DISTANT DRUMMING: MORPHOLOGICAL CORRELATES OF HABITAT AND COURTSHIP BEHAVIOUR IN THE RUFFED GROUSE (*BONASA UMBELLUS*)

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B.Sc., University of Lethbridge, 2011

A Thesis
Submitted to the School of Graduate Studies
of the University of Lethbridge
in Partial Fulfilment of the
Requirements for the Degree

MASTER OF SCIENCE

Neuroscience
University of Lethbridge
LETHBRIDGE, ALBERTA, CANADA

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Date of Defence: August 15, 2014

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ABSTRACT

The Ruffed Grouse (*Bonasa umbellus*) is a resident game bird of North America. Unlike other birds, male Ruffed Grouse do not vocalize during courtship, and are dependent upon 'drumming', a 'wingbeat' display, for the acoustic component of their courtship behaviour. Because this wingbeat display is unique, I investigated morphological correlates that could underlie its production. First, I examined wing shape among grouse from museum specimens using various morphometrics. I found that wing morphology corresponds with habitat, behaviour and phylogentic relationships within Tetraoninae. Next, I examined the brains of male and female Ruffed Grouse. I detected seasonal plasticity between males collected during the breeding and non-breeding seasons; those collected during the breeding season had larger motor regions than those collected during the non-breeding season. My findings indicate that habitat and wing shape are correlated among grouse, and that seasonal changes in brain morphology contribute to the production of the drumming display.

ACKNOWLEDGMENTS

This thesis should stand as a small tribute to all those that have come before me. For without the shoulders upon which we all stand, we would all be lost to ourselves. Foremost, I would like to thank my supervisor Dr. Andrew Iwaniuk for seeing potential and inspiring a love of birds, good beer and the comparative method in me, as well as showing me that it is possible to become a relentlessly multifaceted biologist under the yolk of a discipline as focused as neuroscience. Without his contributions, patience, consideration and tireless dedication to field work this thesis would not have been possible. Thanks Andy, I promise that I am going to pay you back. I also extend my gratitude to Dr. George Iwaniuk for the use of his field site, and Drs. Rob Faucett and John Klicka for access to the ornithology collection at the Burke Museum of Natural History in Seattle, Washington. Next, I thank my committee members, Drs. Drew Rendall and Theresa Burg for their constructive commentary, advice and encouragement. Specifically, I would like to thank Dr. Rendall for dispelling my notion of deserving 'personal time' during graduate school, and Dr. Burg for her continued efforts to motivate me both as a student and a scientist. Without you both I would just be some guy with way too much poorly managed personal time on his hands. Additionally, I thank Dr. Sergio Pellis who has been an ally and inspiration to me throughout my time at the University of Lethbridge. I would also like thank my lab mates Dr. Jeremy Corfield and Danielle Burger (BSc) for teaching me the skills I used in my neuroanatomical studies during graduate school while I was still an undergraduate student. I am also grateful to Doug Bray for his logistical support on a variety of projects. You have all helped me to become a more proficient scientist. I thank you all and am grateful for

your efforts. I also thank my funding source (NSERC Discovery Grant to ANI) and the University of Lethbridge. Hey, somewhere had to take me and someone had to pay my way.

I thank my family for their continued support and for helping shape my world view by allowing me to fail and succeed on my own terms with the knowledge they would be there for me regardless. Specifically, I would like to thank my father Derek for instilling in me a love of science and an appreciation of nature, my mother Donna for showing me what true kindness and perseverance mean, my brother Chad for fortifying me as an individual as we grew up and his friendship to me as an adult, my step-father Grant (and the Lorenz family in general) for his unconditional acceptance of me and his ability to focus on 'the brighter side' on life, my uncle John Schatz for his galvanizing role within my family as well as my cousins John and Joseph Schatz, and finally my grandparents and foregoing generations for their efforts and determination in ages much more inhospitable than ours. I hope that through this small gesture of mine their endeavours are once again vindicated.

Finally I thank all of my friends for the impact they have had on my motivations and for their acceptance of me during stages of my life that were layered with shortcomings. Specifically but not limited to: Jeremy Waldner, Amy Arsene & the Waldner family (Adam, Annie, Jerry, and Sharon), Sean Egeland, Erik Wright, Zach Randall, Neil and Brain Mazurek, Jeremy Remesovff, Cameron Kneen, Cody Bolt, Matthew Schier, Michael Taitenger, Mitchell Dirkach, Maggie Bozyk, Jordan Schneider-Crane, Dylan McGillis and Sarah Plumer. Additionally, I would like to thank my girlfriend Janet Poplawski who has been a bright light to me throughout the completion of my Master of Science degree.

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LIST OF ABBREVIATIONS

A – arcopallium
ANCOVA – analysis of covariance
ANOVA – analysis of variance
AR – aspect ratio
BMNH – Burke Museum of Natural History
C – camber
Cb – cerebellum
CE – coefficients of error
CNS – central nervous system
CV – canonical variate
CVA – canonical variate analysis
DLM - dorsolateral anterior thalamic nucleus
DF – degree of freedom
F – F ratio
*f*prime – primary feather
GM – geometric morphometrics
gm – gram
GPA – generalized Procrustes analysis
HA – hyperpallium
HF – hippocampal formation
HSD – honestly significant difference
Hz – hertz
ICo - nucleus intercollicularis
IEG – immediate early gene
kg – kilogram
L – wing length
LM – landmark
LMAN - lateral magnocellular nucleus of the anterior nidopallium
M – mesopallium
M_b – body mass in grams
mg – milligram
Mg_n – body mass in kilograms
nRt – nucleus rotundus
Nt – nucleus taeniae
nXIIts - tracheosyringeal portion of the hypoglossal nucleus
P – porosity
P1: P10 – Primary feathers 1-10
PBS – phosphate buffered saline
PC – principal component
PCA – principal component analysis
PFA – paraformaldehyde
PNS – peripheral nervous system

PSA – potential surface area
RA – robust nucleus of the arcopallium
S – total area of both wings and the body between the wing's leading and trailing edges
S1 – first secondary feather
SA – surface area
SPC – striatopallidal complex
spp. – species
T – Testosterone
TELE – telencephalon
TM – traditional morphometrics
TPS – thin-plate spline
Tr – nucleus triangularis
UWBM: University of Washington Burke Museum
v. – versus, or, in opposition to
WB – whole brain
WC_m – maximum wing chord
WC_x – chord length at that point
WD_m – maximum wing depth
WL – wing loading
WS – wing span
WW – wing width

CHAPTER ONE: GENERAL INTRODUCTION

Grouse (Aves: Tetraoninae) are galliform birds related to chickens and quail that comprise a monophyletic group within the broader family Phasianadae (Figure 1.1) (Drovetski, 2002; Wang et al., 2013). The 18 recognized extant grouse species are thought to have evolved over the past 3.2 million years, and speciation in this group is associated with dispersal across much of the Northern hemisphere (Figure 1.2) (Drovetski, 2002, 2003; Lucchini et al., 2001). Throughout this broad distribution, grouse are primarily terrestrial or partly arboreal resident species, though some do engage in forms of migration (e.g., *Lagopus* spp., *Dendragapus* spp.) (Johnsgard, 1983; Hjorth, 1970). Grouse have adapted to a number of broadly defined habitats: forest (e.g., early, mid-successional, and old growth), tundra (e.g., upland arctic, montane and alpine regions) prairie (e.g., North American grasslands), and sagebrush (e.g., North American sagebrush-steppe, *Artemisia* spp.) and share a variety of systematic characters related to feeding, thermal regulation and movement in harsh climates, such as convex beaks, scaled toes, and feathered tarsi and nostrils (Drovetski, 2002; Lucchini et al., 2001; Hjorth, 1973). Further, grouse share a range of life history variables including diet, predators, reproductive rate and nesting habits (Hjorth, 1970; Johnsgard, 1983). Despite these and other similarities, grouse are highly diverse in habitat preference, secondary sexual characters, courtship behaviours and mating systems. Among North American grouse for example, species may be monogamous or exhibit variable levels of polygamy (Drovetski et al., 2006). Similarly, they may display collectively in tight or exploded leks, or be territorial and display solitarily (Hjorth, 1970; Johnsgard 1983; Drovetski et al., 2006). This behavioural diversity includes the production of a

variety of non-vocal acoustic signals, one of particular interest being the drumming display of the Ruffed Grouse.

The Ruffed Grouse is found throughout boreal, montane and temperate North American forests, occurring in subarctic areas from the Pacific Northwest across Canada to Labrador, with populations extending into the United States along the Appalachians, Rockies and the Cascade ranges (Figure 1.3). It is the only species of its genus endemic to North America and it differs in a number of respects from its congeners, most notably in plumage. For example, male Ruffed Grouse have dark, 'ruffled' neck feathers as their name suggests, while both the Hazel (*Bonasa bonasia*) and Chinese Grouse (*Bonasa sewerzowi*) are sexually dichromatic (Johnsgard, 1983). Behaviourally, male Ruffed Grouse differ from their close relatives and the majority of birds because they engage in a mechanical wingbeat display known as "drumming" during their courtship display. Drumming is typically performed atop a fallen log and consists of rapid wingbeat movements that produce a stereotyped low frequency sound (Garcia et al., 2012; Figure 1.4). Other grouse species use both wingbeat and flutter displays for courtship, including both Hazel and Chinese grouse (Johnsgard 1983; Scherzinger et al., 2006), but always in conjunction with vocalizations. Drumming is, by comparison, a much more frequent, extended and highly structured behaviour, performed in the absence of vocalizations. Thus, the Ruffed Grouse has evolved a markedly different courtship display compared to related species.

Although uncommon, many avian species produce mechanical sounds (i.e., Apodiformes, Caprimulgiformes, Charadriiformes, Galliformes, Passeriformes, Piciformes) (Bostwick, 2006). While an array of these so-called 'sonations' exist (Murphy et al., 2003; Eda-Fujiwara et al.,

2004; Clark & Feo, 2008), the majority are made by the wings and feathers (Bostwick, 2006). These non-vocal acoustic signals tend to be used during courtship displays, as in the case of the Ruffed Grouse, though they are occasionally utilized for other purposes (Hjorth, 1970; Johnsgard, 1983). For example, the Crested Pigeon (*Ocyphaps lophotes*) has one modified primary feather on each wing (P8) that is significantly ($p = 0.001$) more tapered than the other primaries (Hingee & Magrath, 2009). When they take off under duress, during risk of predation for example, this narrowed feather produces a 'whistle' that conspecifics recognize as an alarm signal (Hingee & Magrath, 2009). Those differences in wing and feather morphology that are associated with courtship, however, tend to be more pronounced (Bostwick, 2006). For example, the Club-winged Manakin (*Machaeropterus deliciosus*) has highly modified 1^{st}-7^{th} secondary feathers (Bostwick et al., 2010) that are likely important for producing sustained harmonic tones during their courtship display (Bostwick & Prum, 2005). Thus, there is ample evidence for a relationship between wing and feather morphology and the expression of specific acoustic behaviours (Bostwick, 2006). Whether Ruffed Grouse also have different wing and/or feather morphology compared to other grouse species in relation to habitat type, courtship behaviour or phylogeny, however, has not been investigated. Similarly, little is known about the neural control of non-vocal courtship displays in birds. For example, it is likely that sex differences in arcopallium volume (i.e., a telencephalic motor nucleus) underlie the production of wing-snapping courtship displays made by male Golden-collared Manakins (*Manacus vitellinus*) during the breeding season, but it remains uncertain if this variation occurs seasonally as it does in the brains of oscine songbirds (Day et al., 2011; Schlinger, 2013; Tramontin & Brenowitz, 2000). Although the drumming display of Ruffed Grouse was first documented centuries ago

(Linnaeus, 1766) and the drumming sound is used to census grouse populations (Gullion, 1966; Jones, 2005; Hansen et al., 2011), few attempts have been made to understand fundamental aspects of how the display is produced. The primary objectives of my thesis are therefore to:

1. Assess the extent to which wing shape varies with habitat type and phylogeny in grouse and determine whether the Ruffed Grouse differs in wing shape from other grouse species.
2. Determine the extent to which brain regions associated with motor control differ in size relative to sex and season in the Ruffed Grouse.

Wing shape

Historically, avian wing shape has been assessed using 'traditional' morphometrics (TM). A host of different wing shape measurements fall under the category of TM, such as aspect ratio (AR), surface area (SA), camber (C) and wing loading (WL). Primary feather lengths (*f*prime$^{1\text{-}10}$) are also commonly used to assess avian wing shape. Measurements of *f*prime$^{1\text{-}10}$, and principal components analyses (PCA) of those lengths, provide a proportional estimate of wing length (WL), width (WW), wing tip shape, and are correlated with a range of traits including sex, age, flight speed and style, migratory distance, clinal variation, habitat type and other factors (Norberg, 1990; Borras et al., 1998; Wang et al., 2011; Senar et al., 1994; de la Hera et al., 2012; Rising, 1988). Collectively, TM measurements have been greatly informative in avian biology, but are limited in a number of respects. For example, a lack of uniform size correction methods and slight variations in their application and interpretation has resulted in

inconsistencies across many TM studies (Adams et al., 2004; Zelditch, 2010). Further, they often lack the power to discern between closely related species, such as those that comprise Tetraoninae (Adams et al., 2004; Maderbacher et al., 2007; Drovetski, 2002). Moreover, many TM studies have focused on broad aspects of wing shape and, as a result, may have largely overlooked subtle variation in wing shape that nevertheless may be important (Adams et al., 2004; Monteiro & Abe, 1999; Perez et al., 2006; Drovetski, 1996). Because of these and other shortcomings, I used not only TM, but also landmark-based geometric morphometric analysis to assess wing shape variation among grouse.

Geometric morphometrics (GM) is a diverse collection of multivariate shape assessment techniques underscored largely by the comparison of sets of distances between anatomical landmarks across a group of specimens (Adams et al., 2004; Zelditch, 2010). One of the primary advantages of GM is that it allows for the analysis of shape independent from the distorting effects of size, scale and orientation and provides a means to quantify and visualize fine scale changes in shape that TM methods do not. Additionally, semi-landmark outline analyses can be used in the absence of well defined landmarks (Zelditch, 2010). GM has been applied widely in the life sciences and is increasingly used to assess shape differences in a range of biological structures (Adams et al., 2004). For example, recent GM studies have examined differences in the shape of bat and insect wings, leafs and flowers, beaks, vibrissae, and the brains of birds and humans (de Camargo & de Oliveira, 2012; Klingenberg et al., 2010; Viscosi & Cardini; 2011; van der Niet et al., 2010; Foster et al., 2008; Ginter et al., 2012; Bookstein et al., 2002; Kawabe et al., 2013). GM has also been applied in recent studies of avian wing (Brewer & Hertel, 2007)

and feather (Sheets et al., 2006; Bendoy et al., 2010; Albutra et al., 2011) shape, but is still infrequently used in comparison to TM methods, and rarely to analyze wing shape (Adams et al., 2004; Ginter et al., 2012; Parsons et al., 2003).

In Chapter 2, I used a combination of TM and GM to assess variation in wing morphology among grouse in relation to habitat type, phylogeny and courtship behaviour; sex differences were also investigated. To do so, I measured and photographed spread wing specimens (Figure 1.5) prepared from most extant grouse species representative of each habitat type (i.e., forest, prairie, sagebrush and tundra). Then, using a variety of TM measurements: AR, C, porosity (P), WL and PCA of *f*prime[1-10], I tested for species and sex differences and associations with habitat preference. Next, I applied a landmark configuration to the photographs using the tips of feathers and structural boundaries of the wing as points of interest. A canonical variate analysis (CVA) of the Procrustes coordinates was then used to test for differences between sexes, among species and habitat preferences. Finally, I used squared-change parsimony to map GM shape data onto a molecular phylogeny (Drovetski, 2002; Figure 1.1), and in conjunction with permutation tests of Procrustes coordinates, quantified phylogenetic signal within my GM data set. Based on these analyses, I show that wing shape varies along a number of axes among grouse genera, species, and the sexes. Further, my data indicate that this variation corresponds well with differences in habitat type and strongly reflects phylogenetic relationships within Tetraoninae. Additionally, I show that neither gross features of wing morphology nor fine scale geometric variations in wing shape differ among the Ruffed Grouse

and other North American forest grouse. In other words, the Ruffed Grouse does not appear to have a unique wing shape that could be used to enhance or produce the drumming display.

Courtship Behaviour and the Avian Brain

Specialized wing and/or feather morphology may be important for the production of the drumming display, but the behaviour itself is a product of the brain. The evolution of sex-specific behaviours or sensory abilities is inherently dependent on evolutionary changes in the central (CNS) and/or peripheral nervous systems (PNS) between the sexes (Striedter, 2005; Balthazart & Ball, 1995). With respect to courtship behaviours, the evolution of learned song and at least one form of non-vocal display is associated with anatomical and physiological changes in the nervous system (Tramontin & Brenowitz, 2000; Liu et al., 2013; Schlinger et al., 2013). For example, the learning and production of song by oscine songbirds (Aves: Passeriformes) is dependent upon specialized forebrain circuitry. This so called 'song-circuit', consists primarily of three vocal control nuclei (i.e., HVC, Area X and the robust nucleus of the arcopallium (RA)), their intrinsic connections and the nuclei to which they project (e.g., lateral magnocellular nucleus of the anterior nidopallium (LMAN), hypoglossal portion of the principal nucleus of the 12th cranial nerve (nXIIts)) (Figure 1.6) (Nottebohm, 2005). These song control nuclei undergo pronounced seasonal variation in cytoarchitecture (i.e., cell size, spacing, connectivity) that commonly equates to changes in their volume (Tramontin & Brenowitz, 2000). For example, seasonal increases in HVC volume result primarily from neurogenesis, while in RA, the cell bodies and dendritic arbors of neurons become larger (Tramontin & Brenowitz, 2000). Similarly, the extent of this plasticity is proportional to song complexity (i.e., number of

syllables, repertoire size) and rate of song such that species, sexes or individuals with more frequent and/or variable song types typically have more, larger, better connected cells in their vocal control nuclei than those with relatively simple or infrequent song, (DeVoogd et al., 1993; Moore et al., 2011; MacDougall-Shackleton & Ball, 1999; DeVoogd, 2004; Kirn et al., 1988). In contrast, the neural basis of other forms of courtship behaviour is infrequently studied among non-vocal learners and even more so among non-passerine species. Further, little is known about the occurrence of associated seasonal variation in brains of most non-oscine lineages (Panzica et al., 1991; Beani et al., 1995; Fusani et al., 2003).

The Golden-collared Manakin (Aves: Tyranni) is a well studied, lekking suboscine with a physically elaborate courtship display that includes wing-snapping and acrobatic jumps punctuated by vocalizations (Ericson et al., 2003; Schlinger et al., 2013). Suboscines are a group within the Passeriformes that includes both New (i.e., Tyrannoidea, Furnarioidea) and Old World (i.e., Eurylaimidae, Pittidae, Philepittidae) species that are closely related to songbirds, but differ in a number of systematic characters (e.g., syrinx morphology andinner ear structures), distribution and behaviour (i.e., vocal learning) (Ericson et al., 2003; Reiner et al., 2004). The manakin's display nevertheless shares similarities with oscine song: it is sexually dimorphic, varies among individuals, is performed exclusively in the breeding season and is thought to be an indicator of fitness to conspecifics (Schlinger et al., 2013). The physiological mechanisms that underlie courtship in this species are numerous and include specializations at multiple levels (Schlinger et al., 2013). For example, considerable amounts of aromatase are present in, and thought to mediate the effects of testosterone on the skeletal muscles (i.e., wing and leg) and

spinal cord (e.g., cell bodies of motor neurons). Androgen receptors and testosterone are also present at high densities in other tissues including various regions of the brain (i.e., arcopallium, cerebellum, preoptic area) (Fusani et al., in press). Further, the volume of the arcopallium is sexually dimorphic, being larger in males than females (Day et al., 2011). Taken together with our understanding of neuromuscular control of the wings (Feenders et al., 2008; Schlinger et al., 2013), this implicates the arcopallium in production of the Golden-collared Manakin's display, and perhaps those of other birds as well.

Because male Ruffed Grouse are reliant upon drumming for the acoustic component of their courtship behaviour, I examined seasonal changes in the volume of brain regions that could be involved in its production. Drumming shares many characteristics with both birdsong and the displays of manakins. For example, the Ruffed Grouse is sexually dimorphic and occurs seasonally, there is both spectral and temporal variation among individuals, it is thought to function as both a territorial and sexual signal, and like the Golden-collared Manakin, it is reliant upon highly coordinated wing movements (Hjorth, 1970; Johnsgard, 1983; Garcia et al., 2012; Prum, 1990; Schlinger et al., 2013). Moreover, because drumming does not incorporate vocalizations, it provides a novel opportunity to gain some insight into the neural substrates underlying non-vocal communication in birds.

In Chapter 3, I examine the brains of male Ruffed Grouse collected in the breeding (April-May) and non-breeding (September-December) seasons as well as non-breeding females to test whether there are sex differences and seasonal variation in the size of brain regions putatively involved in the production of the drumming display. Using unbiased stereology, I

tested for differences in the absolute and relative volumes of general brain areas (telencephalon (TELE), cerebellum (Cb)), as well as motor (arcopallium, striatopallidal complex (SPC)), spatial (hippocampal formation (HF)), and sensory processing regions (mesopallium (M), nucleus rotundus (nRt)) between males (breeding/non-breeding) and females (non-breeding only). My analyses indicate that males collected in the breeding season have larger relative arcopallium and SPC volumes than males or females collected in the non-breeding season, while relative telencephalic volume did not vary seasonally or between the sexes. Further, absolute arcopallium volume was largest in breeding season males. While all telencephalic regions scaled with telencephalic size, only the HF and SPC scaled with whole brain size. Regardless of the scaling variable, arcopallium and SPC were significantly larger in breeding season males. Together, this strongly suggests that both the arcopallium and SPC play a role in producing and/or modulating the drumming display and is the first demonstration of seasonal plasticity in the TELE of a galliform species.

