        col = rug.col, lwd = 0.4)
    box()
}
if (neighbors) {
    fun <- function(x, coo, col, lwd) {
        segments(coo$x[x[1]], coo$y[x[1]], coo$x[x[2]],
                 coo$y[x[2]],
                 col = col, lwd = lwd)
    }
    neig <- nb2neig(tri2nb(pca$S[, c(xax, yax)]))
    coo <- list(x = pca$S[, xax], y = pca$S[, yax])
    apply(unclass(neig), 1, fun, coo = coo, col = col.nei,
          lwd = lwd.nei)
}
if (star & !is.null(fac)) {
    s.class(pca$S, xax = xax, yax = yax, fac = fac, clabel = 0,
            cpoint = 0, add.plot = TRUE, cstar = cstar, col = col.star,
            cellipse = 0)
}
if (ellipses & !is.null(fac)) {
    s.class(pca$S, xax = xax, yax = yax, fac = fac, clabel = 0,
            cpoint = 0, add.plot = TRUE, cstar = 0, col = col.ellipse,
            cellipse = cellipse, axesell = axesell)
}
if (chull & !is.null(fac)) {
    s.chull(pca$S, xax = xax, yax = yax, fac = fac, col =
            col.chull,
            optchull = optchull, add.plot = TRUE)
}
if (arrows) {
    arr.2 <- apply(pca$L.full[, c(xax, yax)], 2, function(x) x^2)
    arr.len <- apply(arr.2, 1, function(x) sqrt(sum(x)))
    if (maxnb.arrow > ncol(pca$mean.shp)) {
        maxnb.arrow <- ncol(pca$mean.shp)
    }
arr.sorted <- order(arr.len, decreasing = TRUE)[1:maxnb.arrow]
arr.disp <- if (missing(dratio.arrow)) {
  arr.len[arr.sorted] > 0
} else {
  arr.len[arr.sorted] > d * dratio.arrow
}
if (sum(arr.disp) > 0) {
  arr.co <- pca$L.full[names(which(arr.disp)), c(xax, yax)]
  s.arrow(arr.co, 1, 2, label = rownames(arr.co), edge = edge.arrow,
          add.plot = TRUE, boxes = box.arrow, clabel = clabel)
}
if (shapes) {
  if (!is.matrix(pos.shp)) {
    if (pos.shp == "full") {
      w <- par("usr")
      pos.shp <- as.matrix(expand.grid(seq(w[1] + d/2,
                                          w[2] - d/2, len = nr.shp),
                                          seq(w[3] + d/2,
                                          w[4] - d/2, len = nc.shp)))
    }
  }
  shapes <- phyl.efa.morphospace(pca, xax = xax, yax = yax,
                                 plot = FALSE, layer = TRUE, pos.shp = pos.shp, nr.shp =
                                 nc.shp = nc.shp, amp.shp = 1, scale.shp = d * scale.shp,
                                 rotate.shp = rotate.shp, circle.nb.shp = circle.nb.shp,
                                 circle.r.shp = circle.r.shp, col.shp = "#70809011",
                                 border.shp = "#708090", first.point = first.point.shp,
                                 pch.pts = NA)
}
if (points) {
  repeach <- function(x, each) {
    if (length(x) != length(each))
      return(rep(x[1], sum(each)))
    res <- vector(mode = class(x[1]))
    for (i in seq(along = x)) {
      res <- append(res, rep(x[i], each[i]))
    }
    return(res)
  }
  if (!is.null(fac)) {
    nb <- table(fac)
    pch.points <- repeach(pch.points, nb)
    if (missing(col.points)) {
      col.points <- palette(nlevels(fac))[fac]
    }
    cex.points <- repeach(cex.points, nb)
  }
  points(pca$S[, c(xax, yax)], pch = pch.points, col =
         col.points,
         cex = cex.points)
}
if (labels) {
s.label(pca$S, xax = xax, yax = yax, clabel = clabel,
cpoint = 0, boxes = boxes, add.plot = TRUE)
}
if (ellipses & !is.null(fac)) {
s.class(pca$S, xax = xax, yax = yax, fac = fac, clabel =
clabel,
cpoint = 0, add.plot = TRUE, cstar = 0, col = NA,
cellipse = 0, axesell = FALSE)
}
if (eigen) {
  par(mar = op)
  add.scatter.eig(pca$Eval, nf = pca$sig.PCs, xax = xax,
yax = yax, eigen.ratio, posi = "bottomright")
}
if (draw.tree) {
  tree <- pca$phy
  X <- pca$S
  if (class(tree) != "phylo")
    stop("tree object must be of class 'phylo.'")
  if (nrow(X) != length(tree$tip))
    stop("X must contain the same number of rows as species in
tree.")
  if (is.null(rownames(X))) {
    warning("X is missing row names; assuming order of tip
table.")
    rownames(X) <- tree$tip.label
  } 
  X <- X[, c(xax, yax)]
  A <- apply(X, 2, fastAnc, tree = tree)
  con = list(col.edge = setNames(rep("black", nrow(tree$edge)),
as.character(tree$edge[, 2])), col.node =
setNames(rep("black",
  max(tree$edge)), as.character(1:max(tree$edge))))
  con[(namc < names(control))] <- control
  if (!is.null(tree$maps))
    colors <- setNames(palette()[1:ncol(tree$mapped.edge)],
sort(colnames(tree$mapped.edge)))
  lwd <- 1
  aa <- setNames(c(X[tree$tip.label, 1], A[, 1]),
c(1:length(tree$tip.label),
  rownames(A)))
  bb <- setNames(c(X[tree$tip.label, 2], A[, 2]),
c(1:length(tree$tip.label),
  rownames(A)))
  XX <- matrix(aa[as.character(tree$edge)], nrow(tree$edge),
2)
  YY <- matrix(bb[as.character(tree$edge)], nrow(tree$edge),
2)
  points(x = A[1, 1], y = A[1, 2], pch = pch.points, col =
col.points,
cex = cex.points)
  if (is.null(tree$maps)) {
    for (i in 1:nrow(XX)) lines(XX[i, ], YY[i, ], col =
    con$col.edge[as.character(tree$edge[i, 2])], lwd = lwd)
  }
else {
    for (i in 1:nrow(XX)) {
        xx <- tree$maps[[i]]/sum(tree$maps[[i]]) * (XX[i, 2] - XX[i, 1])
        yy <- tree$maps[[i]]/sum(tree$maps[[i]]) * (YY[i, 2] - YY[i, 1])
        cc <- names(tree$maps[[i]])
        x <- XX[i, 1]
        y <- YY[i, 1]
        for (j in 1:length(xx)) {
            lines(c(x, x + xx[j]), c(y, y + yy[j]), col = colors[cc[j]],
            lwd = lwd)
            x <- x + xx[j]
            y <- y + yy[j]
        }
    }
    points(c(XX[i, 1], XX[tree$edge[, 2] > length(tree$tip.label), 2]), c(YY[i, 1], YY[tree$edge[, 2] > length(tree$tip.label), 2]), pch = 16, cex = 1)
    points(XX[tree$edge[, 2] <= length(tree$tip.label), 2], YY[tree$edge[, 2] <= length(tree$tip.label), 2], pch = pch.points, cex = cex.points)
    zz <- sapply(1:length(tree$tip.label), function(x, y) which(x == y), y = tree$edge[, 2])
    if (tree.lab)
        textxy(XX[zz, 2], YY[zz, 2], labs = tree$tip.label, cx = 0.5)
    par(mar = op)
}