Summary

Overall, my analyses of wing shape and neuroanatomical variation provide novel insights into the production of the drumming display. Specifically, the wings of Ruffed Grouse do not differ in shape from other grouse in relation to production the drumming display, and that telencephalic motor regions implicated in the display's production are larger during the breeding season in males. In chapter 4, I discuss the implications of my results for understanding Ruffed Grouse behaviour and the correlated evolution of wings, brains and courtship behaviours in grouse. Last, I provide some future directions for my research that could lead to a much more

integrative understanding of how non-vocal components of courtship behaviours are produced evolve in birds as a whole.

CHAPTER TWO

STUDYONE: MORPHOMETRIC ANALYSIS OF WING SHAPE VARIATION AMONG GROUSE

INTRODUCTION

Wing shape varies significantly among birds (Norberg, 1990; Marchetti et al., 1995; Copete et al., 1999; Pérez-Tris et al., 2001). The shape of the wing primarily reflects aerodynamics such that wing shape and size vary with flight behaviour across species. For example, American Kestrels (*Falco sparverius*) have long, pointed wings that facilitate gliding, high speed and level flight (Meyers, 1992; 1993) whereas Black-billed Magpies (*Pica hudsonia*) have relatively short, broad wings better suited for producing vertical lift and intermittent, manoeuvrable flights (Tobalske & Dial, 1996; Tobalske et al., 2003). Although the bulk of studies on wing shape are based upon broad comparisons of species across orders and families (Norberg, 1990; Wang et al., 2012; Lockwood et al., 1998; Dial et al., 2006; Nudds & Bryant, 2000; Tbalske, 1996), similar patterns of wing shape, flight behaviour and habitat occur in more restricted taxonomic comparisons. For example, the wing shape of North American hummingbirds varies both among and within species in age-sex groupings that reflect nectar site defense and nectar acquisition strategies (Stiles et al., 2005). Similarly, among *Phoebastria* albatross species, WL and AR scale with wind speeds and wave height, allowing species to fly

more efficiently within their distributional limits of their home range (Suryan et al., 2008). Comparisons within other avian species demonstrate geographical variation in wing shape consistent with population differences in habitat, migration and/or flight behaviour (Chandler & Mulvihill, 1990; Tellería & Carbonell, 1999; Förschler & Bairlein). Thus, regardless of the taxonomic level of analysis, wing shape varies with habitat and flight behaviour.

Although these patterns of wing shape variation are highly consistent across studies, they are often limited with respect to the measurement of wing shape itself. Avian wing shape is typically assessed through the use of TM (Rayner, 1988; Nudds et al., 2011; Marcus, 1990), which are largely based upon 'simple' linear measurements and ratios thereof. For example, AR, a measure defined as the ratio of wingspan (WS) squared to SA, is used ubiquitously as a measurement of wing shape (Rayner, 1988). Although AR is an important determinant of aerodynamic performance (Norberg, 1990; Rayner, 1988), it is insensitive to fine scale differences in shape across the leading and trailing edges of the wing. Composite measurements, like wingtip shape indices, can estimate the pointedness and convexity of the wing (Swaddle & Lockwood, 2003), but different wingtip shape indices measure different components of wing shape (Chandler & Mulvihill, 1988). Therefore, without prior knowledge of what morphological variation might be relevant, the selection of a given wingtip shape index is somewhat arbitrary (Chandler & Mulvihill, 1988). In addition to these issues associated with specific morphometric measurements, there are several other problems that these TM share in common. First, wing shape varies with size, but there is no standardized means of size correction. Second, TM does not necessarily take into consideration the position of where the distance measurements were made relative to one another. This is problematic because exact sets of distance measurements

(i.e., maximum length and width) can be obtained from differently shaped structures (Adams et al., 2004). Third, TM are largely based upon gross differences in overall wing shape and are therefore potentially ignoring subtle variations in the shape of the wing that are nevertheless functionally important (Adams et al., 2004; Zelditch et al., 2012).

A solution to these problems associated with the use of TM to examine wing shape is GM. GM is a multivariate technique that relies upon the identification of homologous anatomical loci across a group of specimens, which can then serve as landmarks in Cartesian space (Zelditch et al., 2012). Landmark configurations are then translated to, and superimposed upon a common location, the centroid (Zelditch et al., 2012). Shape can then be compared across individuals, free from the effects of orientation, scale and size, while preserving size information separately in the form of centroid size (Sadeghi et al., 2009). Therefore, unlike TM, GM methods significantly reduce shape distortion stemming from size differences and offer an accurate means of visualizing and comparing shape differences (Adams et al., 2004; Zelditch et al., 2012; Birch, 1997). GM also provides a means of quantifying shape variation in complex and/or curved structures whose shape cannot be effectively estimated with TM, such as insect (Sadeghi et al., 2009) and bat wings (de Camargo & de Oliveira, 2012). Avian wing shape shares many similarities with these other structures, but to date there are only a few studies using GM to investigate avian wing and feather shape (Sheets et al., 2006; Brewer & Hertel; Bendoy et al., 2010; Albutra et al., 2011; Moneva et al., 2011).

Here, I use a combination of TM and GM to examine interspecific variation in wing shape among grouse species (Tetraoninae, Galliformes). Grouse are monophyletic (Drovetski et al., 2003) and share similar life histories (Johnsgard, 1983; Hjorth, 1970; Atwater & Schnell,

1989), but vary significantly from one another in habitat preference and the use of the wings (Johnsgard, 1983; Hjorth, 1970; Atwater & Schnell, 1989). Four of the seven genera occupy forested habitats (*Falcipennis, Dendragapus, Bonasa, Tetrao*) while shifts to tundra (*Lagopus*), sagebrush (*Centrocercus*) and the prairies of North America (*Tympanuchus*) have occurred more recently in their evolution (Drovetski, 2003; Drovetski & Rohwer, 2006). The flight demands of these habitats are inherently different, so wing shape likely varies with habitat, as it does in other species (Tobalske, 1996; Stiles et al., 2005; Suryan et al., 2008; Shaffer et al., 2001; Tobalske et al., 2003; Altshuler et al., 2004). In addition, grouse vary significantly from one another in the use of the wings during courtship (Johnsgard, 1983; Hjorth, 1970; Atwater & Schnell, 1989), which could also influence wing shape. Variations in wing size and shape among Neotropical manakins (Pipridae) reflect both habitat and male courtship displays (Théry, 1997), but the extent to which courtship behaviour varies with wing shape among grouse, or most other avian taxa, has yet to be tested.

Based on previous studies of wing shape variation in birds (Lockwood et al., 1998; Drovetski, 1996; Heers et al., 2011), I made several predictions regarding the relationship between wing shape, habitat and behavior. First, TM measurements of wing size and shape (e.g., AR, P) would vary among grouse species according to habitat type. For example, rounded and highly slotted wings assist in production of the vertical left necessary to escape predation in areas of dense vegetation while tapered and less slotted wings facilitate high-speed and level flight, which is necessary to escape terrestrial predators in open-field scenarios. Specifically, forest dwelling species would have more elliptical, less pointed and deeply slotted wings (i.e., high porosity, see below) with lower AR and WL and greater C when compared with tundra and

prairie dwelling species (Norberg, 1990; Rayner, 1988; Heers et al., 2011; Gamauf et al., 1998; Hendenström, 2008; Vanhooydonck et al., 2009; Desrochers, 2010). Second, I predicted that GM analysis of wing shape would also reflect habitat preference among grouse (Drovetski & Rohwer, 2006). That is, species inhabiting forests, tundra, prairie and sagebrush would occupy different parts of a multivariate morphospace based upon GM analyses of wing shape (Klingenberg, 2011). Third, because speciation events in grouse are associated with shifts in habitat preference (Drovetski, 2003), I also predicted that wing shape would express a significant phylogenetic signal (Klingenberg & Gidaszewski, 2010). Last, because male Ruffed Grouse (*Bonasa umbellus*) produce a unique wingbeating courtship display (Johnsgard, 1983; Hjorth, 1970; Atwater & Schnell, 1989), I predicted that its' wings would differ in shape from other forest grouse species (i.e., *Bonasa bonasia*, *Falcipennis canadensis, Dendragapus* spp., *Tetrao* spp.).

METHODS

All measurements were taken from spread wing specimens in the Burke Museum of Natural History (BMNH, Seattle, WA) collection (Table 2.1). Each spread wing was articulated with the leading edge fully extended, preserved in a natural flight position (Stiles et al., 2004; Drovetski, 1996) (Figure 2.1) and the collection is commonly used for studies of wing morphology (Drovetski, 1996; Rohwer & Manning, 1990; Young, 1991). Moulting or damaged wings (i.e., feathers broken and/or missing) were excluded from all multivariate analyses, while intact and fully developed feathers from these wings were used to calculate wing lengths and widths where possible. In total, 212 specimens from 16 species were used in this study (Table

2.1). In addition to linear measurements taken directly from the specimens (see below), each spread wing was also photographed in the dorsal and anterior position, with a scale bar, using a Cannon PowerShot SX30 IS. All measurements described below were made by a single observer (JMK).

Traditional Morphometrics (TM)

The length of $f_{prim}{}^{1\text{-}10}$ was measured directly with digital hand calipers, to the nearest 0.1 mm. f_{prim} was defined as the distance between the distal tip of the rachis to the point of calamus emergence from the skin (Wang et al.., 2012; Lukas & Raffael, 1989). Each was numbered conventionally from 1 (nearest to the secondary feathers) to 10 (nearest the leading edge of the wing); this ordination differs from the labeling in the GM component of this study which was chosen for ease of analysis. Total L, the distance from the wrist of the wing to its tip (carpometacarpus + 1st and 2nd phalanx length + max f_{prim} = L), was also measured to the nearest 0.1 mm (Marchetti et al., 1995; Stiles & Altshuler, 2004; Nudds, 2007; Nudds et al., 2011). Maximum wing chord (WC_m) was measured as the distance from the wrist of the wing to the tip of the first secondary feather (S1) (also numbered conventionally) (Drovetski, 1996; Stiles & Altshuler, 2004) and was only recorded from wings where S1 was present and fully developed. Coefficients of variation were calculated from repeated linear measurements to assess measurement error (Albert & Zhang, 2010). To do so, C, L, WC_m and $f_{prim\ (1\text{-}10)}$ were measured five times from the right wing of a male Ruffed Grouse. All coefficients of variation were less than 3% of the mean (range = 0.62 - 2.40%), indicating that the measurements are highly repeatable. SA is defined as total wing area, which I calculated to the nearest 0.1 mm^2

from the digital photographs using ImageJ (Rasband, 1987-2011). Measurements of SA were then used to calculate porosity, a ratio that describes the transmissivity of a wing (Heers et al., 2011; Müller & Patone, 1998). Following Heers et al. (2011), I calculated P using the following equation: $P = 100 (PSA / SA) – 100$ where PSA is measured as the area of a stylized wing, outlined by the leading edge and the tips of all primary and secondary feathers (Figure 2.2). Using this measurement, wings lacking space between feathers would have a WP of 0, while wings with space between feathers would have a WP greater than 0 (Heers et al., 2011).

AR is commonly described as the ratio WS squared to surface area ($WS^2 / SA = AR$) (Drovetski, 1996; Heers et al., 2011; Marden, 1987; Winkler & Leisler, 1992), but WS data were unavailable for the majority of spread wing specimens so AR was estimated using L and SA instead. Measurements of SA and WL were used to calculate AR (dimensionless), defined here as the ratio of wing length squared to maximum wing chord ($L^2 / SA = AR$) (Videler, 2005).

C was calculated from measurements taken from photographs using ImageJ (also in a similar fashion to that in Heers et al. (2011)). Each wing was placed dorsal side up on a level tray and then photographed with a scale bar from an anterior position. Photographs were used to measure maximum wing depth (WD_m) and wing chord length at that point (WC_x). Thus, C (dimensionless) is defined here as maximum wing depth divided by chord length at that point ($WD_m / WC_x = C$) (Heers et al., 2011).

WS, in mm and body masses (M_b) in grams (g) were documented from museum records, although this information was generally unavailable for the specimens used in this study (Table 2.2). WL is typically defined as $WL = Mg_n / S$, where M is body mass in kilograms (kg), g_n is acceleration due to gravity (i.e., 9.81 m/s^2) and S is the total area of both wings and the body

between the wing's leading and trailing edges (Drovetski, 1996). Because I did not have S data for the majority of the specimens, I used a proxy measurement instead; measurements of M_b were used in concert with measurements of SA of single wings to calculate wing loading (WL), defined here as: M_b / SA= WL (Van den Berg & Rayner, 1995; Taylor et al., 2012). Statistical analyses of TM measurements were carried out using JMP v10 (SAS Institute Inc., Cary, NC, 1989-2013). A two-way analysis of variance (ANOVA) was performed on TM measurements (i.e., AR, C, P, and WL) with species, sex and their interaction as effects. Similarly, a one-way ANOVA was performed on species means of each TM measurement by habitat type. Although grouse inhabit a variety of temperate, arctic and mountainous areas, their habitat preferences can be generalized into four types: forest, prairie, sagebrush and tundra (Table 2.1) (Drovetski & Rohwer, 2006). While forest, tundra and prairie habitat groupings each included three or more species, only one sagebrush species was available, the Greater Sage-Grouse (*Centrocercus urophasianus*). Because I used species means to test for differences in wing shape among habitat types, I had to exclude 'sagebrush' from our ANOVAs, but show the intraspecific variation in the Greater Sage-Grouse for comparison in all of the scatter plots.

To measure variation in wingtip shape, I performed a PCA on $f_{\text{prim}}^{1\text{-}10}$ in a similar fashion to previous studies on avian wingtip shape (Marchetti et al., 1995; Wang et al., 2012; Senar et al., 1994; Neto et al., 2013; Borras et al., 1998; Pérez-Tris & Tellería, 2002). Principal components (PCs) were derived from a PCA on the correlation matrix of all $f_{\text{prim}}^{1\text{-}10}$ lengths (Marchetti et al., 1995; Chandler & Mulvihill, 1990; Senar et al., 1994). A two-way ANOVA was then performed on the first three principal components (PC1-3) with species, sex, and their

interaction as effects to test for differences among species and between sexes. I also used a one-way ANOVA of species averages of PC1-3 grouped by habitat type to test for differences in wingtip shape among prairie, tundra and forest habitats.

Landmark-based Geometric Morphometrics (GM)

To be included in our GM analysis, spread wings needed to be complete (i.e., no damage to primaries or secondary feathers of interest) and have all landmarks visible. Thus, some specimens that could be used for the linear measurements described above could not be used for the GM analysis. This reduced our overall sample size to 100 spread wing specimens representing 15 species (Table 2.1).

A set of 26 unambiguous landmarks was identified across all specimens (Figure 2.3). The distal tips of $f_{\text{prim}}^{1\text{-}10}$ and the first and last secondary feathers served as 'Type 1' landmarks in this study (LM: 10-1, 11-12 respectively). The inverse numbering of the primary feathers was chosen for ease of GM analysis. Each of these landmarks occurs along the edge of the specimen (i.e., locally defined) and can be precisely defined across all specimens and therefore satisfies the criteria of Bookstein's (1991) 'Type 1' landmarks. I also used 12 'Type 2' or 'semi-landmarks' (LM: 15-26), to capture the curvature of the leading edge of the wing. In order to do so, I employed the sliding semi-landmark method whereby semi-landmark points are slid along the outline of a curve retaining their relative positions along the leading edge across all specimens (Adams et al., 2004). Finally, I identified two 'Type 3' landmarks representing the extremes of structural boundaries (Bookstein, 1991); one directly opposite of the first secondary (LM: 13) along the leading edge and the other opposite of P8 along the inner wing (LM: 14). These

landmarks were necessary to adequately describe the overall shape of the wing, including leading and trailing edges as well as the proximal edge, along the midline of the bird.

Prior to landmark digitization, image backgrounds were removed using Photoshop CS6, leaving discrete images of each spread wing specimen with an associated scale bar. TPS (thin-plate spline) files were built for each species from these images using TPSutil (Rohlf, 2006). Landmarks and curves were digitized in tpsDig2 (Rohlf, 2006), while TPS curves were appended to landmarks using TPSutil (Rohlf, 2006). Each of the 26 landmarks used in this study were examined using MorphoJ (1.05a) (Klingenberg, 2011). A generalized Procrustes analysis (GPA) was performed for each species, permitting visual comparison of landmark configurations and calculations of CS (centroid size) as well as Procrustes coordinates. CS is equal to the square root of the sum squared distances of a set of landmarks to their centroid (Zelditch et al., 2012). It is untransformed, thereby preserving information about the size of the landmark configuration for each specimen (Klingenberg & Mcintyre, 1998). Procrustes coordinates were then used to generate the covariation matrices used in subsequent analyses. To examine differences in wing shape among species and genera, I first used a canonical variate analysis (CVA) of Procrustes coordinates as implemented in MorphoJ to generate canonical variates (CVs). This allowed us to examine the distribution of specimens within a multivariate morphospace. In addition, I used permutation tests of Procrustes distances among groups permuted 10,000 times to test for significant differences in wing shape among species and genera.

I also used MorphoJ to determine whether wing shape in grouse carries significant phylogenetic signal. This method permutes shape data across a phylogenetic tree to test the null hypothesis of no phylogenetic signal (Klingenberg, 2011; Klingenberg & Gidaszewski, 2010).

The null hypothesis is rejected if less than 5% of the permutations produce trees with lengths equal to or shorter than the length of the original tree, calculated by squared-change parsimony (Klingenberg & Gidaszewski, 2010). I tested for phylogenetic signal in grouse wing shape using Procrustes coordinates and a rooted tree that was weighted by branch length and permuted 10,000 times. The phylogeny, taken from Drovetski, (2002), was built using the mitochondrial control region (MC) and was chosen because it provided better resolution across all species. The phylogeny and branch lengths were reconstructed in Mesquite (Maddison & Maddison, 2011) and then a NEXUS file was imported into MorphoJ where subsequent analyses were carried out. Finally, to visualize changes in wing shape over the evolutionary history of grouse, I projected Drovetski's phylogeny (2002) into the morphometric space defined by the first two CVs (Lee & Frost, 2002; Oliveira et al., 2011; de la Hera, 2012).

RESULTS

ARs varied from 531.97 in the Hazel Grouse to 1482.85 in the Western Capercaillie (*Tetrao urogallus*) (Figure 2.4A). A two-way ANOVA yielded significant effects of species ($p = <0.0001$) and sex (<0.0001) on AR, and a statistically significant interaction effect was found ($p = 0.0088$) (Table 2.3). In all species but the Rock Ptarmigan, males had higher ARs than females and post-hoc Tukey HSD tests revealed that the only significant within species sex difference was among Black-billed Capercaillie, with males having larger ARs than females ($p = 0.0088$) (Table 2.3). Tukey HSD tests also indicated pairwise differences within *Bonasa* and *Lagopus*. Specifically, that the Hazel Grouse had significantly lower AR than its congener, the Ruffed

Grouse, while the Rock Ptarmigan had significantly larger AR than the Willow, but not White-tailed Ptarmigan, although this difference was driven largely by sex differences in AR among Rock Ptarmigan. A one-way ANOVA of mean AR for each species grouped according to habitat revealed no significant effect of habitat ($p = 0.669$, table 2.4, (Figure 2.5A). It should, however, be noted that this was based on species averages and there was significant variation between the capercaillie species and North American forest species. Specifically, three of the four *Tetrao* species had larger mean aspect ratios than the other forest, tundra and prairie species as well as the sage-brush species not included in this analysis of habitat type (Figure 2.5A).

Camber also varied among species, from 0.18 in the Caucasian Grouse to 0.28 in the Hazel Grouse (*Bonasa bonasia*) (Figure 2.4B). A two-way ANOVA of the effects of species and sex on camber revealed a significant effect of species ($p < 0.0001$), but no significant effects of sex ($p = 0.82$) or the interaction between species and sex ($p = 0.49$) (table 2.3). Post-hoc Tukey Honestly Significant Difference (HSD) tests revealed that there were several pair-wise species differences in C. For example, Rock Ptarmigan (*Lagopus mutus*) wings are significantly less cambered than other ptarmigan. Similarly, Ruffed Grouse wings are significantly less cambered than the Hazel Grouse and the Western Capercaillie (*Tetrao urogallus*) has significantly more cambered wings than other *Tetrao* species. No significant differences were detected among *Tympanuchus* species. A one-way ANOVA of mean camber of each species by habitat yielded no significant effect of habitat preference (table 2.4). An inspection of Figure 2.5B reveals that camber varied greatly among forest grouse (0.18- 0.28) and there is substantial overlap in camber across all habitat types.

The Western Capercaillie and the Caucasian Black Grouse could not be included in our analysis of porosity because there were an insufficient number of specimens with intact feathers to measure porosity. Among the remaining 14 species, average porosity ranged from 2.98 in the Black-billed Capercaillie (*Tetrao parvirostris*) to 5.67 in the Black Grouse (Figure 2.4C). A two-way ANOVA revealed a significant effect of species on porosity ($p = 0.0009$), but no significant effects of sex ($p = 0.49$) or species-sex interaction ($p = 0.60$) was found (table 2.3). Post-hoc tests indicated that the Black-billed Capercaillie (*Tetrao parvirostris*) had significantly lower porosity (i.e., a less heavily slotted wing) than Rock Ptarmigan and Black Grouse, but no other significant species differences were found. A one-way ANOVA of porosity by habitat yielded no significant effect of habitat preference ($p = 0.57$) (table 2.4; Figure 2.5C).

Due to a lack of specimen specific body mass data, wing loading could only be analyzed across both sexes for 12/16 species. Among these 12 species, wing loading ranged from 7.73 in the Hazel Grouse to 28.68 in the Greater Sage-Grouse (*Centrocercus urophasianus*). A two-way ANOVA yielded significant effects of species ($p = < 0.0001$), sex ($p = 0.0001$) and the interaction between the two ($p = <0.0001$) on wing loading (table 2.3). Overall, males had significantly higher WL than females. In both Greater Sage and Black Grouse, males have significantly higher WL than females, but no other significant sex differences were found within species. Post-hoc tests did yield a single pairwise difference among Hazel and Ruffed Grouse (*Bonasa* spp.) where the former had significantly lower wing loading. In contrast, no significant difference in WL was detected among *Tympanuchus* spp., *Dendragapus* spp. or *Lagopus* spp. A one-way ANOVA of WL with species grouped by habitat revealed no significant effect of

habitat preference (table 2.4). As with C, forest species varied the most in WL (7.73 – 26.39), whereas tundra and prairie species differed only marginally (Figure 2.5D).

Principal Component Analysis

The first PC explained 93.04% of the total variance among measurements of $f_{prim}^{1\text{-}10}$ whereas PC2 and PC3 accounted for 2.84% and 1.47% of the total variance respectively (Figure 2.6). All feather lengths were strongly and positively loaded on PC1 (table 2.5), which suggests that it reflects overall size (Förschler & Bairlein, 2011; Borras et al., 1998; de la Hera et al, 2012; Kralj et al., 2010; Maggini et al., 2013). This is further supported by the distribution of species in our scatterplots of PC1; the largest species (*Tetrao* and *Centrocercus*) have the largest PC1 scores and the smallest species (*Bonasa bonasia* and *Lagopus leucurus*) have the smallest PC1 scores (Figure 2.6A; 2.6C). In contrast, factor loadings on PC2 and PC3 were mixed (i.e., positive and negative loadings), and appear to reflect differences in wing shape (Marchetti et al., 1995; Vanhooydonck et al., 2009; Senar et al., 1994). More specifically, the distal primaries ($f_{prim}^{6\text{-}10}$) were positively loaded onto PC2 whereas the proximal primaries ($f_{prim}^{1\text{-}5}$) had negative loadings (table 2.5). Thus, relatively longer, pointed wings will have larger PC2 scores due to lengthening $f_{prim}^{6\text{-}10}$ and shortening of $f_{prim}^{1\text{-}5}$. The loadings on PC3 were also mixed; of the loadings > 0.1, f_{prim}^{10} was positively loaded and $f_{prim}^{5\text{-}7}$ were negatively on PC3 (table 2.5).

A two-way ANOVA of PC1 yielded significant effects of species, sex and their interaction. Post-hoc tests supported the association between PC1 and overall body size. For example, Black-Billed Capercaillie and Greater Sage-Grouse had significantly larger PC1 scores than all other species, and, in both species, male PC1 scores were significantly larger than

females. *Tympanuchus* species did not differ significantly from one another or exhibit sex differences, but they did have significantly larger PC1 scores than *Lagopus, Bonasa,* and *Falcipennis* species. Within *Lagopus* and *Bonasa*, there were no significant species differences and sex differences were detected only within Willow Ptarmigan.

I also detected significant effects of species, sex and their interaction on PC2 (table 2.3). Across all species, males tended to have higher PC2 scores and therefore more pointed wings than females with the exceptions of the Willow Ptarmigan, Greater Sage and Sooty Grouse. In terms of species differences, post-hoc tests revealed that *Tympanuchus* and *Lagopus* species had significantly larger PC2 scores than *Bonasa*, *Dendragapus*, *Falcipennis* and *Tetrao* species. That is, the ptarmigan and prairie species had relatively longer and more pointed wings than forest species. PC3 did not, however, differ significantly between sexes or among species.

A one-way ANOVA of species PC scores and habitat revealed a significant effect of habitat preference on PC2, but not on PC1 or PC3 (table 2.4). Tundra and prairie species did not differ significantly from one another, but both tundra and prairie species had significantly larger PC2 scores than forest species (Figure 2.6B), as suggested by our analyses across all species. Thus, forest species have significantly less pointed wings than prairie and tundra species.

Geometric Morphometrics

Mean centroid size ranged from 293.21 in the White-tailed Ptarmigan to 672.30 in the Greater Sage-Grouse (Figure 2.7). A two-way ANOVA yielded significant effects of species and sex on centroid size, but no significant interaction effect (table 2.3). Males had significantly larger centroids than females, but intraspecific sex differences were only significant within

Greater Sage-Grouse. Post-hoc tests revealed that Greater Sage-Grouse have significantly larger centroids than all other species. *Tetrao*, *Tympanuchus* and *Bonasa* species did not differ significantly within their own genera, but White-tailed Ptarmigan had a significantly smaller centroid than Willow Ptarmigan.

Our canonical variate analysis of Procrustes coordinates yielded six CVs that explained 100% of interspecific variation in wing shape (table 2.6). The first CV accounted for 57.62% of the total variance among specimens while CV2 and CV3 explained 19.01% and 9.47% of total variance respectively (table 2.6). A scatter plot of CV1 against CV2 shows a clear separation of most genera (Figure 2.8A). Note that only genera are shown here for clarity, but comparisons were also made across species. With the notable exception of the Spruce Grouse, all other forest species had positive CV1 scores. There was also a clear separation along the CV2 axis with *Tympanuchus*, *Centrocercus* and *Tetrao* having positive CV2 scores and all other genera having negative CV2 scores (Figure 2.8 A; B). An examination of CV1 and CV3 revealed comparable CV3 scores among most genera except for *Centrocercus* and *Dendragapus*, which represented the upper and lower limits of CV3 scores respectively (Figure 2.8C).

Permutation tests of Procrustes coordinates across species yielded many significant pairwise differences (table 2.7). *Bonasa* differed from most other genera and there were significant differences among *Lagopus*, *Tympanuchus* and *Tetrao* species. *Centrocercus* was also significantly different from most of the ptarmigan and prairie chickens. Three species did not, however, significantly differ from most other grouse species: both *Dendragapus* species, Spruce Grouse and the Western Capercaillie. In the scatterplot, there was a lot of variation across *Dendragapus* and *Tetrao* specimens (Figure 2.8). The Spruce Grouse, however, appears

to occupy a unique position in morphospace that is intermediate between ptarmigan and smaller bodied forest grouse (e.g., *Bonasa*, *Dendragapus*). Finally, in terms of variation within genera, no significant differences were found within *Tetrao*, *Lagopus* or *Dendragapus*. In fact, the only significant pairwise differences detected within a genus were between Ruffed and Hazel Grouse and between the Sharp-tailed Grouse and Greater Prairie Chicken.

A one-way ANOVA of the effect of habitat type on mean CV1-3 scores detected significant differences among habitats for CV1 and CV2, but not CV3 (table 2.4). Forest species had significantly larger CV1 scores than prairie and tundra species, but there was no significant difference between prairie and tundra species. As mentioned previously, this differentiation is apparent in our scatter plots (Figure 2.8) and parallels the differences in wing pointedness between forest and tundra/prairie species (Figure 2.6B). In the case of CV2, tundra and forest species did not differ from each other and, when grouped by habitat, both had significantly lower mean CV2 scores than the prairie species. This is in contrast to our analysis of CV1 and suggests that CV2 may represent wing shape changes related to body size (Figure 2.8C).

As expected from the evolutionary history of grouse (Drovetski, 2002; 2003; Drovetski & Rohwer, 2006) and similarities in wing shape within most genera (see above), permutation tests yielded a significant phylogenetic signal in our data set (both weighted and unweighted models $P < 0.001$). Thus, wing shape is significantly affected by phylogenetic relatedness. To visualize evolutionary changes in wing shape with respect to phylogenetic relatedness, I projected Drovetski's (2002) molecular phylogeny into multivariate space using mean species CV1-2 scores, which represents the majority of variation within our data (76.63% of total variance). As shown in Figure 2.9, the direction and magnitude of evolutionary changes in wing shape vary

throughout the phylogenetic history of grouse species. For example, *Dendragapus* species are closely related to *Tympanuchus* and *Centrocercus*, but have a wing shape more similar to that of the basal *Bonasa* species. Similarly, *Centrocercus* have evolved a wing shape more similar to *Tetrao* species even though they are not sister-genera. This could reflect, in part, shared similarities in wing shape associated with large body size as both *Centrocercus* and *Tetrao* are by far the largest taxa in our dataset. Last, *Falcipennis* differs markedly from its closest relatives, the *Tetrao* species, and occupies a unique position in morphospace with a wing shape that is intermediate between forest species (*Dendragapus*, *Bonasa*) and ptarmigan.

DISCUSSION

Wing shape and size varies tremendously among and within avian taxa (Norberg, 1990; Marchetti et al., 1995; Copete et al., 1999; Pérez-Tris & Tellería, 2001; Lockwood et al., 1998; Tobalske, 1996; Stiles et al., 2005; Suryan et al., 2008; Rayner, 1988; Mönkkonen, 1995; ; Lee et al., 2009; Livezey, 1988), and, as I have shown here, this also holds true for grouse. As detailed in our TM and GM analyses, some aspects of wing shape in grouse vary among habitats in a similar fashion to other birds (Marchetti et al., 1995; Gamauf & Preleuthner, 1998; Winkler & Leisler, 1992; Müller & Patone, 1998, Livezey, 1988) and wing shape varied significantly among and within genera in multivariate space. Although I sampled most extant grouse species, our sample sizes were limited for several species (table 2.1) and I were unable to access spread wing specimens of Siberian Grouse (*Falcipennis falcipennis*), Gunnison's Sage-Grouse (*Centrocercus minimus*) and Chinese Grouse (*Bonasa sewerzowi*). The addition of these species could potentially affect our analyses, particularly the distribution of genera in multivariate space

based on GM (Figure 2.9). Indeed, the inclusion of the Siberian Grouse could yield insight into the apparently unique wing shape of Spruce Grouse (see below).

It should also be noted that our analyses reflect only those components of wing shape that can be readily derived from static, spread-wing preparations. All of the grouse wings were prepared in a standardized way by the staff at the Burke Museum, greatly limiting the potential effects of variation in preparation on our results. Nevertheless, the avian wing is a dynamic structure and it can vary in shape depending on what a bird is doing. For example, the shape of the leading edge differs drastically when a bird is in flight compared with when the wing folded at rest. Similarly, the trailing edge of the wing may change shape in flight as a bird articulates its flight feathers to change course or correct for wind (Lentink et al., 2007). In addition to shape changes associated with behaviour, the shape of the wing can vary according to age and moulting stage. For example, feather length is highly variable over the lifetime of birds (Dial et al., 2006; Heers et al., 2011) and can vary intraspecifically with age, sex and season, between geographical regions and among populations with different foraging strategies and migratory patterns (Marchetti et al., 1995; Chandler & Mulvihill, 1990; Vanhooydonck, 2009; Peirò, 2003; Romero et al., 2005; Winkler & Leisler, 1992). Despite all of these sources of potential variation in our data, there was generally less variation within species than among species, which strongly suggests that the values I report are representative of each species. Details of some of these differences are discussed in detail below.

Traditional Morphometrics

AR is a determining factor in the cost of flight (Drovetski, 1996). For example, the same speed of horizontal flight can typically be obtained using less energy per unit distance by birds of the same size with high AR wings compared to low AR wings (Rayner, 1988). AR varied significantly among grouse species, between the sexes and significant interaction between the two was found (tables 2.3, 2.4). Although I used a proxy measurement of AR, the overall pattern I observed across species was similar to that found in other studies of wing shape (Lockwood et al., 1998; Rayner, 1988; Drovetski, 1996). For example, males had significantly larger AR than females, which likely stems from sexual dimorphism in body size within Tetraoninae (Norberg, 1990; Johnsgard, 1983; Heers et al., 2011). Further, species differences also appeared to largely reflect variation in body size, and to a lesser extent habitat preference and movement patterns. For example, the capercaillie are significantly larger than any of the North American forest grouse, and although both groups generally inhabit areas of dense vegetation, capercaillie have significantly larger mean ARs, even when compared to prairie and tundra species. Relatively short, broad wings, of low AR are well suited to fly short distances and facilitate vertical, rather than horizontal, flight to escape predators, and are characteristic of the North American forest species, none of which differed significantly in AR. However, due to the large body size of capercaillie, their ARs remain comparatively high throughout Tetraoninae. This is different from other avian taxa whereby species occurring in forested habitats generally have lower AR than those in open habitats or those that engage in migration (Förschler & Bairlein, 2011; Vanhooydonck et al., 2009; Desrochers, 2010). This pattern is evident again among the ptarmigan, which move over larger distances than the other species (Cade & Hoffman, 1993; Herzog & Keppie, 1980; Schroeder & Braun, 1993; Irving et al., 1967; Hoffman & Braun, 1975;

Stokkan, 1992) but did not differ significantly in mean AR from forest species in either of these analyses (Figure 2.5A).

C is also a key component of wing aerodynamics (Warrick et al., 2005), but in contrast to AR, C did not vary by habitat type or sex (table 2.3; 2.4) and only a few pairwise differences among species were detected. Grouse are largely terrestrial and are not as heavily reliant on flight as other birds (Johnsgard, 1983; Bergerud & Gratson, 1988). Even species that feed in trees (i.e., most forest grouse) generally move from branch to branch by walking or hopping (Johnsgard, 1983; Hjorth, 1970). As a result of this reliance on walking, C might not be subject to change as much as in other avian taxa that are heavily reliant on flight for prey capture or other behaviors. This would at least partially explain the overall lack of significant variation in C across grouse species. It should, however, also be noted that C is a difficult measurement to obtain accurately. Here, I followed the technique outlined in Heers et al. (2011), which is a relatively crude measurement. In addition, I relied on spread wing specimens in which C could be varying among specimens due to inconsistencies in the drying process. Thus, the lack of variation in C in our present study could also reflect limitations in our measurement. An alternative means of measuring C that could prove to be more accurate is 3D laser imaging. This would have provided an accurate measure of the C gradient along the length of the wing (Liu et al., 2006), which could yield differences among species. Future studies of interspecific variation in avian wing shape should use multiple methods to measure C in order to determine whether C varies in a predictable fashion with body size, habitat preference and other variables.

Similar to C, P varied only marginally among species and neither sex nor habitat differences were found (table 2.3). Wing P influences lift-to-drag ratio regardless of the species, and varies intraspecifically by season and age relative to moulting stage (Norberg, 1990; Rayner, 1988; Müller &Patone, 1998; Heers et al., 2011; Tucker, 1991; Hendenström & Sunada). All grouse, and indeed most galliforms, have slotted wings and deep trailing edge notches (Parsons et al., 2003) that increase the degree of P and, based on our analyses, do so in a relatively uniform fashion. As discussed previously, grouse are primarily terrestrial and use flight to escape predation or, in the case of forest species, to forage in trees (Van der Niet et al., 2010; Ginter et al., 2012). In a similar fashion to C then, there may be little need for P to vary significantly among grouse species.

WL varied among species, between the sexes and an interaction between the two was detected (table 2.3). Although I used a proxy measurement of WL, the overall pattern I observed across species was similar to that found in other studies of galliforms (Dial et al., 2006; Drovetski, 1996; Tobalske & Dial, 2000). For example, males typically had greater WL than females and WL tended to increase with body size. However, in contrast to our prediction, there were no significant differences in WL across habitat types (table 2.4). WL is associated with manoeuvrability, flight speed, and the amount of energy used during flight, all features that are associated with habitat preference (Norberg, 1990; Rayner, 1988; Hails, 1979; Nudds & Bryant, 2000). The lack of such a difference across habitat types in the current study could be because WL is largely a function of body size in grouse (Norberg, 1990; Suryan et al., 2008). Body size varies significantly among grouse species and this is most notable among forest species, which vary from around 300-4,000g (Dunning, 1993). Although I did not specifically test for a scaling

relationship between WL and body size, wing loading was lowest in the smallest species (*B. bonasia*, *L. leucurus*) and greatest in the largest species (*C. urophasianus*, *T. tetrix*) for which I had data, supporting our contention that wing loading at least partially reflects body size.

The final component of our TM analyses was the PCA of primary feather lengths. In contrast to all of the other TM measurements that are bivariate measurements of gross variation in wing shape, the PCA is meant to reflect multivariate variation in wingtip shape (Förschler & Bairlein, 2011; Senar et al., 1994; Borras et al., 1998; de la Hera et al., 2012; Kralj et al., 2010; Maggini et al., 2013). As with other studies that have used PCA on feather lengths, PC1 primarily reflects size variation across species. In our analyses, this was clearly shown by both the factor loadings (table 2.5) and distribution of species along the PC1 axis (Figures 2.6). PC2 and PC3, however, reflect components of wing shape (tables 2.3, 2.4) (Förschler & Bairlein, 2011; Senar et al., 1994; Borras et al., 1998; de la Hera et al., 2012; Kralj et al., 2010; Maggini et al., 2013). As with similar studies of other avian taxa, PC2 reflected wing pointedness and our analyses showed that wing pointedness varies among sexes, species and habitat types. Across species, the forest grouse had significantly lower PC2 scores that ptarmigan or *Tympanuchus* species. In other words, the ptarmigan and *Tympanuchus* species had significantly more pointed wingtips than forest species. Analyzing species means with respect to habitat corroborated this finding; prairie and tundra species have significantly more pointed wings than forest grouse. The more rounded wings of forest species would enable greater manoeuvrability when flying through dense vegetation (Johnsgard, 1983; Théry, 1997) whereas the more pointed wings of tundra and prairie species likely assist in short distance migrations (Marchetti et al., 1995; Johnsgard, 1983) and open-field predator avoidance strategies respectively (Johnsgard, 1983; van den Hout et al.,

2010). Thus, our PCA of primary feather length indicates a habitat specific gradient of wing shape that varies among grouse in a similar manner as in other birds (Förschler & Bairlein, 2011; Senar et al., 1994; Borras et al., 1998; de la Hera et al., 2012; Kralj et al., 2010; Maggini et al., 2013).

Geometric Morphometrics of Wing Shape

GM analysis is increasingly used to examine shape variation in biological structures, but there have been relatively few attempts to apply this method to avian wing and feather shape (Sheets et al., 2006; Brewer & Hertel, 2007; Bendoy et al., 2010; Albutra et al., 2011; Moneva et al., 2011). Our use of GM to analyze the morphology of the avian wing is among the first of its kind (i.e., combined use of landmarks and semi-landmarks) and offers a simple and convenient way to quantify and compare fine scale morphological differences in the wing shape of birds from 2D images. Because GM is based on landmark configurations, it samples wing shape broadly, unlike the uni and bivariate measurements that characterize the majority of TM measurements. Additionally, GM only required imaging and landmark placement as opposed to time consuming measurements taken by hand, and because of the phylogenetic and statistical tools built into MorphoJ (and other GM software), GM analysis was by comparison a much more streamlined process than the TM methods used here. That said, our PCA of *f*prime $^{1\text{-}10}$ was also informative and its results paralleled our GM analysis in a number of respects including species differences and habitat associations. Thus, in the case of grouse both GM and TM measurements yielded similar results. The advantages of the GM analysis in this context, however, was that I

could map shape changes readily on top of a phylogeny and some species differences were revealed that were not readily apparent using TM measurements.

Based on my permutation test of Procrustes coordinates, wing shape has a significant phylogenetic signal. Most of the speciation events in grouse occurred in forested habitats with the other three habitat types occupied by individual genera. Thus, the prairie (*Tympanuchus*), tundra (*Lagopus*) and sagebrush (*Centrocercus*) habitats are occupied by a single genus each. Given that wing shape varies with habitat (Norberg, 1990; Desrochers, 2010; Alistair, 2005) and this pattern of a single genus occupying each of the non-forest habitats, the fact that wing shape is significantly affected by phylogeny is unsurprising. Phylogenetic history is not, however, the only factor that affects wing shape evolution in grouse. Body size, for example, appears to have played some role in evolution of wing shape in *Centrocercus* and *Tetrao*. These two genera live in completely different habitats and are not closely related to one another (Bookstein et al., 2002), but occupy the same morphospace (Figure 2.9) and are the largest species in our dataset with body masses of 900-5,000 g (Dunning, 1993). Although this is correlative evidence, it suggests that at least some aspects of wing shape appear to be dependent on body size.

Further, our GM analysis of wing shape also shows a clear separation of grouse genera that corresponds well with their respective habitat types (Figure 2.8A; 2.8B). CVA of Procrustes coordinates provided a conservative estimate of the among group variance scaled by the inverse of the within-group variation that is embodied in the wing shape of grouse (table 2.6). Two of the factors produced (CV1+2) were significantly associated with habitat type, and significant differences were detected among species as determined by permutation tests of Procrustes distances (table 2.4; 2.7; 2.8). In other words, not only did I find significant interspecific

differences in wing shape, wing shape also varied among habitat types. For example, there was significant variation within and among genera and the Spruce Grouse had a unique wing shape for a forest dwelling species (Figure 2.8).

One of our predictions was that the Ruffed Grouse would have a different wing shape from other forest grouse because of its reliance on the drumming display as part of its courtship (Johnsgard, 1983; Atwater & Schnell, 1989; Hjorth, 1970). Although the Ruffed Grouse does have a significantly different wing shape from its sister species, the Hazel Grouse, as well as *Lagopus, Tympanuchus* and *Tetrao* spp., it did not differ significantly from *Dendragapus* spp, Spruce Grouse or the Greater Sage-Grouse. Thus, although I predicted that the Ruffed Grouse would be different from all other forest grouse species, they differed from some, but not others. The Ruffed Grouse is the only species that 'drums', but most grouse engage in some form of flutter jump or other wing-based display (e.g., wing claps), including several of the species that did not differ significantly from the Ruffed Grouse in our analyses. For example, *Centrocercus*, *Dendragapus* and *Falcipennis* spp. use their wings in a variety of courtship behaviours including display and drumming flights, drumming jumps, wing claps, and drumming-like asymmetrical striking during territorial interactions (Hjorth, 1970; Andreev et al., 2001; Pellis et al., 2013). Although I cannot conclude that differences in its wing shape are specialized to produce the drumming display, this does not necessarily mean that the behaviour is not associated with wing morphology or feather shape at some level. For example, hummingbird feathers that differ in shape produce different frequencies and modes of vibration in wind tunnel experiments (Clark et al., 2011). Similarly, Club-winged Manakins (*Machaeropterus deliciosus*) have enlarged, club shaped rachi that stridulate to produce various sounds used in their courtship display (Bostwick

& Prum, 2005). Similar wing and feather specializations related to acoustic communicative signals may also be present grouse, but this has remained untested to date.