###
# phylo.pca.contrib generates a plot illustrating the shape change along a given PC axis
###

phylo.pca.contrib <-
function (pca, PC.r = 1:pca$sig.PCs, sd = 2, cols = rep(NA, 3),
    borders = c("#000080", "#000000", "#EE0000"), lwd = 1, nb.pts = 300,
    plot = TRUE, legend = TRUE)
{
    if ((length(PC.r) > pca$sig.PCs) | (max(PC.r) > pca$sig.PCs)) {
        stop("The PC.r must correspond to PC axes present in the phylo pca object")
    }
    res <- list()
    for (i in seq(along = PC.r)) {
        pos.i <- sd * sd(pca$sS[, PC.r[i]])
        shp.i <- pca2shp.new(pos = matrix(c(-pos.i, 0, pos.i), nrow = 3),
        }
rot = as.matrix(pca$Evec.full[, PC.r[i]]), mean.shp =
 pca$mean.shp,
 method = "efourier", trans = FALSE, nb.pts = 300)
 shp.i <- a2l(shp.i)
 names(shp.i) <- paste0(rep(paste0("PC", PC.r[i]), 3),
 c("-", "m", "+"))
 res <- append(res, shp.i)
}
if (plot) {
  op <- par(no.readonly = TRUE)
  on.exit(par(op))
  par(mar = c(1, 2, 1, 1), xpd = NA)
  n <- length(PC.r)
  pos <- cbind(1:n, matrix((n + 1):(4 * n), nrow = n, ncol = 3,
  byrow = TRUE))
  plot(NA, asp = 1, xlim = c(0, 4), ylim = c(0, n), xaxs = "i",
  yaxs = "i", frame = FALSE, ann = FALSE, axes = FALSE)
  res.t <- lapply(res, coo.template, size = 0.9)
  for (i in 1:n) {
    coo.draw(coo.trans(res.t[[i]], 0.5, n =
      ((i - 1) + 0.5)), col = cols[1], border = borders[1],
    lwd = lwd, points = FALSE, first.point = FALSE)
    coo.draw(coo.trans(res.t[[((i - 1) + 2)]], 0.5, n =
      ((i - 1) + 0.5)), col = cols[2], border = borders[2],
    lwd = lwd, points = FALSE, first.point = FALSE)
    coo.draw(coo.trans(res.t[[((i - 1) + 3)]], 0.5, n =
      ((i - 1) + 0.5)), col = cols[3], border = borders[3],
    lwd = lwd, points = FALSE, first.point = FALSE)
  }
  for (i in 1:(n * 3)) {
    pos.x <- rep(0:2 + 1.5, times = n)
    pos.y <- rep((n - 1):0 * 1 + 0.5, each = 3)
    coo.draw(coo.trans(res.t[i], pos.x[i], pos.y[i]),
      col = cols[((i - 1)%%3) + 1], border = borders[((i -
      1)%%3) + 1], lwd = lwd, points = FALSE, first.point =
      FALSE)
  }
  if (legend) {
    text(1.5, n, labels = paste("-", sd, "s.d.", sep = ""),
      adj = 0.5)
    text(2.5, n, labels = "Mean", adj = 0.5)
    text(3.5, n, labels = paste("+", sd, "s.d.", sep = ""),
      adj = 0.5)
    text(0, (n:1) - 0.5, labels = paste("PC", PC.r),
      adj = 1)
  }
  invisible(res)
}
Step-By-Step Guide to R Script Usage:

**Step 1: Open R:**
Start R and execute the script above to load all of the required functions. Be sure to load the required libraries (Momocs, ape, geiger, phytools, and calibrate)

**Step 2: Input and Prepare data:**
efa.phyl.pca.R can utilize EFA data from two different sources: either a Coe object created with the Momocs R package (Bonhomme et al., 2013) or a .nef file of normalized elliptical Fourier descriptors produced by the SHAPE software package (Iwata and Ukai, 2002). If a .nef file is to be used, it must be imported and converted to a Coe object as follows. With normalized elliptical Fourier data, the coefficients A1, B1, and C1 are constants and should be deleted before analysis using the neftrimmer function.

```r
coeA <- NEF2COE("your_file_name_here.nef")
data <- coeA@coe
dataA <- neftrimmer(data)
```

**Step 3: Import Phylogenetic Tree:**
A phylogenetic tree should then be imported as a phy object. One way to do this to import a topology from a Nexus file as shown below. The topology must have tip labels and branch lengths.

```r
tree1 <- read.nexus("your_file_name.nex")
```

**Step 4: Estimate phylogenetic signal:**
efa.phyl.pca.R contains a function for estimating phylogenetic signal in EFA shape data using the consistency index (Klingenberg and Gidaszewski, 2010). Note that this function can take a very long time to execute, especially on older computers.

```r
efa.phylo.signal(tree1, dataA, nharm=8, iter=1000)
```

**Step 5: Calculate Phylogenetic PCA**
Phylogenetic PCA of elliptical Fourier shape data can now be calculated. The number of harmonics to retain must be specified. This should be calculated using the Fourier power equation (Crampton, 1995; Claude, 2008). In this example, 8 harmonics will be retained.

```r
PCA <- efa.phyl.pca(tree1, dataA, nharm=8)
PCA will be a list containing the following objects:
  mean.shp matrix of the NEF descriptors of the mean shape
  num.harms the number of harmonics retained
  Eval the eigenvalues
  Evec eigenvectors, without values for A1, B1, and C1, the harmonic coefficients that were removed for the PCA
  S principal components scores
  L loadings on each axis, without values for A1, B1, and C1, the harmonic
coefficients that were removed for the PCA

\textbf{L.full} loadings on each axis WITH values for A1, B1, and C1. this can be used for plotting and subsequent analyses

\textbf{Evec.full} Eigenvectors WITH values for A1, B1, and C1. this can be used for plotting and subsequent analyses

\textbf{var.contrib} a vector showing the percent of total variance explained by each principal component axis

\textbf{sig.PCs} the number of PC axes that explain 5 percent or greater of the cumulative variance

\textbf{phy} the phylogenetic tree used for the analysis

**Step 6: Plot Results:**

The \texttt{phylo.efa.plot} function, a derivation of the \texttt{dudi.plot} function of the Momocs package (Bonhomme et al., 2013), is an extremely powerful and customizable tool for plotting the results of a phylogenetic PCA of EFA data in phylomorphospace

\begin{verbatim}
# phylomorphospace plots
# just points for tip taxa:
phylo.efa.plot(PCA2,shapes= FALSE,star=FALSE,ellipses=FALSE,
pch.points=20)
# place taxon names centered over the appropriate points on the plot:
phylo.efa.plot(PCA2,shapes= FALSE,star=FALSE,ellipses=FALSE, labels
=TRUE, pch.points=20)
# Include phylogenetic tree, ancestral taxa, and reconstructed ellipses
# on the plot:
phylo.efa.plot(PCA2,shapes=TRUE,star=FALSE,ellipses=FALSE,
draw.tree=TRUE)
# same as previous plot but with tip labels adjacent to data points
phylo.efa.plot(PCA2,points=FALSE,
shapes=TRUE,star=FALSE,ellipses=FALSE, draw.tree=TRUE, tree.lab=TRUE)
\end{verbatim}