Perhaps the most unexpected result from our GM analyses was that Spruce Grouse have a markedly different wing shape than that predicted by phylogeny or habitat. The Spruce Grouse wing differs in shape from *Tetrao*, its sister genus, and the other forest species (i.e., *Dendragapus*, *Bonasa*). Instead, the wing shape of Spruce Grouse is intermediate between that of forest species (*Dendragapus* spp.) and the ptarmigan. However, unlike ptarmigan, Spruce Grouse are generally not found in open areas, like tundra or alpine habitats, do not undergo large migratory movements and rarely fly long distances. In fact, Spruce Grouse tend to be far more arboreal than many other forest grouse species (Johnsgard, 1983). Why Spruce Grouse have evolved such a unique wing shape compared to other forest species is unclear. Although the Franklin's subspecies (*Falcipennis canadensis franklinii*) of Spruce Grouse does produce a wing clap display during courtship (Hjorth, 1970), all of the Spruce Grouse used in the GM component of this study were collected in Western Alaska, far north of the known distributional limits of the Franklin's Grouse subspecies (Barry & Tallmon, 2010). As indicated above, I was unable to examine the Siberian Grouse, so it remains unclear whether this wing shape characterizes all Spruce Grouse subspecies or even both *Falcipennis* species. Regardless of variation within or among *Falcipennis* species, the divergent wing shape revealed by our GM analysis warrants further investigation.

Conclusions

Wing shape varies greatly among grouse species and habitat types in a similar fashion to other birds (Norberg, 1990; Stiles et al., 2005; Rayer, 1988; Gamauf et al., 1998) and this variation was apparent using TM, including our PCA of *f*prime$^{1\text{-}10}$, and GM methods. However, across these analyses, the wing shape of the Ruffed Grouse did not vary from other grouse species in a manner commensurate to production of the drumming display. That said, our GM analyses did identify species differences that were not apparent using TM measurements and I therefore recommend that future studies incorporate both TM and GM approaches to better examine species, and even population level, differences in wing shape. Through the combination of methods, such as that provided herein, future studies will be able to develop a more integrative view of avian wing evolution and diversification.

CHAPTER THREE

STUDY TWO: SEASONAL VARIATION IN THE BRAINS OF RUFFED GROUSE (*Bonasa umbellus*)

INTRODUCTION

Sexual dimorphism and seasonal plasticity of nuclei within the song system are characteristic of the brains of songbirds (Tramontin & Brenowitz, 2000). Both sex differences and seasonal plasticity occur at a number of anatomical levels: brain region volume, cell number and density, soma size, synapse number and dendritic branching (Brenowitz, 2013; Tramontin 1998; Freas et al., 2013; Tramontin & Brenowitz, 2000). Among male songbirds, volumes of the various song control nuclei (e.g., HVC, robust nucleus of the arcopallium (RA), Area X, nXIIts) can increase as much 200% during the breeding season across species while the number of HVC neurons can rise from approximately 150,000 to 250,000 between the non-breeding and breeding seasons (Brenowitz, 2013; Tramontin & Brenowitz, 2000). Similarly, Area X can undergo seasonal changes in volume as large as 75% due to increases in soma size and cell spacing (Thompson & Brenowitz, 2005) while the number of synapses on RA neurons can increase roughly 50% under spring like-conditions (DeVoogd et al., 1985). This neuroanatomical variation is correlated with song complexity (i.e., syllable number, repertoire size) and amount of singing behavior such that nuclei volume, the number, size and density of cells, and connectivity of song control nuclei vary seasonally among and within species, between the sexes and among

individuals relative to singing behaviour (DeVoogd, 2004; Ward et al., 1998; Brenowitz & Beecher, 2005; Gahr et al., 2008).

Although examining seasonal plasticity in the song system has provided much insight into the mechanisms and processes underlying seasonal variation in the brain and corresponding differences in the behaviour of birds, there are very few studies that have examined seasonal plasticity in the brains of birds outside of oscine songbirds. Further, it is unclear if seasonal plasticity in the brain accompanies non-vocal components of courtship behavior, which are diverse and occur in many avian lineages. For example, breeding male Palm Cockatoos (*Probosciger aterrimus*) use sticks or hard fruit to rhythmically 'drum' on prospective nest-hollows, behaviour thought to indicate both mate and nesting territory quality (Murphy et al., 2003). Conversely, in place of vocalizations during courtship, both male and female Oriental White Storks (*Ciconnia boyciana*) clap their mandibles to produce a clattering sound which differs between the sexes (Eda-Fujiwara et al., 2004). Despite these and other examples, non-vocal acoustic courtship behaviours in most lineages (i.e., Apodiformes, Caprimulgiformes, Charadriiformes, Galliformes, Passeriformes, Piciformes) involve the production of mechanical sounds using feathers (Bostwick, 2006). For instance, male Magnificent Riflebirds (*Ptiloris magnificus*) perform a highly structured acoustic wing fanning display after attracting females by vocalizing (Frith & Cooper, 1996). Similarly, the tail feathers of Anna's Hummingbird (*Calypte anna*) produce a 'chirp' or 'squeak' during the descent of their display flights (Clark & Feo, 2008). Despite this breadth of acoustic courtship behaviour across birds, the vast majority of neuroanatomical studies on the subject are focused on the song of oscine songbirds.

One notable exception to the focus on song and oscines is the work by Schlinger and colleagues on the courtship display of the Golden-collared Manakin (*Manacus vitellinus*), a suboscine species found in Central and South America (Schlinger et al., 2013). Suboscines are closely related to oscine songbirds (Chesser, 2004), but differ from them with respect to their neuroanatomy and vocalizations. Instead of complex, learned vocalizations, manakin (Pipridae) males engage in elaborate, acrobatic wing-snapping displays exclusively during the breeding season. Similar to the courtship behaviour of oscine songbirds, these displays include complex acoustic and visual features that are thought to be a result of intense male competition for mates. The Golden-collared Manakin is particularly well studied and has a wide range of anatomical and physiological specializations that enable them to produce their wing snapping display (Schlinger, 2013). For example, their wing muscles, and the spinal motor neurons that mediate their control, express relatively high levels androgen receptors (Schlinger, 2013; Fusani et al., in press). Golden-collared Manakins also express significant levels of androgen receptors in the arcopallium (Schlinger et al., 2013) and males have larger relative arcopallium volumes than females (Day et al., 2011). The arcopallium is a major source of premotor output from the TELE (Reiner et al., 2005; Shanahan et al., 2013) and combined with this data suggests that the arcopallium likely plays a key role in performing the complicated wing movements involved in their display. The extent to which there are seasonal changes in the brains of male manakins that parallel those of oscines, however, remains unknown. Similarly, whether seasonal plasticity or sex differences in brain regions related to non-vocal courtship in species outside of oscines and suboscines has not, to our knowledge, been investigated.

Although many non-passerine species produce non-vocal courtship displays, one species of particular interest is the Ruffed Grouse. Ruffed Grouse are residents of North American woodlands, occurring in subarctic areas from the Pacific Northwest across Canada to Labrador, with populations extending south into both the Eastern and Western United States (Hjorth, 1970; Johnsgard, 1983) (Figure 1.3). Unlike most other birds, male Ruffed Grouse do not vocalize during courtship, but rather are reliant upon a mechanical wingbeat display known as "drumming" for the acoustic component of their courtship display (Figure 1.4A-D). Drumming is typically performed atop a platform (e.g., fallen tree, stump or stone) from a stationary position and consists of 40-50 wingbeat movements performed over 8-11 seconds, with most of the energy produced concentrated under 100 Hz (Garcia et al., 2012). Other grouse species use wingbeats and "flutter jumps" for courtship, but always in conjunction with vocalizations (Hjorth, 1970; Johnsgard, 1983). Drumming is by comparison a much more frequent, protracted and elaborate behaviour, performed in the absence of any vocalization whatsoever (Hjorth, 1970; Johnsgard, 1983). This unique suite of traits renders the Ruffed Grouse dependent upon drumming, and to a lesser extent visual displays, during courtship, and makes it an ideal model species in which to examine the neural mechanisms that underlie the production of non-vocal courtship behaviours.

As with other courtship behaviour, drumming varies seasonally with males barely drumming or not drumming at all in the fall and winter months. Here, I test whether there are corresponding seasonal changes in the brains of male Ruffed Grouse. More specifically, I focus upon motor regions that are likely involved in producing the drumming display (arcopallium, Cb, SPC) in comparison with non-motor regions of the TELE and thalamus as well as WB and TELE

volumes. The arcopallium is of particular interest because it is a premotor area implicated in the production of most motor behaviour (Shanahan et al., 2013; Reiner et al., 2005; Feenders et al., 2008) and, as mentioned above, courtship behaviour in manakins (Schlinger, 2013; Day et al., 2011). In addition, the RA nucleus within the oscine song system is nested within the A and undergoes seasonal changes in neuron size, spacing, dendritic arborization and synaptogenesis in relation to song production during the breeding season (Tramontin and Brenowitz, 2000). I therefore predict that the arcopallium will be larger in spring males that are actively drumming daily than fall males that are not drumming. I similarly predicted that the SPC would vary with season because of the roles that the basal ganglia play in motor coordination and execution (Kuenzel et al., 2012). The Cb, however, is not known for undergoing seasonal plasticity in size, despite the presence of androgen receptors in the cerebellar nuclei and Purkinje cells (Mirzatoni et al., 2010; London et al., 2006), so I predicted no seasonal difference in cerebellar size. I also measured the HF because of its role in spatial memory and differences between males and females in home-range size (Maxson, 1977; 1978; Whitaker et al., 2007). For example, females occupy larger home-ranges than adult males throughout the year, but this sex difference is even greater in the breeding season when males reduce their home range size to maintain and defend drumming sites and females move over larger distances among drumming sites (Maxson, 1977; 1978; Whitaker et al., 2007). Therefore, our final prediction was that males collected in the breeding season would have hypotrophied HFs when compared to both males and females collected in the fall. These predictions were tested by using unbiased stereology to measure brain region volumes of grouse collected throughout the year. Although I did not collect females

during the breeding season, I speculate that this reverse sexual dimorphism would persist throughout the year relative to differences in home range size (Whitaker et al., 2007).

METHODS

Specimens

Male Ruffed Grouse (n=6) were tracked to drumming logs in their natural habitat (Buck Lake, Alberta, Canada, 52.97°N, 114.77°W) during the breeding season (April-May) between 2010-2012. Following several days of observations and/or audio recordings, a mirror trap (Gullion, 1965) was secured to the drumming log. Grouse that were trapped were then weighed and euthanized by intraperitoneal injection of sodium pentobarbital (100 mg/kg). Although some methods have proven effective for trapping female Ruffed Grouse during the breeding season, (Maxson, 1977) they are extremely difficult to trap (Atwater and Schnell 1989; Bump et al. 1947) and I have been unable to trap or collect any breeding season females over the past five years. Male (n=8) and female (n= 6) Ruffed Grouse were obtained from hunters elsewhere in Alberta during the non-breeding season (September-November) between 2012 and 2013 and the sex of each bird was confirmed by dissection. All of the specimens (n = 20) were decapitated and the head immersion fixed in 4% paraformaldehyde (PFA) in a phosphate buffered saline (PBS) solution within minutes of collection in the field. Immersion fixation was used in all instances to ensure that no differences arose from using perfusion for some birds and immersion fixation for others. In addition, unfixed muscle samples were required for a parallel study of

seasonal changes in muscle fiber histochemistry (Welch, Malik and Iwaniuk, in prep) and therefore systemic perfusions could not be used for any of the birds collected.

Histology and volumetric measurements

Each brain was carefully dissected out of the skull following several weeks of fixation and placed in 30% sucrose in 0.01 M PBS until it sunk in order to cryoprotect the tissue. The brains were embedded in gelatin and sectioned in the coronal plane at a thickness of 40µm on a freezing stage microtome. All sections were collected and stored in PBS with 0.01% sodium azide and every second section (i.e., a 1:2 series) was mounted onto gelatinized slides. The only exception to this sampling interval was a single male that was mounted in 1:6 series (RUGR144) for a previous study (Corfield et al., 2013). The slides were air dried, stained with thionin for Nissl substance, coverslipped with Permount (Fisher Scientific) and allowed to dry.

To obtain TELE, Cb and post-processing WB volumes, sections were imaged with a digital camera and then the area of each region was outlined using Image J. The volume of each region was calculated by multiplying its total area by section thickness (40µm) and then by the sampling interval. For the TELE, I measured both hemispheres of every 4th section (i.e., every 160 µm) while Cb and WB volumes were measured from every 8th section (i.e., every 320 µm). For the smaller brain regions of interest, I used unbiased stereology to estimate the volumes of the A, SPC, HF, M, and nRt (Figure 3.1 A-E) using a Zeiss Axio Imager MT microscope (Carl Zeiss, MicroImaging GmBH, Germany) and the Cavalieri estimator, as implemented in StereoInvestigator (Microbrightfield Inc., Colchester, VT, USA). For each structure, I used the 2.5x objective, and the size of counting frame varied among structures. An evaluation interval of

8 (i.e., every 320 µm) was used for all structures except in the case of the 1:6 series mentioned above. In this instance, an evaluation interval of six (i.e., every 240 µm) was used for each structure. Cytoarchitectural boundaries for each region are provided below and coefficients of error (CE) and other stereological parameters for all measurements shown in table 3.1.

Cytoarchitectural borders

The boundaries of the TELE, Cb and all other structures measured were based on the brain atlas of Karten and Hodos (1967), using the revised nomenclature of Reiner et al. 2004 (Figure 3.2A-C). In the Ruffed Grouse, the arcopallium begins lateral to the SPC and more caudally, it is bordered by the nucleus taeniae (Nt). The arcopallium terminates as it runs alongside the piriform cortex, which I used as its lateral boundary (Figure 3.2A). Although the arcopallium is comprised of numerous subregions (Shanahan et al., 2013), these were not readily discernible in our tissue and therefore I did not measure the subregions individually. The HF in birds is bordered by the midline, the ventricle, the brain surface and by apical regions of the hyperpallium (HA). In the Ruffed Grouse, the boundary of the hippocampus proper (HP) was not clearly delineated throughout the caudal extent of the telencephalon by changes in cytoarchitecture with our Nissl stain. However, the lateral boundary where the area parahippocampalis meets the lateral corticoid area nearest the lateral aspects of the ventricle was clearly defined throughout (Figure 3.2A). Therefore, our measurement of the HF includes both the hippocampus proper and area parahippocampalis, in accordance with most volumetric studies (Sherry et al., 1989; Ward et al., 2012; Corfield et al., 2012; Abott et al., 1999). The SPC is first evident in a relatively rostral position bordered medially by the ventricle. More caudally, it is

encapsulated by the lamina medullaris dorsalis and bordered by the entopallium, terminating near dorsal aspects of the A (Figure 3.2A). The rostral extent of the M is clearly bordered by the cortex prepiriformis and moving caudally, the ventricle forms its medial boundary. As the M extends throughout the telencephalon, expanding laterally, it is bordered by the HA and hippocampus proper then shrinks towards the caudal pole of the TELE (Figure 3.2A). The borders for nucleus rotundus (nRt) follow that of previous studies (Iwaniuk et al., 2010; Gutierrez-Ibanez et al., 2014) and include nucleus triangularis (Tr) because Tr could not be reliably differentiated from nRt across all sections or specimens (Figure 3.2B).

Statistical analyses

To examine variation in the relative size and scaling of each of the brain region, I performed analyses of variance (ANOVAs) on ratios and analyses of covariance (ANCOVAs) on log-transformed volumes. Although the use of ratios is problematic for a variety of reasons (Arndt et al., 1991; Nevill and Holder, 1995; Baur and Leuenberger, 2011; Lefebvre, 2012), I include it here because it is one of the most commonly used methods to 'correct' for overall brain or telencephalon size, especially in studies of songbirds (e.g., DeVoogd et al., 1993; MacDougall-Shackleton et al., 2003; Moore et al., 2011). For all sets of analyses, I examined variation across three groups in our sample: breeding season males, non-breeding season males and non-breeding season females. The ratios were calculated by simply dividing the volume of each brain region by whole brain volume or, in the case of telencephalic regions, by telencephalic volume (DeVoogd et al., 1993; MacDougall-Shackleton et al., 2003; Ward et al.,

2012; Schmidt et al., 2013). For the ANCOVAs, I first subtracted the volume of each brain region from WB volume, following Deacon (1990). In the case of telencephalic regions, I subtracted each region's volume from TELE volume as well. The data were then $\log_{10}$-transformed and I ran an ANCOVA of brain region volumes with group (spring male, fall male, fall female), the scaling variable, either WB or TELE volume minus that of the region of interest, and their interaction. In all instances where significant effects were detected, I used post-hoc Tukey-Kramer HSD tests to assess specific differences among groups.

RESULTS

Absolute volumes

No significant differences among breeding season males, non-breeding season males and non-breeding season females were detected for WB ($F = 0.82$, $df = 2, 17$, $p = 0.82$) or TELE volumes ($F = 0.37$, $df = 2, 17$, $p = 0.70$). Similarly, there were no significant differences among the three groups for nRt ($F = 1.44$, $df = 2, 17$, $p = 0.26$) or Cb volumes ($F = 1.23$, $df = 2,17$, $p = 0.31$). Within the TELE, HF ($F = 0.22$, $df = 2, 17$, $p = 0.80$), M ($F = 0.23$, $df = 2, 17$, 0.80) and SPC ($F = 1.58$, $df = 2, 17$, $p = 0.23$) also did not differ significantly among groups. The only brain region that varied significantly in absolute volume among the three groups of birds was A volume ($F = 3.75$, $df = 2, 17$, $p = 0.04$). Post-hoc tests indicated that this was due to breeding males having significantly larger A volumes than non-breeding males (Table 3.2).

Ratio analyses

Although WB size did not vary significantly among the three groups, I did detect significant differences in the size of two brain regions, relative to WB size. The A ($F=7.57$, $df= 2, 17$, $p=0.005$) and SPC ($F = 4.64$, $df = 2, 17$, $p = 0.02$) varied significantly among groups and post-hoc tests indicated that this was due to breeding season males having relatively larger A and SPC volumes than either non-breeding season males or females (Figure 3.3B,C). This translates to a 21.4% and 10.1% increases in the relative sizes of A and SPC, respectively, in breeding season males compared to non-breeding season males. The relative sizes of the Cb ($F= 0.75$, $df = 2, 17$, $p = 0.49$), HF ($F = 0.08$, $df = 2, 17$, $p = 0.92$), M ($F = 0.47$, $df = 2, 17$, $p = 0.63$), nRt ($F = 1.98$, $df = 2, 17$, $p = 0.17$) and TELE ($F = 0.73$, $df = 2, 17$, $p = 0.50$) did not, however, vary significantly among groups (Figure 3.3D-H).

For all four telencephalic regions measured, the results were qualitatively the same when examining brain region volume relative to TELE volume. For example, both the A ($F = 10.55$, $df = 2, 17$, $p = 0.001$) and SPC ($F = 7.09$, $df = 2, 17$, $p = 0.006$) were significantly larger, relative to TELE volume, among breeding season males than non-breeding season males or females, which again did not differ significantly for either measurement (Figure 3.4A, B). Effect sizes for the A and SPC volume relative to TELE volume were similar to those reported above, each having undergone 28.6% and 14.1% increases in volume respectively. Last, the sizes of the HF ($F = 0.05$, $df = 2, 17$, $p = 0.95$) and M ($F = 1.02$, $df = 2, 17$, $p = 0.38$), relative to TELE size, also did not differ significantly among groups (Figure 3.5C, D).

ANCOVAs

An ANCOVA of TELE yielded no significant group effect ($F = 0.80$, $df = 2, 14$, $p = 0.47$), and neither the WB volume ($F = 1.99$, $df = 2, 14$, $p = 0.18$) nor the interaction between groups and WB size were significant ($F = 0.53$, $df = 2, 14$, $p = 0.60$) (Figure 3.5A). The same was also true of the Cb, which did not vary significantly either by group ($F = 2.12$, $df = 2, 14$, $p = 0.16$) or WB size ($F = 2.60$, $df = 1, 14$, $p = 0.13$) and no significant interaction between the two was detected ($F = 0.53$, $df = 2, 14$, $p = 0.60$) (Figure 3.5B). Similarly, nRt did not vary significantly by group ($F = 1.29$, $df = 2, 14$, $p = 0.31$) or WB size ($F = 0.15$, $df = 1, 14$, $p = 0.70$) and no significant interaction ($F = 0.10$, $df = 2, 14$, $p = 0.91$) was found (Figure 3.5C). Although no significant group differences were detected in HF ($F = 0.16$, $df = 2, 14$, $p = 0.85$), it did scale with WB size ($F = 13.91$, $df = 1, 14$, $p = 0.002$). That is, HF size increased as WB size increased. There was, however, no significant interaction detected ($F=5.56$, $df = 2, 14$, $p = 0.50$) (Figure 3.5D). M volume, did not yield significant differences among groups ($F = 0.33$, $df = 2, 14$, $p = 0.73$), WB size ($F = 3.72$, $df = 1, 14$, $p = 0.07$) or an interaction between the two ($F = 1.42$, $df = 2, 14$, $p = 0.28$) (Figure 3.5E). Unlike HF or M, SPC volume differed significantly among groups ($F = 5.35$, $df = 2, 14$, $p = 0.02$) and varied significantly with WB size ($F = 29.05$, $df = 1, 14$, $p < 0.0001$), but no significant interaction was detected ($F = 1.36$, $df = 2, 14$, $p = 0.29$) (Figure 3.5F). The effect of group was due to SPC volume being significantly larger in breeding season males compared to non-breeding season males or females. Last, A volume differed significantly among groups ($F = 5.18$, $df = 2, 14$, $p = 0.02$), but did not scale with WB size ($F = 2.78$, $df = 1, 14$, $p = 0.12$) and no significant interaction was found ($F = 0.97$, $df = 2, 14$, $p = 0.40$) (Figure 3.5G). Again, this difference was due to A volumes being significantly larger in breeding season males. The ANCOVAs that examined the relationship between telencephalic

regions and TELE size corroborated the results described above for WB size. The HF had a significant relationship with TELE size ($F = 33.00$, $df = 1, 14$, $p<0.0001$), but no significant group ($F = 0.07$, $df = 2, 14$, $p = 0.93$) or interaction effects ($F = 3.06$, $df = 2, 14$, $p = 0.08$) were detected (Figure 3.6A). Similar to the HF, M volume scaled with TELE size ($F = 6.10$, $df = 1, 14$, $p= 0.027$), but there was no significant difference among groups ($F = 0.65$, $df = 2, 14$, $p = 0.54$) and no statistically significant interaction was detected ($F= 1.62$, $df = 2, 14$, $p= 0.232$) (Figure 3.6B). In contrast, the SPC shared a significant scaling relationship with the TELE (TELE-SPC: $F = 46.65$, $df = 1, 14$, $p<0.0001$) and varied significantly among groups ($F = 8.91$, $df = 2, 14$, $p = 0.003$) with breeding season males having a larger SPC than both non-breeding season males or females. As with the other brain regions, no significant interaction effect on SPC volume was found ($F = 0.201$, $df = 2, 14$, $p =0.82$) (Figure 3.6C). Finally, our analysis of A volume yielded a significant relationship with TELE size ($F = 9.38$, $df = 1, 14$, $p = 0.008$) and a difference among groups ($F = 7.66$, $df = 2, 14$, $p = 0.006$), but no significant interaction effect ($F =1.57$, $df = 2, 14$, $p = 0.243$) (Figure 3.6D). In a similar fashion to our other analyses of A size, breeding season males had significantly larger A volume, relative to TELE size, than non-breeding season males or females.

DISCUSSION

Because I was unable to collect the brains of female Ruffed Grouse during the breeding season, it remains uncertain whether the patterns of seasonal plasticity detected here are specific to males or occur in both sexes. Despite the lack of breeding season females, I was able to test for sex differences in the non-breeding season. I found no significant sex differences in the

volume of any brain region that I measured or overall brain size during the non-breeding season. In the fall, the only behavioural sex differences appear to be related to dispersal distance and habitat selection (Small & Rusch, 1989), but these sex differences are relatively minor and unlikely to be related to the sizes of the brain regions that I examined. Given that males and females exhibit greater behavioural differences in the breeding season, such as drumming and home range size, I predict that some brain regions will be sexually dimorphic in the breeding season, but testing this will be dependent on acquiring a sufficient number of females during the breeding season.

With respect to seasonal differences in male Ruffed Grouse, I found no significant differences in WB, Cb, TELE, HF, M and nRt volumes between breeding and non-breeding seasons. WB size was unlikely to vary with season independent of increases in TELE volume because there is limited seasonal plasticity in brain region volumes outside the forebrain in birds (Ball et al., 2004; VanMeir et al., 2006). Additionally, I am unaware of any seasonal variations in their Cb morphology and several studies have shown no effect of season on nRt size (Riters et al. 2000; MacDougall-Shackleton et al., 2003; but see Smulders 2002). Although seasonal changes in TELE volume have been reported in oscine songbirds (VanMeir et al., 2006), these likely arise from large increases in the volume of song control nuclei (e.g., 100-200%), other auditory forebrain regions and/or the HF (Tramontin & Brenowitz, 2000; DeGroof et al., 2009; Clayton et al., 1997). This seasonal variation underlies song learning, food caching or brood parasitism in songbirds, but these behaviours are absent in the Ruffed Grouse. Within the TELE, M is a multisensory, multimodal telencephalic region implicated in a wide range of behaviours (Atoji & Wild, 2011; Jeanne et al., 2011; Avey et al., 2011; Timmermans et al., 2000; Nakamori

et al., 2010) and, as a result of its multifunctional nature, is unlikely to be tied to the expression of highly specific, seasonally variable behaviours, like drumming. That said, I did not measure individual subregions within the M, which could vary by sex (Day et al., 2011) and potentially with season.

In contrast to the TELE and M, I predicted a sex difference and a seasonal difference in males in the HF based upon seasonal variation and sex differences in dispersal patterns and home range size of Ruffed Grouse (Rusch & Keith, 1971; Maxson, 1977; 1978; Thompson & Fritzell, 1989). Although home range size varies among populations and age groups as well as across the breeding and non-breeding seasons, female home ranges are 2-2.5x larger than that of males throughout the year (Maxson, 1978; Thompson & Fritzell, 1989; Archibald, 1975; Frearer & Stauffer, 2003; Whitaker et al., 2007). Despite this sex difference in home range size, there is little information on the possible relationship between home range and HF size in birds. HF volume is only infrequently assessed outside the context of food caching or brood parasitism in birds (Healy et al., 1996; Abott et al., 1999; Day et al., 2005; Rehkamper et al., 2008; Melhorn et al., 2010; Ward et al., 2012; Cnotka et al., 2008; Cristol et al., 2003) and there are no published tests of the relationship between home range size and HF volume in birds. Further, HF volume itself has been criticized as a measurement because it might not be as important as neuron numbers or sizes (Roth et al., 2010) and questions have been raised about the relationship between sex differences in home range size and corresponding sex differences in spatial ability (Clint et al., 2012). Thus, the lack of sex difference in HF volume in male Ruffed Grouse could be due to relying on volume rather than cell counts and/or a lack of seasonal variation in spatial ability, despite major differences in home range size.

Seasonal plasticity in galliforms

Seasonal plasticity is typical of the song system of songbirds and, to a lesser extent, the HF (Tramontin and Brenowitz, 2000; Sherry & Hoshooley, 2010; Yaskin, 2011), but it has only rarely been documented in non-songbirds. In galliforms, the only evidence, apart from that presented herein, are cell size changes in the midbrain nucleus intercollicularis (ICo). For example, in the Grey Partridge (*Perdix perdix*) both males and females experimentally treated with testosterone (T) have lower pitched vocalizations, more robust syringeal labia and increased cell sizes in ICo (Beani et al., 1995). ICo also undergoes T-induced seasonal plasticity in Japanese Quail (*Coturnix coturnix*) (Panzica et al., 1991) as well as songbirds (Gurney and Konishi, 1980). ICo neurons, especially those within the dorsomedial region, have a high concentration of androgen receptors (Balthazart et al., 1992), which makes them responsive to the effects of increased T levels and thereby modulate vocal behaviour in galliforms. Outside of ICo, I am unaware of other examples or attempts to test for seasonal variation in brain region sizes in relation to courtship in galliforms or other non-songbirds. Here, our results clearly demonstrate that in male Ruffed Grouse, both SPC and A undergo seasonal changes in volume such that both are larger in the breeding season and this is correlated with drumming behaviour. In the fall, few males drum at all in our population and when they do, it is infrequent. In contrast, during the spring breeding season, males will drum at 2-7 minute intervals for several hours in the morning for weeks in a row (Archibald, 1976; Atwater and Schnell; Rusch et al. 2000). At the peak of their drumming behaviour, a male can produce upwards of 350 drumming displays in a single day (Iwaniuk, unpublished data). These same actively drumming males have

significantly larger SPC and A volumes than males in the non-breeding season, which suggests that both structures play a role in producing or modulating drumming behaviour.

In birds, the SPC supports and modulates behaviour primarily by initiating voluntary movements and suppressing involuntary ones (Kuenzel, 2012; Butler & Hodos, 2005). The medial and lateral striatal components (i.e., the dorsal striatum) project to the ventral striatum and have reciprocal connections with somatosensory areas of the HA and the ventrointermediate area, a dorsal thalamic nucleus (Steiner & Tseng, 2010; Butler & Hodos, 2005). The dorsal striatum also projects to the pretectum to subserve visual orientation (Butler & Hodos, 2005). At the molecular level in songbirds, the transcription factor ZENK is heavily expressed within the anterior striatum, adjacent to Area X, during flight and wing movements independent of flight, such as wing whirring (Elmen & Elmen, 1996; Mouritsen, 1988; Feenders et al., 2008). During wing whirring, the level of ZENK expression is also proportional to the number of wingbeats produced, indicating the functional involvement of the SPC in the anterior forebrain motor pathway during wing movement (Feenders et al., 2008). Seasonal changes in SPC volume and drumming beahviour in the Ruffed Grouse parallel increases in ZENK immunoreactivity in the anterior striatum during both flight and wing whirring, and taken together with its hodology, implicate the SPC in the production of the drumming display. Putatively, the function of seasonal plasticity within the SPC of male Ruffed Grouse could be processing proprioceptive feedback from the limbs, thereby facilitating the maintenance of balance and synergistic movements during the drumming display (Feenders et al., 2008).

The seasonal increase in SPC volume of Ruffed Grouse is an interesting parallel with Area X in the songbird song system. Area X is a region critical for learning vocalizations in

songbirds, is located within the medial striatum, undergoes seasonal neurogenesis and exhibits corresponding changes in volume (Tramontin & Brenowitz, 2000). That said, the amount of variation I observed in SPC volumes in Ruffed Grouse were much lower than that reported for Area X (Smith 1996; 1997; Brenowitz et al., 1998; Gulledge & Deviche, 1999) and I only detected significant differences in relative volumes (both WB and TELE) and not absolute volumes. Further, there is a large amount of overlap between breeding and non-breeding season males in both the relative and absolute SPC volumes (Figure 3.4C, 3.5F; Table 3.2). Despite these caveats, the SPC might be involved in producing or modulating Ruffed Grouse drumming behaviour because of its prominent role in coordinating movements (Reiner, 2013).

Like the SPC, A plays a role in motor function, but is also important for sensory, somatosensory and limbic functions in birds (Kass, 2009; Jarvis et al., 2013; Feenders et al., 2008; Saint-Dizier et al., 2009). The SPC is thought to be homologous to parts of the claustroamygdaloid complex in mammals based on its hodology (Reiner, 2013). It also gives rise to telencephalic projections to the brainstem from the dorsal ventricular ridge (DVR) and possesses neuron types and connections typical of the mammalian neocortex (Shanahan, 2013). The A, especially the intermediate and dorsal subregions, can be described as nodes within a premotor circuit that mediate various streams of the information flow within the avian forebrain (Shanahan, 2013; Reiner, 2013) and form the primary source of output from the avian telencephalon (Kass, 2009; Jarvis et al., 2014). Together, with our understanding of neuromuscular control of the wings (Feenders et al., 2008; Liu et al., 2012; Schlinger, 2013), this suggests a prominent role of the A along the descending motor pathway, which is why I predicted that A size would vary seasonally in male Ruffed Grouse. The degree of volumetric

change in arcopallium I observed is not as great as those in RA, which is nested within the arcopallium of songbirds, but it is larger than that of the SPC and was robust across all of our analyses (Figure 4A, 5G; table 3.1). Akin to the SPC, ZENK immunoreactivity is also proportionally expressed in the lateral intermediate arcopallium during both flight and wing whirring (Feenders et al., 2008). Taken together, this strongly implicates arcopallium as a key region in the production of the drumming display.

Although a promising finding, it is important to note that A is a heterogeneous structure; it is comprised of several anatomically and functionally distinct subregions (e.g., anterior, intermediate and dorsal arcopallial areas) (Shanahan, 2013), the boundaries of which could not be consistently delineated by our Nissl stain (Figure 3.1C; 3.2A). This prevented us from assessing the volume of each subregion relative to arcopallium, TELE and WB volume. Thus, it remains uncertain whether the volumetric differences in arcopallium volume that I found arise from changes across all or just a subset of these subregions. Similarly, it is unclear at this stage what anatomical changes result in the increase in arcopallium volume in breeding season males. In RA, seasonal changes in volume are largely an effect of increased neuron size and spacing as opposed to an increase in cell numbers (Tramontin & Brenowitz, 2000). Further, synaptic morphology changes (i.e., pre and postsynaptic terminals increase in size), dendritic arborization and spine density of RA neurons is greatest under spring like conditions (Tramontin & Brenowitz, 2000). If a similar mechanism underlies seasonal plasticity in the arcopallium of Ruffed Grouse, then I should observe an increase in neuron size and spacing, including dendritic arborization, in breeding season males.

Conclusions

Seasonal variation in the brains of oscine songbirds is among the most pronounced, consistent and well-studied examples of neuroplasticity in any vertebrate lineage (Tramontin & Brenowitz, 2000; Ball et al., 2004; Garćia-Verdugo et al., 2002; Holmat & Svoboda, 2009; Kolb, 2013). Our results provide clear evidence that the neuroplasticity observed in the songbird telencephalon is not necessarily limited to songbirds or to vocal learning in general. In fact, this is the first evidence of neuroplasticity in the telencephalon of both a non-songbird species and a species that does not engage in vocal learning or vocal courtship behaviours. Whether the same is also true for other species that engage in non-vocal forms of courtship (e.g., manakins) or non-songbirds that incorporate both vocal and non-vocal components in their displays (e.g., other grouse species) remains to be tested. At present, the extent to which the volumetric differences in the SPC and arcopallium of male Ruffed Grouse reflect changes in cell size, dendritic arborization or other anatomical changes is unclear. If these changes are similar to that of songbirds, it could represent a generalized mechanism of neuroplasticity that is shared across many avian taxa and far more widespread than has been recognized.

CHAPTER FOUR: GENERAL DISCUSSION

As discussed in Chapter 1, one of the characteristic features of the Ruffed Grouse is its remarkable drumming display. Despite the large number of publications on drumming log selection and the use of drumming counts as a census technique (Boag & Sumanik, 1969; Schumacher et al., 2001; Buhler & Anderson, 2001; Gullion, 1966; Jones, 2005; Hansen et al., 2011), until recently there was very little known about this behaviour or how it might relate to wing morphology or neuroanatomy. In Chapter 2, I showed that wing morphology varies greatly among grouse species, but I did not find any evidence that the wings of Ruffed Grouse are unique with respect to other forest grouse or other species that engage in some form of wingbeating display. In Chapter 3, my results clearly showed that two motor control regions in the telencephalon vary significantly between breeding and non-breeding seasons in male Ruffed Grouse. This is the first evidence of neural plasticity in the TELE of a non-songbird and strongly implicates both the SPC and arcopallium in the production of the drumming display. Below, I will discuss the implications of these two chapters for our understanding of the coordinated evolution of wing shape, courtship behaviour and the avian brain with special reference to the drumming display. Finally, I suggest future directions for my research with an emphasis on integrative approaches to studying how non-vocal components of courtship behaviours are produced and how they have evolved in birds as a whole.

Wing shape: important or unimportant?

Based on my analyses of wing morphology, some features of wing size and shape vary among grouse and appear to be associated with body size and habitat type, while others differed primarily between the sexes and/or did not vary significantly among species (table 2.3, Figures 2.4-2.8). Additionally, wing shape variation is strongly related to phylogenetic relationships within Tetraoninae (Figures 1.1, 2.9). However, contrary to one of my predictions, the Ruffed Grouse did not differ significantly from most other forest species. Indeed, species that engage in wingbeating displays of various forms did not differ from one another in both the TM and GM analyses. Thus, I concluded that habitat type is a more prominent determinant of wing shape variation among grouse, as it is in other avian lineages (Senar et al., 1994; Gamauf et al., 1998; Suryan et al., 2008; Desrochers, 2010). This conclusion is corroborated by strong phylogenetic signal contained within my wing shape data and the fact that speciation is strongly associated with habitat in Tetraoninae (Lucchini et al., 2001; Drovetski, 2002). That said, this does not necessarily mean that differences in wing and feather shape do not contribute to the production of the display, but rather that they could be occurring at a level that was not examined here (Bostwick, 2010; Clark, 2012). Here, I discuss some aspects of wing and feather shape and wing use in greater detail with respect to Ruffed Grouse and the drumming display.

Specialized feather morphologies (e.g., shape, flexural stiffness, resonant frequency) have evolved independently numerous times and underlie the production of non-vocal sounds or "sonations" in various lineages (Bostwick, 2006; Clark, 2008; Hinge & Mcgrath, 2009; Bostwick, et al., 2009). The sonations and feather morphology of select hummingbird species have been well studied and provide an excellent example of the relationship between feathers

and the sounds that they can produce. For example, tail feather shape is related to the frequencies produced by Anna's Hummingbirds (*Calypte anna*) during their display flights (Clark & Feo, 2008). Further, the manner in which feathers are articulated during displays has also proven to be critically important to their production (Bostwick, 2006; Clark, 2008). For example, the sounds generated during the display flights of Red-billed Streamertail hummingbirds (*Trochilus polytmus*) was previously attributed to elongated tail feathers, but recent high-speed video and experimental manipulation of tail feather length indicate that the articulation of P8 relative to P9 during the display produced the sound (Clark, 2008). Among galliforms, Greater Sage-Grouse rub feathers of their distal wing against specialized stiffened breast feathers in a plucking motion, which produces a "swish" sound (Bostwick, 2006). Whether the primary and/or secondary feathers of Ruffed Grouse wings are similarly stiffened or differ in shape from that of congeners that do not drum (*B. bonasia*, *B. sewerzowi*) has not been tested, but could contribute to the production of the drumming sound and would not necessarily translate to a different overall wing shape.

An alternative explanation to wing and/or feather specialization in relation to the drumming display of the Ruffed Grouse is that many galliforms engage in similar wingbeating or fluttering movements and therefore might share a common overall structure to enable these behaviours. In fact, all grouse species use wing movements, in one form or another, as part of their courtship displays. For example, ptarmigan have "song flights" that combine an aerial wingbeat display and simple vocalizations; *Tetrao*, *Tympanuchus*, and *Dendragapus* species each produce flutter jumps; the Hazel and Franklin's Spruce Grouse incorporate wing claps into

their courtship displays while the Greater and Gunnison Sage-Grouse use wingbeats in territorial interactions that produce similar sounds to the drumming display of the Ruffed Grouse (Hjorth, 1970; Johnsgard, 1983). Even Ring-necked Pheasants (*Phasianus colchicus*) engage in 'drumming' as part of their display (Johnsgard, 1975; 1976). Wingbeating or other types of wing movements could therefore represent a fairly common trait among galliforms that is ancestral within the grouse lineage and the Ruffed Grouse have simply accentuated it.

Given that wingbeating appears to be fairly common among galliforms, perhaps it is not the drumming *per se* that is unique to Ruffed Grouse, but rather the almost exclusive reliance on drumming for courtship that differentiates it from other grouse and galliform species. If true, then drumming could be supported by species-specific musculature adaptations rather than a change in wing or feather shape (Thomas, 1985; Drovetski, 1996; Schlinger et al., 2013; Welch, Malik and Iwaniuk, in prep). Variation in muscle size, type and chemistry vary greatly among bird species (Battley et al., 2000; Lindstrom et al., 2000; Askew & Marsh, 2002; Dietz et al., 2007; Welch & Altshuler, 2009; Groom et al., 2013), and are correlated with gross features of wing morphology among grouse (Thomas, 1985; Drovetski 1996). For example, as wing loading and aspect ratio increase among grouse species, pectoral muscle fibers become darker, indicating a greater density of fast twitch muscle tissue (Drovetski, 1996) and related increases in contractile ability (Barnard et al., 1981). This suggests that species more reliant upon vertical lift (i.e., Ruffed Grouse) than level flight (i.e., White-tailed Ptarmigan) should have lower wing loadings, lower aspect ratios and higher densities of slow twitch muscles (Barnard et al., 1981; Drovetski, 1996). Similarly, the Ruffed and Spruce Grouse differ greatly in various features of

their myology (Thomas, 1985). For example, although the relative mass of the pectorals and wing loading were similar between the two species, the pectorals of Spruce Grouse are more oxidative and contain exponentially higher levels of myoglobin, which together, is thought to facilitate endurance flight and sustained flight (Thomas, 1985; Pagés & Planas, 1983; Butler & Woak; Torella et al., 1998). These corresponding variations between myology and gross wing morphology suggest that the musculature of the Ruffed Grouse likely differs from related species, and potentially, in relation to production of the drumming display.

In sum, the drumming display may still involve adaptations related to wing use that simply did not translate to a difference in overall wing shape. An integrative approach that includes detailed examination of drumming in the Ruffed Grouse as well as interspecific analyses of the wingbeat displays of other galliforms is likely necessary to characterize what, if any, differences in morphology contribute the production of the display and how.

Neural control of drumming and other non-vocal courtship behaviours

The data presented in chapter 3 indicate that seasonal variation in the volume of telencephalic motor regions (i.e., arcopallium, striatopallidal complex) is a prominent feature of the brains of male Ruffed Grouse (table 2; Figures 3.3-3.6). Again, males collected in the breeding season had larger arcopallial volumes across all of my analyses. Similarly, SPC volume was also relatively larger in breeding season males than non-breeding males or females, though not in absolute terms or to the same magnitude as the arcopallium (table 2; Figure 3.3-3.6). Although I was unable to obtain the brains of female Ruffed Grouse in the breeding season, and the brains of male and female Ruffed Grouse collected in the fall did not differ in any of my

measurements, I predict that there are significant sex differences in both the SPC and arcopallium during the breeding season. This prediction is based upon both the behaviour of Ruffed Grouse and comparable studies in other species. For example, the most prominent behavioural difference between males and females is the production of the drumming display by males during the breeding season. In addition, sex differences in brain regions that support courtship behaviours are widespread in birds and occur within the TELE and other brain regions (Panzica et al., 1991; Balthazart & Ball, 1995; Beani et al., 1995; MacDougall-Shackleton & Ball, 1999; Ball &MacDougall-Shackleton, 2001; Day et al., 2011). For these reasons, it is likely that the seasonal variation detected here is specific to males and may be related the production of the drumming display.

One exciting implication of my research is that the seasonal plasticity in the telencephalon may be far more widespread in birds than has been recognized. Furthermore, this seasonal variation is likely tied into courtship behaviours, in a similar fashion to the well documented seasonal variability in birdsong and the song system that controls and modulates song. Because the arcopallium plays a central role in the descending motor system, perhaps any type of seasonal behaviour or courtship display that requires significant increases in sensory motor control is associated with variations in arcopallium volume (Shanahan, 2013; Feenders et al., 2008). This hypothesis could be tested in a variety of ways, both within and across species. For example, based on my findings, arcopallium size likely fluctuates seasonally in male Golden-collared Manakins (Day et al., 2011). Because other manakins also produce similar wing snapping displays exclusively in the breeding season, a pattern of sex specific seasonal

variation may underlie the production of these displays across several manakin species (Prum, 1990; Schlinger et al., 2013; Tramontin & Brenowitz, 2000). Alternatively, arcopallium volume could fluctuate with seasonal increases in wing movements in general. For example, arcopallium volume could be increasing during migration when birds engage in periods of extended flight and various behavioural manifestations of migratory restlessness occur (Sol et al., 2010; Mouritsen, 1988; Elmen & Elmen, 1996; Feenders et al., 2008). Seasonal plasticity of arcopallial volume could also occur in other stereotyped motor behaviours that have little to do with the wings, especially those that are related to courtship. For example, the bill clapping of Oriental White Storks occurs in both sexes, but is sexually dimorphic, spectral features differ among individuals and the rate of clapping varies between the breeding and non-breeding seasons (Eda-Fujiwara et al., 2004). Similarly, during the breeding season, male Ruddy Ducks (*Oxyura jamaicensis*) clap their bills on their breast feathers then produce high pitched vocalizations to advertize to females in addition to other non-acoustic display postures (Johnsgard, 1965). Further, the pathway that mediates movements of the jaw in birds originates within the arcopalliaum (Wild, 1997). Among grouse, both the Greater and Lesser Prairie Chicken stomp their feet rhythmically during displays in a manner reminiscent of drumming (Johnsgard, 1983). These and other non-vocal acoustic courtship behaviours serve similar functions as drumming and wing-snapping displays and similar neural mechanisms, such as seasonal variation in the size of motor regions, could underlie their production.

One means by which the seasonal variation in the Ruffed Grouse TELE could be affected is through the activation of androgen and/or estrogen receptors. A common feature of nuclei

within the song circuit is the presence of androgen and estrogen receptors, as well as the conversion enzyme aromatase (Farley et al., 2010; Remage-Healey et al., 2009). A specialized neuroendocrine system also modulates the Golden-collared Manakin's physiology in a manner that appears to support the wing snapping display (Schlinger et al., 2013). For example, high concentrations of androgen and estrogen receptors and aromatase are found in skeletal muscles of the wings, the spinal cord and the arcopallium in Golden-collared Manakins (Schlinger et al., 2013; Fusani et al., in press). This indicates a key role of steroid hormones in the production of the wing snapping display (which is also shown by behavioural endocrinology studies, Schlinger et al., 2013). Comparable neuroendocrine mechanisms could modulate the drumming display, including the expression of androgen receptors in both the arcopallium and pectoral muscles of the Ruffed Grouse. *In situ*-hybridization of androgen and estrogen receptor mRNA would be able to indicate the presence and concentration of steroid hormone receptors within the arcopallium or other central and peripheral features of the nervous system as has recently been shown in the Golden-collared Manakin (Fusani et al., 2014). However, the neuroendocrine mechanism by which this occurs likely differs from the manakin because aromatase is not expressed in the arcopallium of Ruffed Grouse (Corfield et al., 2012).

Finally, other neuroanatomical parameters like neuronal size, number and spacing would be greatly informative regarding the plasticity detected here. Based on Schlinger et al. (2013), and other studies of songbirds (Tramontin & Brenowitz), I predict that increased arcopallium volume among breeding male Ruffed Grouse would result from increased cell size (e.g., cell body, dendritic arbor) and spacing as opposed to the significant addition of new cells as is seen

in seasonal studies of HVC (Tramontin & Brenowitz, 2000). Additionally, the connectivity of cells within the arcopallium could also become larger during the breeding season as is observed in RA (i.e., more dendrtic spines, larger synaptic terminals). Based on Schlinger et al., (2013), the expression of immediate early genes, like c-fos and ZENK, could be useful in determining the brain regions that are involved in production of the display. Based on the work of Feenders et al., (2008), ZENK expression would likely be high in the arcopallium and anterior regions of the SPC in males following periods of drumming during the breeding season.

Overall, drumming appears dependent upon seasonal increases in the functional capacity of motor regions, as inferred by increased arcopallium and SPC volume in breeding season males. The mechanisms by which this variation occurs as well as extent that it differs among species, including other non-vocal learners, remains uncertain and is an exciting avenue for future research.

Future directions

In order to further assess the extent to which wing and feather specialization and articulation contribute to the production of the display, I put forward several suggestions. First, high-speed video of Ruffed Grouse drumming is an essential step towards understanding both the production of the display and the spectral features that are produced by the feathers in the process (Clark, 2008). Second, examination of feather shape (Bostwick, 2010), other physical properties (Bachmann et al., 2012) and the frequencies at which they produce flight sounds (Clark & Feo, 2008) would be greatly informative. Together, this should indicate the extent and

manner in which feather specialization or manipulation contribute to drumming and perhaps suggest the involvement of other systems in production of the display as well.

Further insight into the neurophysiology of the display, and non-vocal displays in general, could be gleaned by reproducing some of the experimental studies of the Golden Collared Manakin and songbirds on the Ruffed Grouse (Schlinger et al., 2013; Tramontin & Brenowitz, 2000). First, additional stereology and immunohistochemistry should be used to clarify the underlying cellular changes that caused the increase in volume detected here. Second, to better understand whether, and the extent to which, these cellular changes are under hormonal control, I recommend the *in situ*-hybridization of androgen and estrogen receptor mRNA (Fusani et al., *In press*) as well as the use of autoradiography (Nottebohm, 2005). Third, the application of electrophysiology or IEG could potentially indicate the brain regions activated during the display and perhaps those of other non-vocal acoustic behaviours as well (Feenders et al., 2008). Finally, comparative studies of grouse, other galliforms and birds with unique and otherwise comparable courtship behaivours should be conducted to indicate how non-vocal acoustic courtship behaviours have evolved among birds.

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TABLES

Table 2.1. A list of the species examined, abbreviations used in the figures, sample sizes for all males, females and total number of specimens measured (with n for geometric morphometrics in brackets) and habitat type as listed in Drovetski et al. (2006).

Common name	Species	Abb	Male n	Female n	Total n	Habitat
Hazel Grouse	*Bonasa bonasia*	BB	20	10	30 (12)	Forest
Ruffed Grouse	*Bonasa umbellus*	BU	12	8	20 (12)	Forest
Greater Sage-grouse	*Centrocercus urophasianus*	CU	7	6	13 (7)	Sagebrush
Sooty Grouse	*Dendragapus fuliginosus*	DF	4	6	10 (2)	Forest
Dusky Grouse	*Dendragapus obscurus*	DO	17	11	28 (1)	Forest
Spruce Grouse	*Falcipennis canadensis*	FC	6	4	10 (4)	Forest
Willow Ptarmigan	*Lagopus lagopus*	LL	8	8	16 (13)	Tundra
White-tailed Ptarmigan	*Lagopus leucurus*	LA	3	3	6 (4)	Tundra
Rock Ptarmigan	*Lagopus mutus*	LM	9	6	15 (10)	Tundra
Caucasian Black Grouse	*Tetrao mlokosiewiczi*	TM	1	4	5 (0)	Forest
Black-billed Gapercaillie	*Tetrao parvirostris*	TP	4	3	7 (4)	Forest
Black Grouse	*Tetrao tetrix*	TT	4	3	7 (5)	Forest
Western Capercaillie	*Tetrao urogallus*	TU	2	4	6 (1)	Forest
Greater Prairie-chicken	*Tympanuchus cupido*	TC	8	2	10 (5)	Prairie
Lesser Prairie-chicken	*Tympanuchus pallidicinctus*	TD	5	5	10 (7)	Prairie
Sharp-tailed Grouse	*Tympanuchus phasianellus*	TA	12	7	19 (13)	Prairie

Table 2.2. Averages, standard deviations (+/-) and sample sizes (n) of traditional morphometric measurements of the grouse species available in this study. Wing span (WS) and body mass (M_b) were recorded for males and females of each species from Burke Museum of Natural History (BMNH) records in millimeters (mm) and grams (g) respectively. Wing loading (WL) was calculated from surface area (SA) and mass.

Species	Male WS	Female WS	Male M_b	Female M_b	Male WL	Female WL
B. bonasia	528.25 +/- 6.15 (n=13)	509.00 +/- 6.48 (n=4)	353.50 +/- 26.10 (n=12)	375.00 +/- 42.0 1(n=7)	9.93 +/- 1.56 (n=12)	9.35 +/- 1.89 (n=4)
B. umbellus	-	581.30 +/- 8.69 (n=4)	651.60 +/- 64.68 (n=5)	521.80 +/- 81.80 (n=8)	15.18 +/- 1.13 (n=4)	12.36 +/- 1.18 (n=6)
C.urophasianus	-	-	1970.50 +/- 624.78 (n=4)	880.00 +/- 214.27 (n=5)	26.27 +/- 1.13 (n=3)	18.79 +/- 0.73 (n=2)
D. fuliginosus	-	-	502.50 +/- 212.29 (n=4)	800.00 (n=1)	20.84 +/- 2.17 (n=3)	14.48 (n=1)
D. obscurus	726.66 +/- 10.60 (n=3)	-	1055.728 +/- 288.21 (n=2)	660 (n=1)	20.34 +/- 4.35 (n=2)	14.75 (n=1)
F. canadensis	-	-	619.60 +/- 66.05 (n=5)	518.70 +/- 93.39 (n=4)	17.49 +/- 1.61 (n=4)	12.89 +/- 2.21 (n=3)
L. lagopus	676.40 +/- 11.07 (n=8)	622.60 +/- 5.50 (n=4)	663.80 +/- 98.26 (n=7)	607.30 +/- 31.53 (n=4)	13.26 +/- 1.06 (n=7)	13.77 +/- 0.52 (n=3)
L. leucurus	-	-	381.00 +/- 59.39 (n=2)	340.00 (n=1)	9.68 +/- 0.78 (n=2)	9.24 (n=1)
L. mutus	622.70 +/- 38.74 (n=4)	-	460.70 +/- 45.72 (n=4)	418.30 +/- 1.42 (n=3)	12.79 +/- 1.71 (n=3)	11.15 +/- 1.42(n=3)
T. mlokosiewiczi	-	669.70 +/- 76.65 (n=4)	-	750.00 (n=1)	-	15.59 (n=1)
T. parvirostris	-	-	-	-	-	-
T. tetrix	888.60 +/- 7.50 (n=4)	833.00 +/- 53.45 (n=3)	1365.00 +/-115.54 (n=4)	955.00 +/-125.79 (n=3)	24.29 +/- 9.04 (n=3)	18.17 +/- 2.32 (n=3)
T. urogallus	-	-	-	1930.00 +/- 84.85 (n=2)	-	-
T. cupido	-	-	824.20 +/- 84.85(n=11)	-	19.61 +/- 1.77 (n=4)	-
T. pallidinctus	713.30 +/- 11.13 (n=7)	675.50 +/- 9.27 (n=7)	764.50 +/- 60.70 (n=7)	699.60 +/- 25.81 (n=6)	16.91 +/- 1.02 (n=6)	16.69 +/- 0.93 (n=6)
T. phasianellus	-	-	819.00 +/- 158.08 (n=3)	789.50 +/- 43.33 (n=4)	16.65 +/- 4.53 (n=2)	17.69 +/- 0.58 (n=4)

Table 2.3. Results of two-way analyses of variance (ANOVA) of species, sex, and their interaction on traditional morphometric measurements, principal components 1-3 of primary feather lengths and variables from geometric morphometrics (centroid size and canonical variates 1-3).

	Species			**Sex**			**Species x Sex**		
Measurement	F	df	p	F	df	p	F	df	p
Aspect ratio[a]	52.36	13, 158	<0.0001	39.05	1, 158	<0.0001	2.30	13, 158	0.0088
Camber	21.91	15, 152	<0.0001	0.05	1, 152	0.8299	0.96	15, 152	0.4973
Porosity[a]	2.91	13, 133	0.0009	0.47	1, 133	0.4924	0.86	13, 133	0.6003
Wing Loading[b]	34.85	11, 64	<0.0001	42.74	1, 64	0.0001	4.34	11, 64	<0.0001
PC1[c]	252.76	11, 99	<0.0001	59.68	1, 99	<0.0001	6.73	11, 99	<0.0001
PC2[c]	23.37	11, 99	<0.0001	0.82	1, 99	<0.0016	2.59	11, 99	0.0063
PC3[c]	1.49	11, 99	0.1460	0.46	1, 99	0.4970	0.16	11, 99	0.9989
Centroid Size[d]	101.91	9, 86	<0.0001	4.49	1, 86	0.0377	2.16	9, 86	0.0363
CV1[e]	162.28	11, 94	<0.0001	0.01	1, 94	0.9185	1.70	11, 94	0.0917
CV2[e]	40.89	11, 94	<0.0001	0.81	1, 94	0.7768	0.45	11, 94	0.9271
CV3[e]	18.49	11, 94	<0.0001	0.15	1, 94	0.6985	0.72	11, 94	0.7177

[a] must exclude TU and TM because there are insufficient samples per sex
[b] TU, TC, TP and TM excluded because there are insufficient samples per sex
[c] TU, TT, TM, DO excluded
[d] TU, TT, TM, TP, DO, DF excluded because there are insufficient samples per sex
[e] TU, TT, TM, DO, excluded because there are insufficient samples per sex

Table 2.4. Results of one-way analyses of variance (ANOVA) of habitat type on traditional and geometric morphometric (GM) measures of wing shape. Species averages were used in analyses of aspect ratio (AR), camber (C), porosity (P) and wing loading (WL). Species means of principal component (PC) scores 1-3 were used to assess primary feather length ($f_{\text{prim (1-10)}}$) with regard to habitat type while species means of canonical variate (CV) scores 1-3 were used to assess GM variations in wing shape.

Measurement	**F**	**df**	**p**
Aspect ratio [a]	0.4158	2, 14	0.669
Camber [a]	0.099	2, 14	0.905
Porosity [a]	0.588	2, 10	0.577
Wing loading [a]	2.449	2, 11	0.1274
PC1 [a]	1.308	2, 13	0.309
PC2 [a]	21.337	2, 13	0.0002
PC3 [a]	1.292	2,13	0.313
CV1 [a]	29.276	2, 13	<0.0001
CV2 [a]	19.163	2, 13	0.019
CV3 [a]	0.016	2, 13	0.9835

[a] must exclude CU because of insufficient samples per habitat type

Table 2.5. Loadings of the first three principal components (PC's) obtained from a principal component analysis on primary feather lengths and the associated Eigen values and cumulative percentage of variation explained.

Primary feather	PC1	PC2	PC3
1	0.939	-0.182	0.074
2	0.972	-0.176	0.085
3	0.969	-0.198	0.063
4	0.964	-0.194	-0.021
5	0.979	-0.039	-0.157
6	0.983	0.076	-0.129
7	0.968	0.124	-0.176
8	0.96	0.158	-0.039
9	0.963	0.21	0.099
10	0.942	0.225	0.213
Eigen values	9.304	0.2848	0.1473
% cumulative	93.049	95.897	97.370

Table 2.6. Wing shape variation among grouse obtained from a canonical variate analysis (CVA) of Procrustes distances, expressed as Eigen values and cumulative percentage of variance explained.

Factor	Eigen values	% Variance	Cumulative %
1.	17.324	57.621	57.621
2.	5.715	19.010	76.631
3.	2.849	9.475	86.106
4.	1.792	5.962	92.069
5.	1.426	4.743	96.812
6.	0.958	3.188	100.00

Table 2.7. P-values resulting from permutation tests of Procrustes distances among species. For species abbreviations see Table 1. All significant differences (i.e., p = < 0.05) are shown in bold.

	BB	BU	CU	DF	DO	FC	LA	LL	LM	TA	TC	TD	TP	TT
BU	**<0.001**													
CU	**0.001**	0.136												
DF	**0.016**	0.211	0.249											
DO	0.084	0.344	0.143	0.661										
FC	**<0.001**	0.076	0.201	0.468	0.794									
LA	**0.003**	**0.018**	0.240	0.238	0.563	0.157								
LL	**<0.01**	**<0.001**	**0.040**	**0.035**	0.187	0.116	0.334							
LM	**<0.001**	**<0.001**	**<0.001**	**0.013**	0.088	**0.039**	0.167	0.151						
TA	**<0.001**	**<0.001**	**<0.001**	**0.037**	0.228	0.096	**<0.001**	**<0.001**	**<0.001**					
TC	**<0.001**	**0.034**	0.555	0.319	0.401	0.568	0.516	0.525	0.211	**0.036**				
TD	**<0.001**	**<0.001**	**0.033**	0.189	0.128	0.200	0.070	**0.001**	**0.002**	0.099	0.442			
TP	**<0.001**	**<0.001**	**0.009**	0.165	0.181	**0.016**	**0.018**	**<0.001**	**<0.001**	**0.036**	**0.019**	**0.002**		
TT	**<0.001**	**0.045**	0.261	0.466	0.656	0.643	0.149	**0.037**	**0.006**	**0.017**	0.560	**0.041**	0.068	
TU	**0.030**	**0.025**	0.051	1.000	1.000	0.374	0.266	0.056	0.058	0.333	0.074	0.118	0.560	0.28

Table 2.8. Coefficients of variation, standard deviations and means of linear measurements taken from the right wing of a Ruffed Grouse (*Bonasa umbellus*): camber (C), wing length (L), maximum wing chord (WC_m) and primary feather length ($f_{\text{prim (1-10)}}$).

Measure (mm)	Mean	Standard Deviation (+/-)	Coefficient of Variation
Camber (dimensionless)	0.2554	0.3974	1.55%
L	176.61	2.2	1.24%
WC_m	112.9	1.07	0.95%
f_{prim} 1	95.67	2.3	1.29%
f_{prim} 2	102.87	1.84	0.78%
f_{prim} 3	111.8	1.33	0.64%
f_{prim} 4	128.35	2.45	0.62%
f_{prim} 5	141.22	2.63	1.26%
f_{prim} 6	142.4	1.8	1.86%
f_{prim} 7	139.86	0.87	1.91%
f_{prim} 8	137.54	0.89	1.19%
f_{prim} 9	123.46	0.96	1.79%
f_{prim}10	95.54	1.23	2.40%

Table 3.1. Parameters for unbiased stereological assessment of each target nucleus including magnification, grid size (μm^2), evaluation interval (EI), average coefficient of error (CE) and corresponding standard deviation (+/-).

Structure	Magnification	Grid size (μm^2)	Average CE	Range
Arcopallium	2.5	750.00	0.026	0.017 - 0.035
Hippocampal formation	2.5	750.00	0.021	0.018 – 0.025
Mesopallium	2.5	1000.00	0.019	0.015 – 0.022
Striatopallidal Complex	2.5	1000.00	0.020	0.017 – 0.024
Nucleus Rotundus	2.5	500.00	0.043	0.034 – 0.053

Table 3.2. Average measurements of body and brain mass in grams (g) and the absolute volume (mm^3) of each general area and specific brain region: whole brain (WB), telencephalon (TELE), cerebellum (Cb), nucleus rotundus (nRt), arcopallium (A), hippocampal formation (HF), mesopallium (M), and striatopallidal complex (SPC).

Group	Body Mass (g)	Brain mass (g)	WB (mm^3)	TELE (mm^3)	Cb (mm^3)	nRt (mm^3)	A (mm^3)	HF (mm^3)	M (mm^3)	SPC (mm^3)
Spring Male	568.33	2.65	2177.30	1142.04	335.56	9.71	64.92	74.70	193.02	156.50
Fall Male	608.12	2.68	2233.40	1211.72	349.19	8.72	53.46	78.24	187.30	147.05
Fall Female	543.83	2.54	2141.60	1138.52	311.13	9.63	54.15	75.13	182.84	138.15

FIGURES

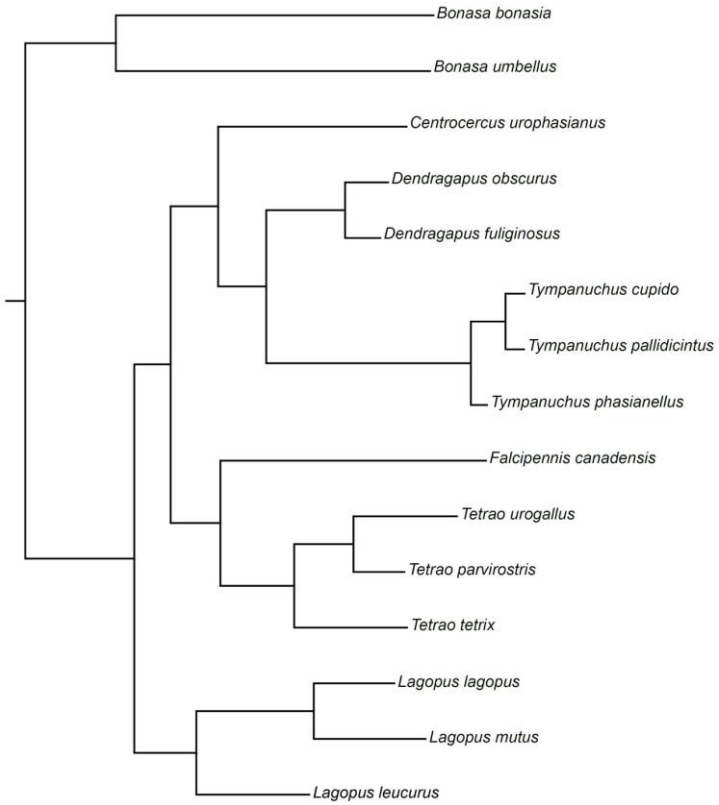

Figure 1.1. A phylogenetic tree of Tetraoninae based on mitochondrial CR sequences including each of the species used in this study, based on the molecular phylogeny of Drovetski (2002) and reconstructed in Mesquite (Madden & Madden, 2011).

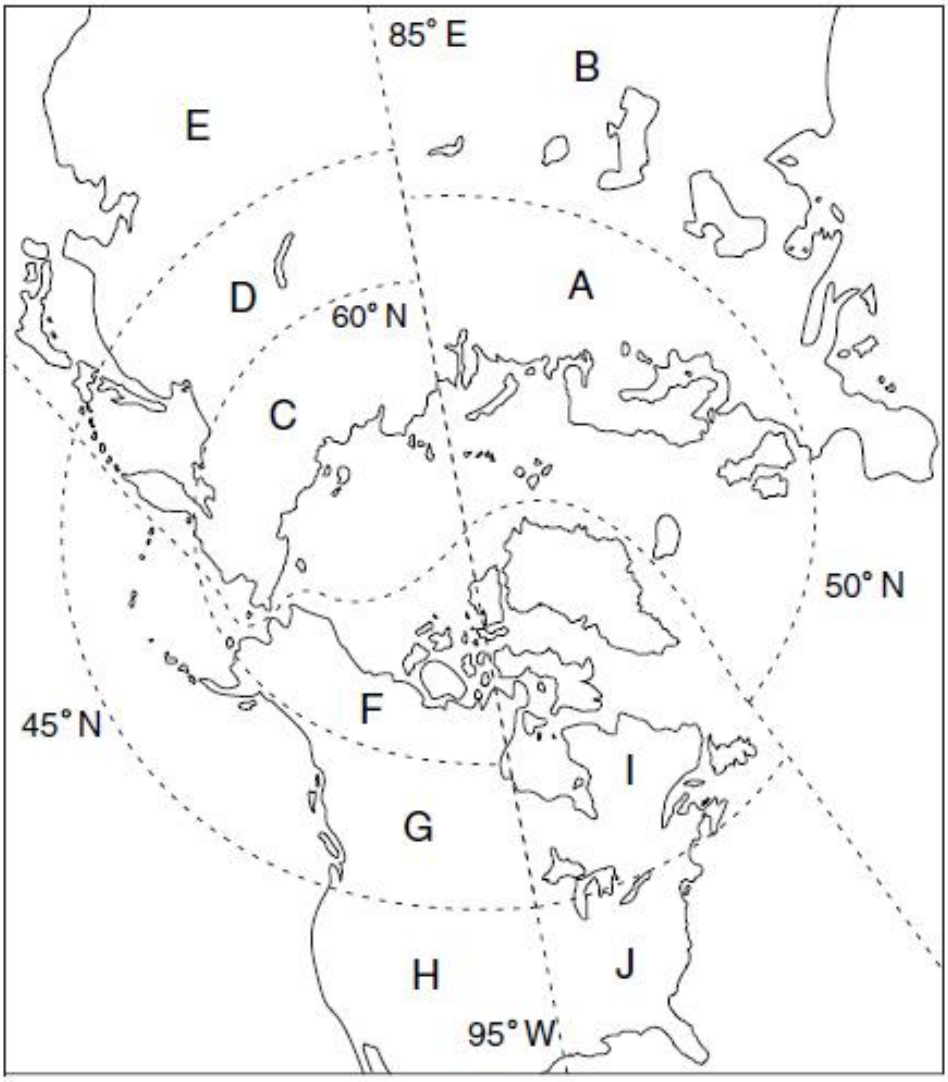

Figure 1.2. A schematic representation of the historical Holarctic areas inhabited by grouse taken from Drovetski (2003). A, north-western Palearctic, B, south-western, Palearctic, C, north-eastern Palearctic, D, central-eastern Palearctic, E, south-eastern Palearctic, F, north-western Nearctic, G, central-western Nearctic, H, south-western Nearctic, I, north-eastern Nearctic, J, south-eastern Nearctic.

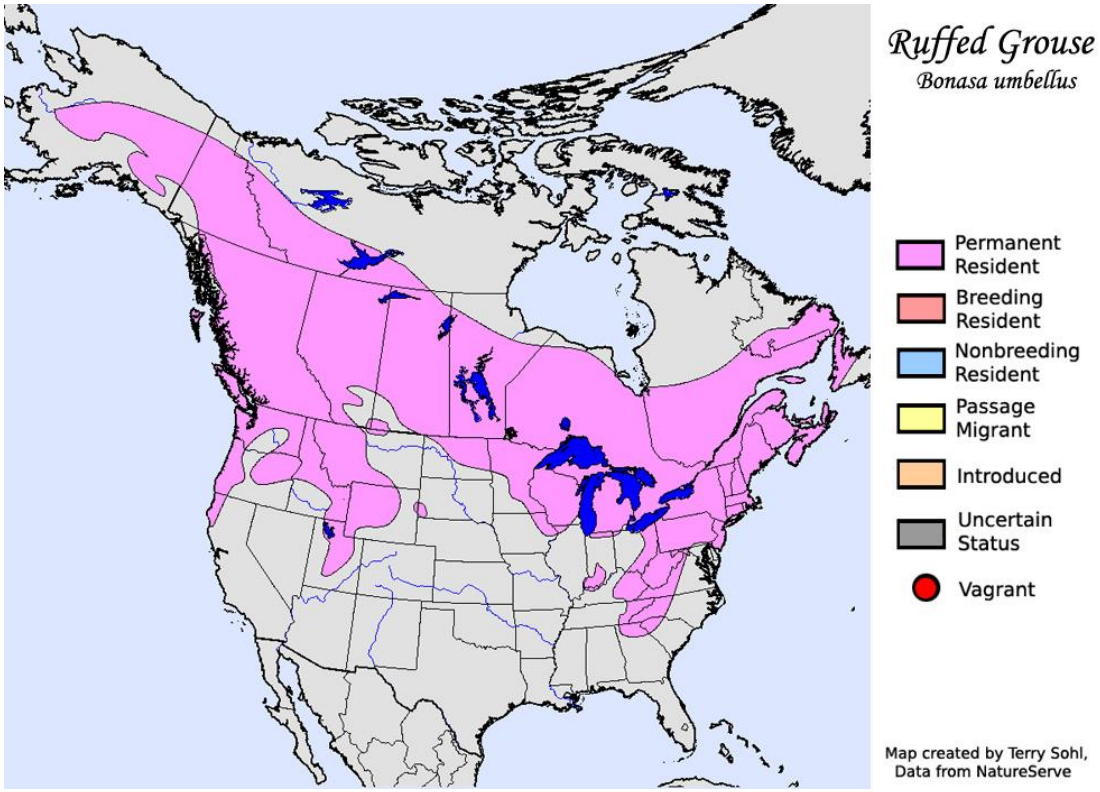

Figure 1.3. Ruffed Grouse distribution map created with Arc GIS based on species data obtained from Ridgely et al. 2003. Map was taken from: http://sdakotabirds.com/species/spruce_grouse_info.htm

Figure 1.4. Photographs of a Ruffed Grouse (*Bonasa umbellus*) drumming on a drumming log (photos courtesy of Dr. Andrew Iwaniuk). Pannel A): Male Ruffed Grouse standing atop drumming log; B-D) wing movements characteristic of the drumming display.

Figure 1.5. Photograph of a spread wing preparation (*Tympanuchus cupido* [UWBM: 76798]) at the Burke Museum of Natural History (Seattle, WA).

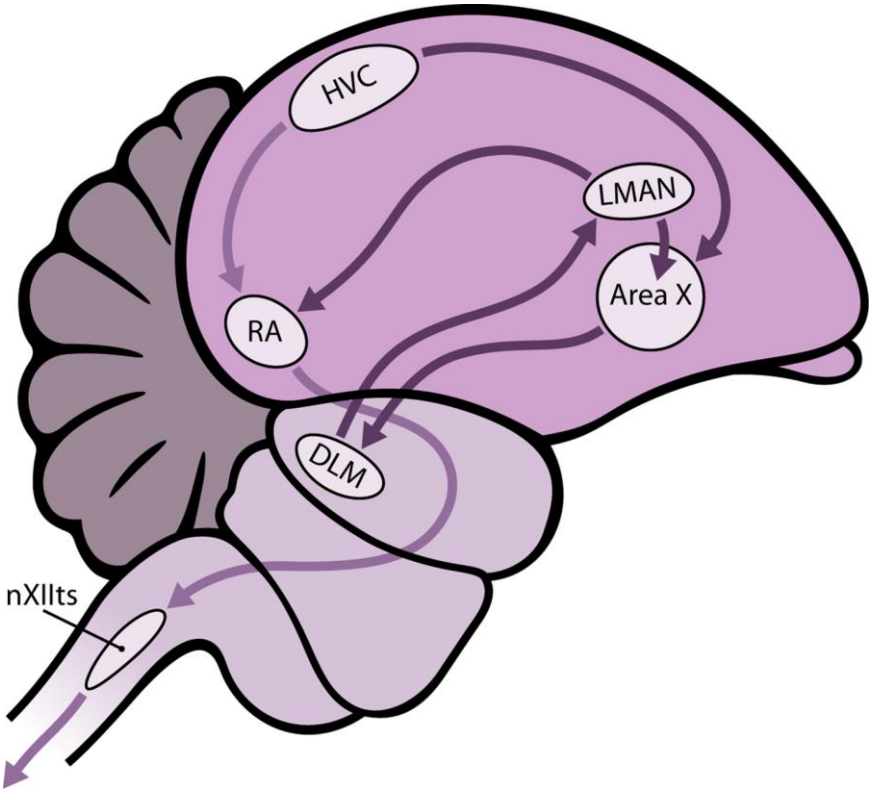

Figure 1.6. The song system of a typical song bird, taken from Nottebohm (2005). Nucleus HVC feeds information into two pathways that ultimately lead to the neurons in the tracheosyringeal half of the hypoglossal nucleus (nXIIts) that project to vocal muscles. HVC projects to nucleus RA directly (PDP), and indirectly via Area X, the dorsolateral anterior thalamic nucleus (DLM), and LMAN (AFP) in a manner that shares similarities with the mammalian pathway cortex→basal ganglia→thalamus→cortex.

Figure 2.1. Depictions of the spread wing specimens used in this study. Spread wing specimens representative of species from each genus examined in this study with associated scale (5 cm): (**A**) *Bonasa umbellus* [UWBM: 51048], (**B**) *Lagopus lagopus* [UWBM: 58891], (**C**) *Dendragapus obscurus* [UWBM: 63812], (**D**) *Tetrao tetrix* [UWBM: 57213], (**E**) *Falcipennis canadensis* [UWBM: 53855], (**F**) , *Centrocercus urophasianus* [UWBM: 84466], (**G**) *Tympanuchus phasianellus* [UWBM: 63835].

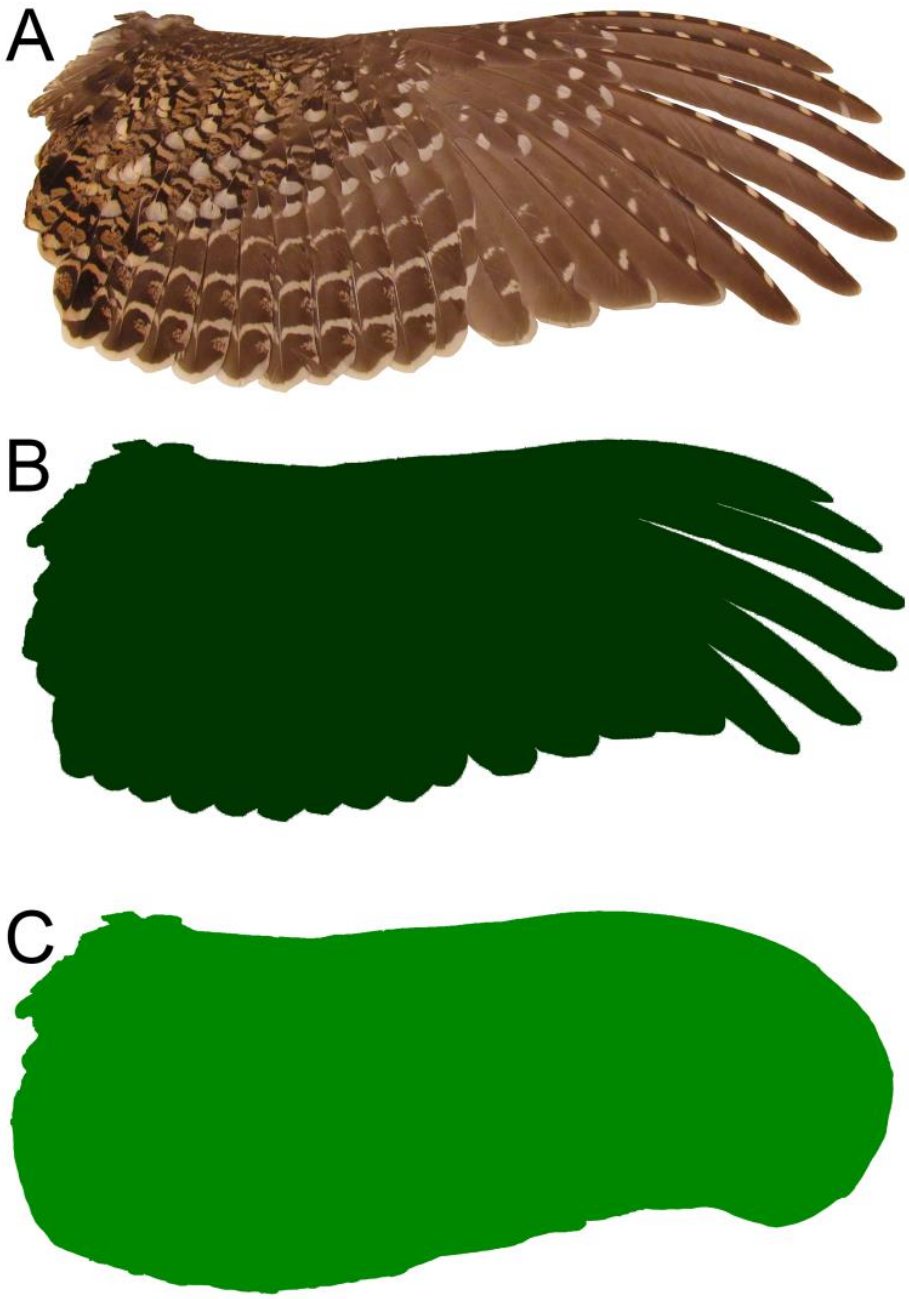

Figure 2.2. Graphic representation of the calculation of wing porosity. Images of a female Sharp-tailed Grouse (*Tympanuchus phasianellus*) spread wing delineating the process of defining porosity (P): (**A**) a photograph of the dorsal surface of the wing is taken, (**B**) surface area (SA) is derived from **A**; (**C**) potential surface area (PSA) rendered from **B**.

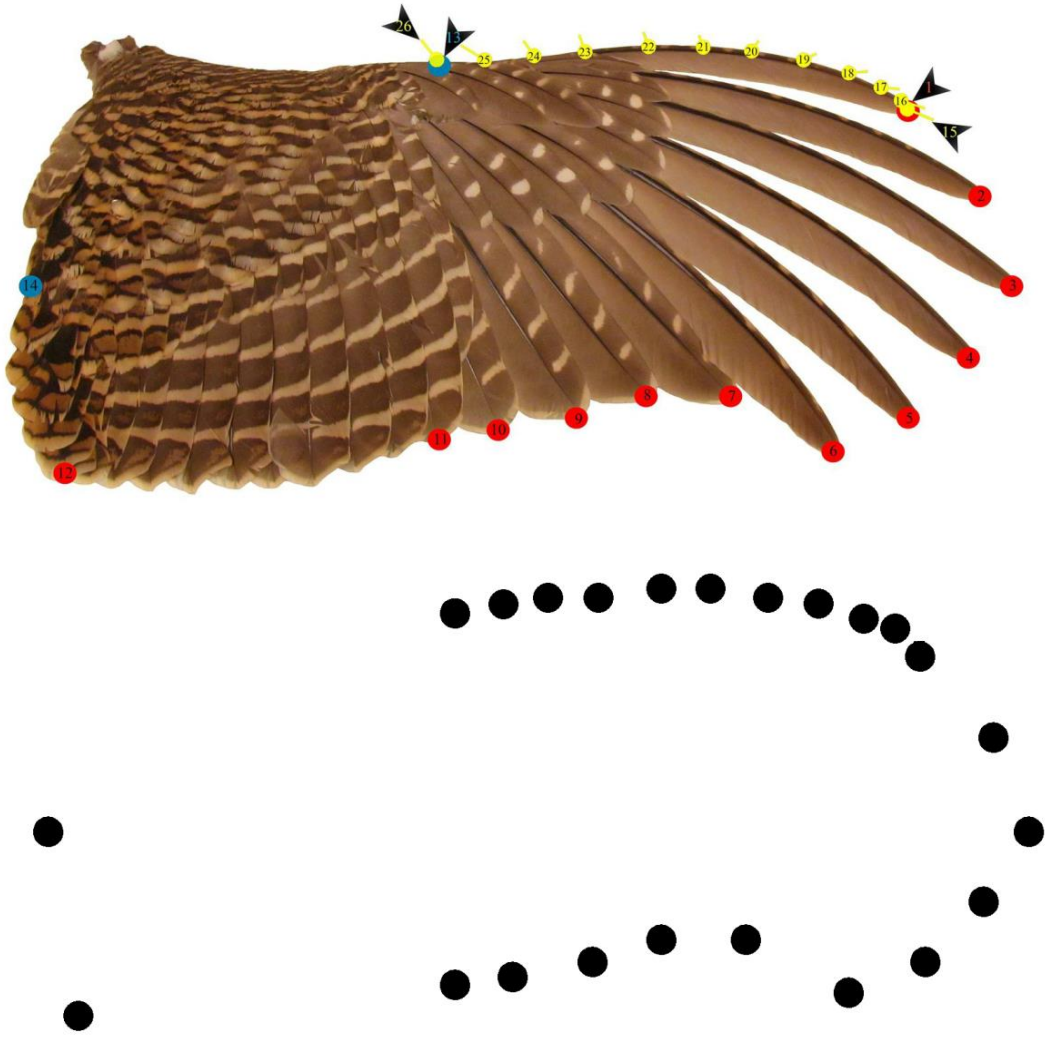

Figure 2.3. Schematics of the landmark configuration used in this study. The circles depict the 26 landmarks used in our geometric morphometrics analyses, superimposed on the right wing of a male Sharp-tailed Grouse (*Tympanuchus phasianellus*, [UWBM: 63835]. (1) distal tip of P10, (2) distal tip of P9, (3) distal tip of P8, (4) distal tip of P7, (5) distal tip of P6, (6) distal tip of P5, (7) distal tip of P4, (8) distal tip of P3, (9) distal tip of P2, (10) distal tip of P1, (11) distal tip of S1, (12) distal tip of S^{Ω}, (13) landmark paired with S1(LM 11) along the leading edge, (14) landmark paired with P8 (LM 3) along the wing pit. Landmarks (15:26) consists of equidistant points along a curve standardized across all specimens (red dots, 'Type 1' LM, yellow dots, 'Type 2' LM; blue dots, 'Type 3' LM). Landmarks and curves were digitized in TPSdig2 before analysis in MorphoJ.

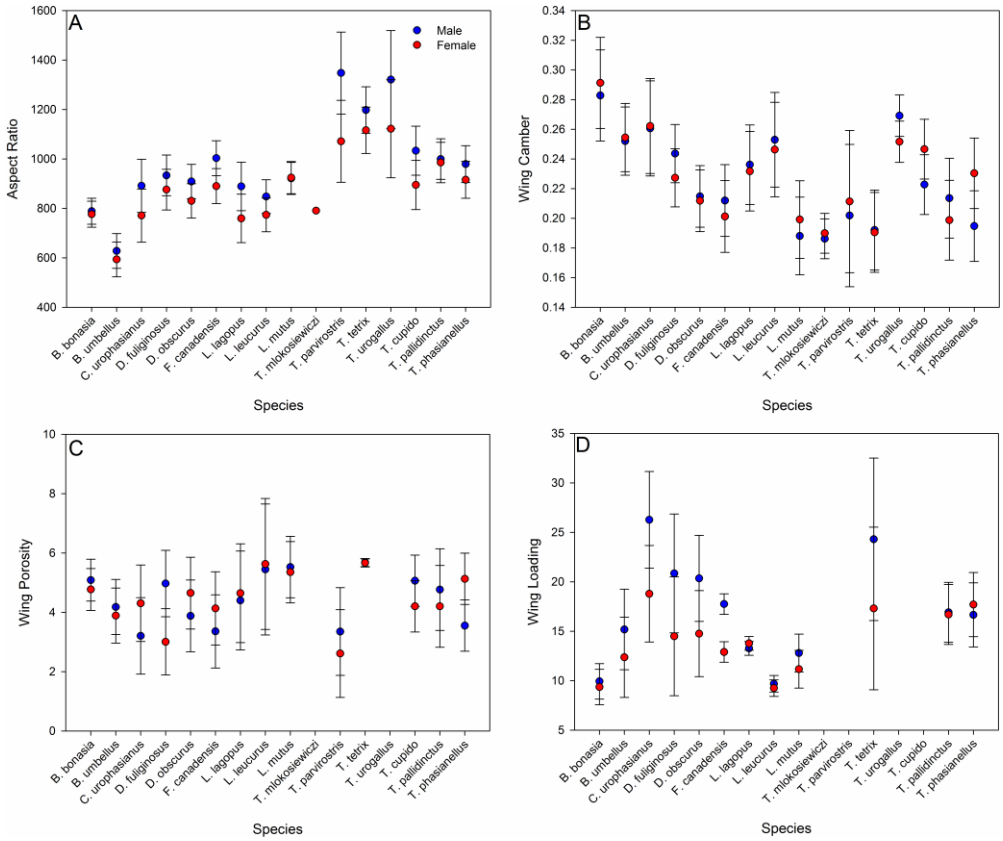

Figure 2.4. Plots of traditional morphometric measurements taken from grouse wings. Values are species means plotted by sex with error bars showing +/- standard deviation calculated from species means: (**A**) aspect ratio of the wing (AR); (**B**) maximum wing camber (C); (**C**) wing porosity (P); (**D**) wing loading (WL), refer to table 2.1 for sample sizes.

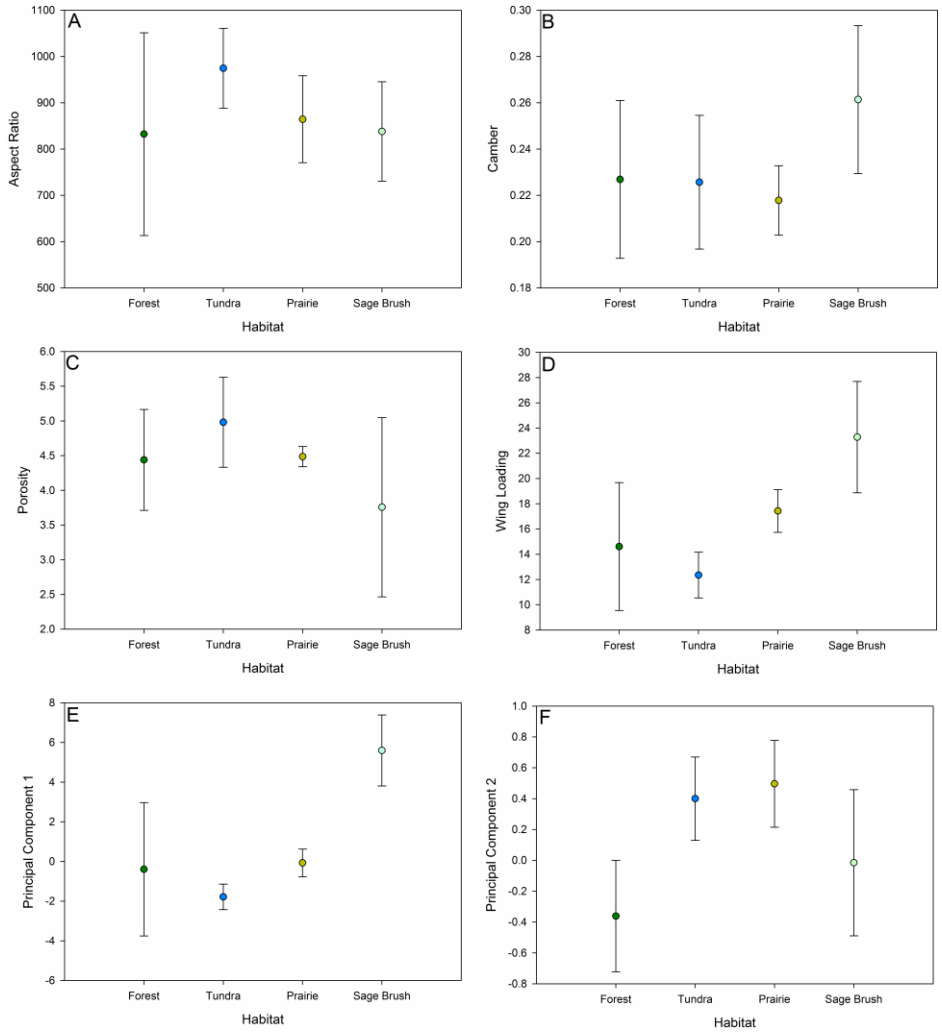

Figure 2.5. Traditional morphometric analyses of wing shape by habitat preference. Plots of traditional morphometric measurements taken from grouse wings plotted by habitat preference. Values are means calculated from habitat groupings with error bars showing +/- standard deviation calculated from habitat means: (**A**) aspect ratio of the wing (AR); (**B**) maximum wing camber (C); (**C**) wing porosity (P); (**D**) wing loading (WL); (**E**) Principal Component 1 of primary feather length; (**F**) Principal Component 2 of primary feather length.

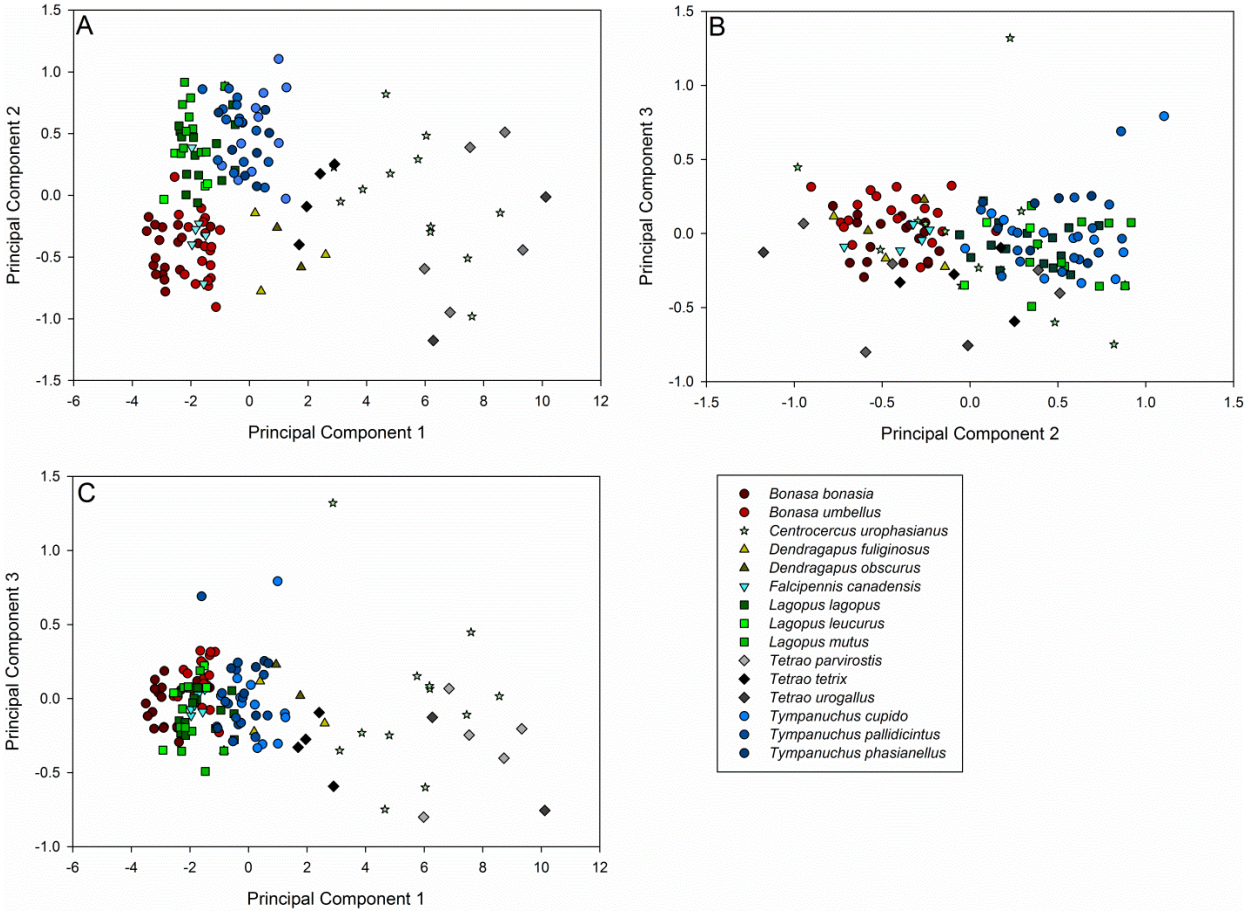

Figure 2.6. Principal component analysis of primary feather length among grouse species. Scatter plots of principal components (PCs) 1-3 resulting from a principal component analysis (PCA) on length measurements of primary feathers (*f*prime$_{1\text{-}10}$). (**A**) PC1 (93.04%) plotted against PC2; (**B**) PC2 (2.84%) plotted against PC3; (**C**) PC1 plotted against PC3 (1.47%).

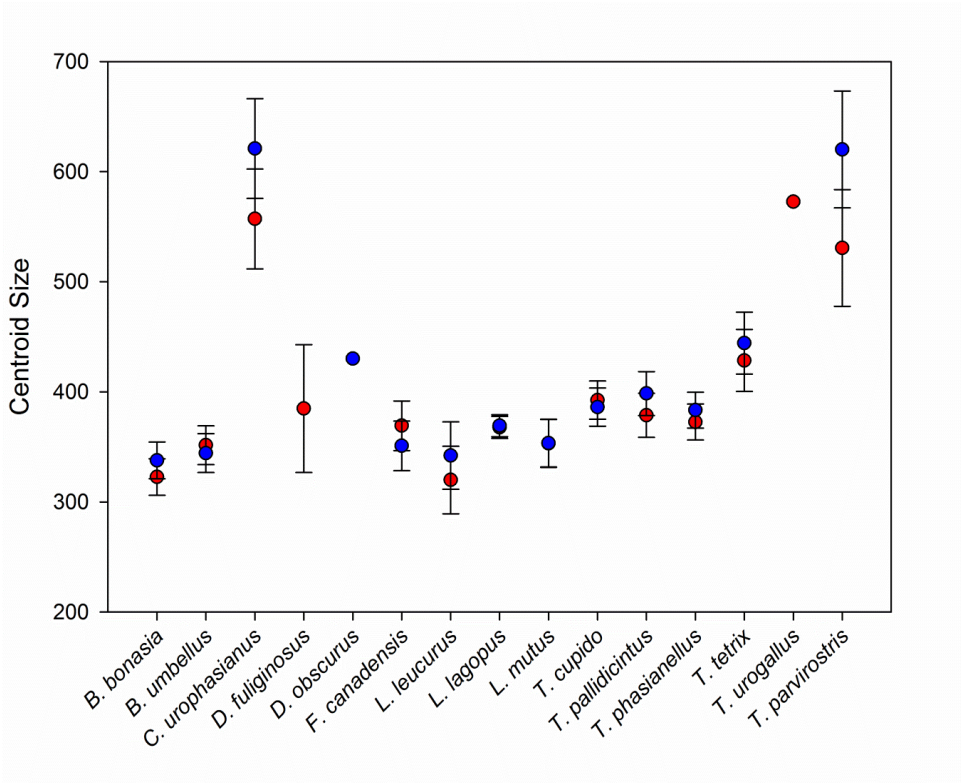

Figure 2.7. Geometric morphometric analysis of centroid size. Plot of centroid size (CS) as calculated from our landmark configuration. Values are species means plotted by sex with error bars showing +/- standard deviation calculated from species means.

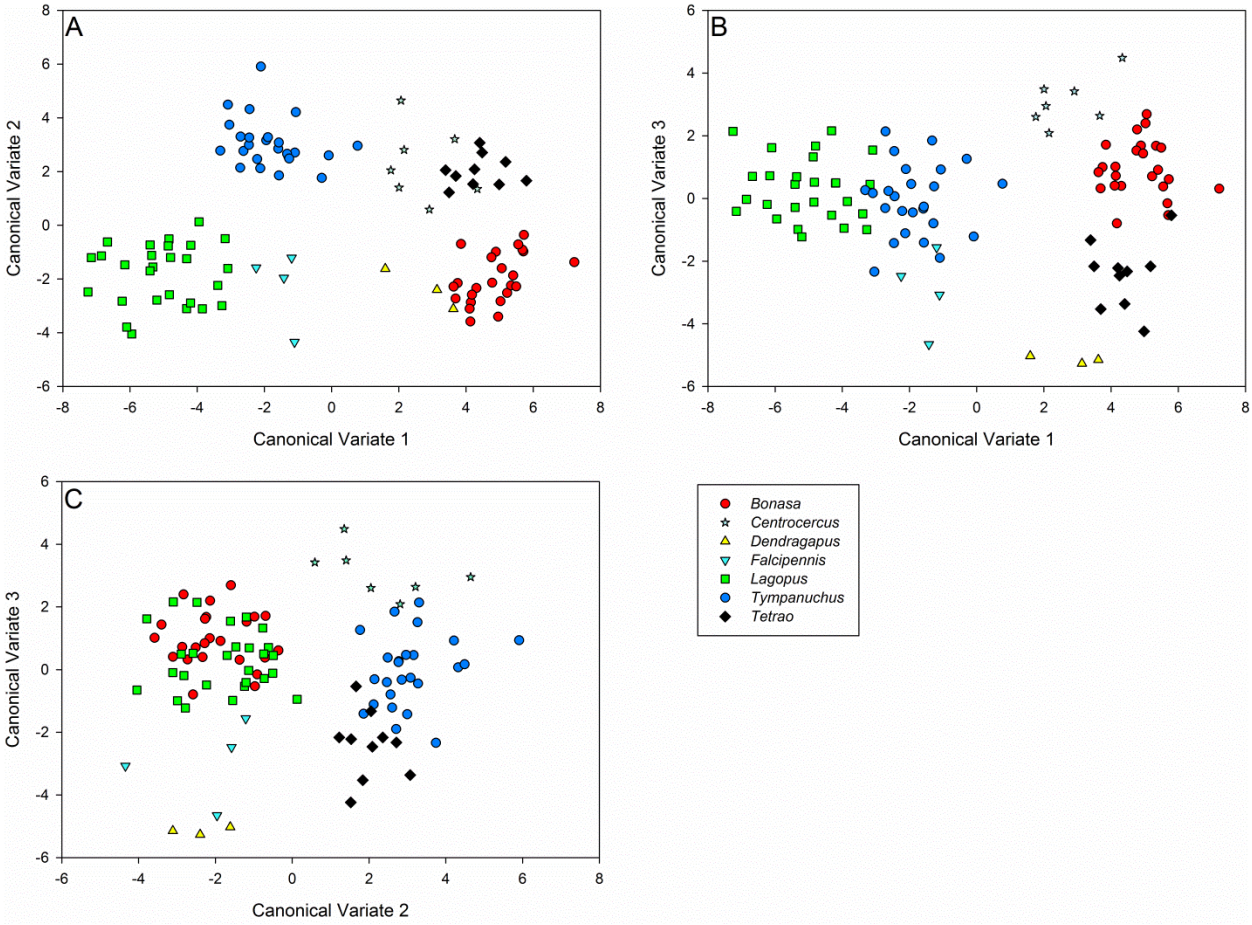

Figure 2.8. Canonical variate analysis of Procrustes coordinates among grouse genera. Scatter plots of canonical variates (CVs) resulting from a canonical variate analysis (CVA) of Procrustes coordinates. (**A**) CV1 plotted against CV2; (**B**) CV1 plotted against CV3; (**C**) CV2 plotted against CV3. Note that only genera are shown here for clarity, but comparisons were made across species.

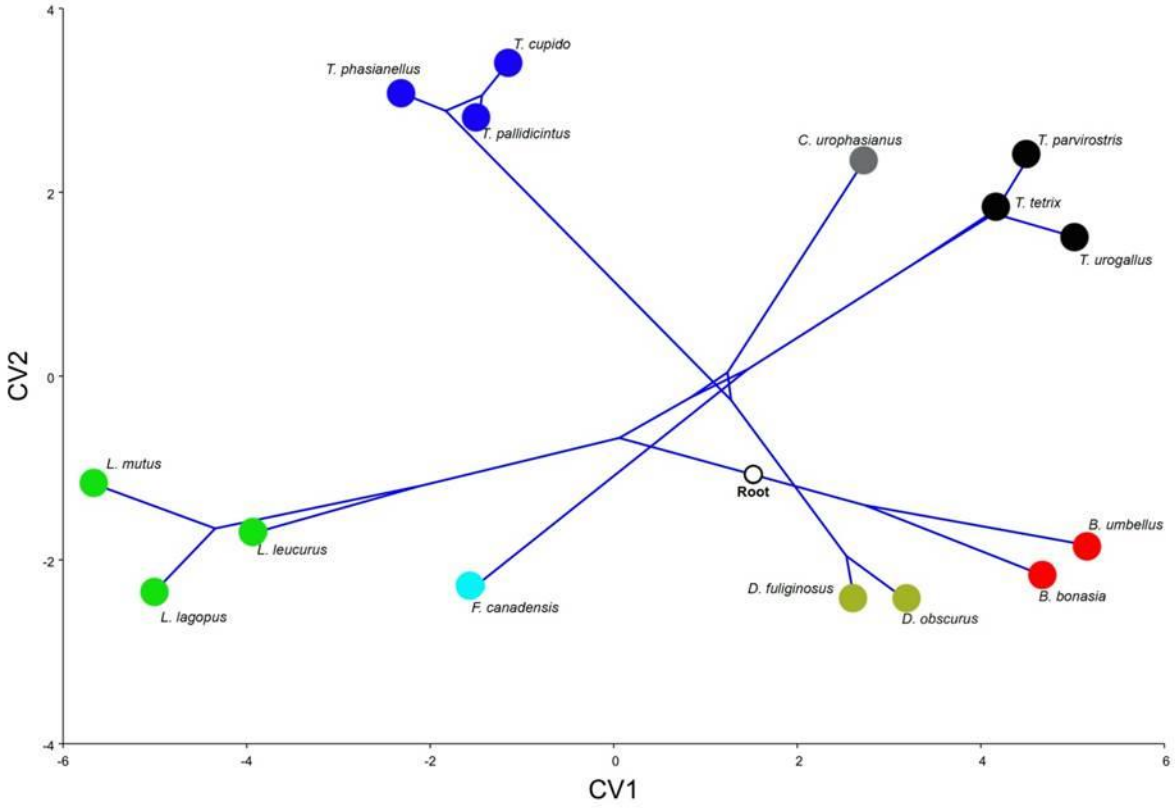

Figure 2.9. Reconstruction of phylogenetic variation in wing shape. A plot of species means of the first two canonical variates (CV1-2) from a canonical variate analysis (CVA) of Procrustes distances superimposed over top of Drovetski's (2002) grouse phylogeny. The positions of internal nodes were reconstructed with squared-change parsimony and branch tips correspond to species means but have been exaggerated and colour coded for visibility. This reconstruction depicts the best estimate of variation among species mean CV scores, representing 76.63% of the total variance.

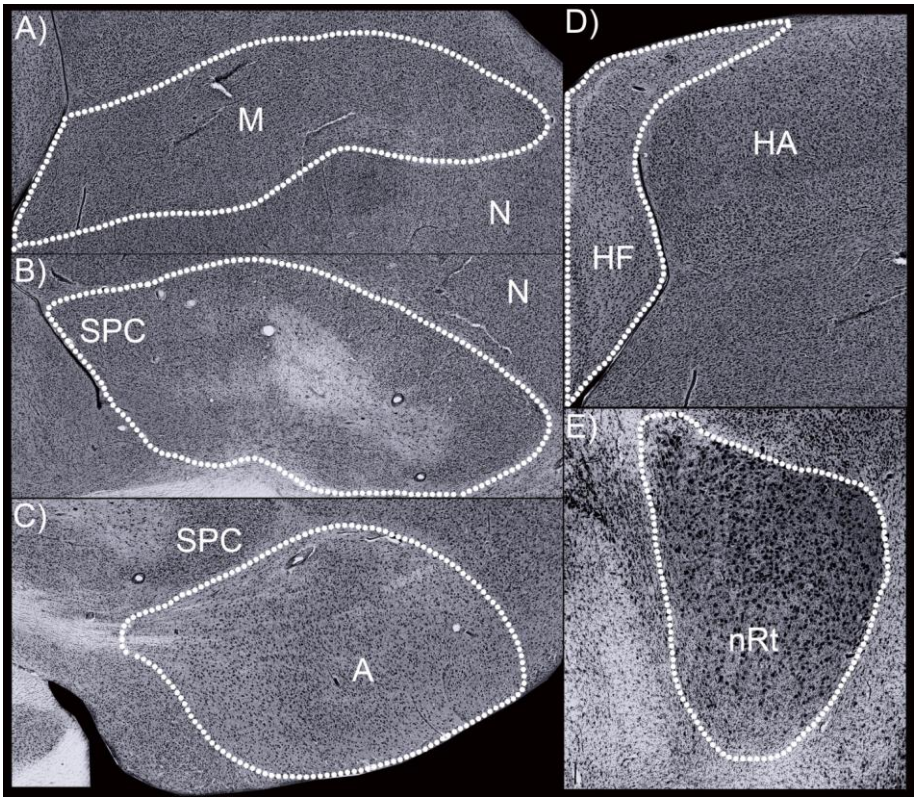

Figure 3.1. Photomicrographs of each target nucleus measured here, taken from Nissl stained sections from the brain of a male Ruffed Grouse (*Bonasa umbellus*). A) Mesopallium (M), nidopallium (N); B) Striatopallidal Complex (SPC); C) Arcopallium (A); D) Hippocampal Formation (HF), hyperpallium (HA); E) Nucleus Rotundus (nRt).

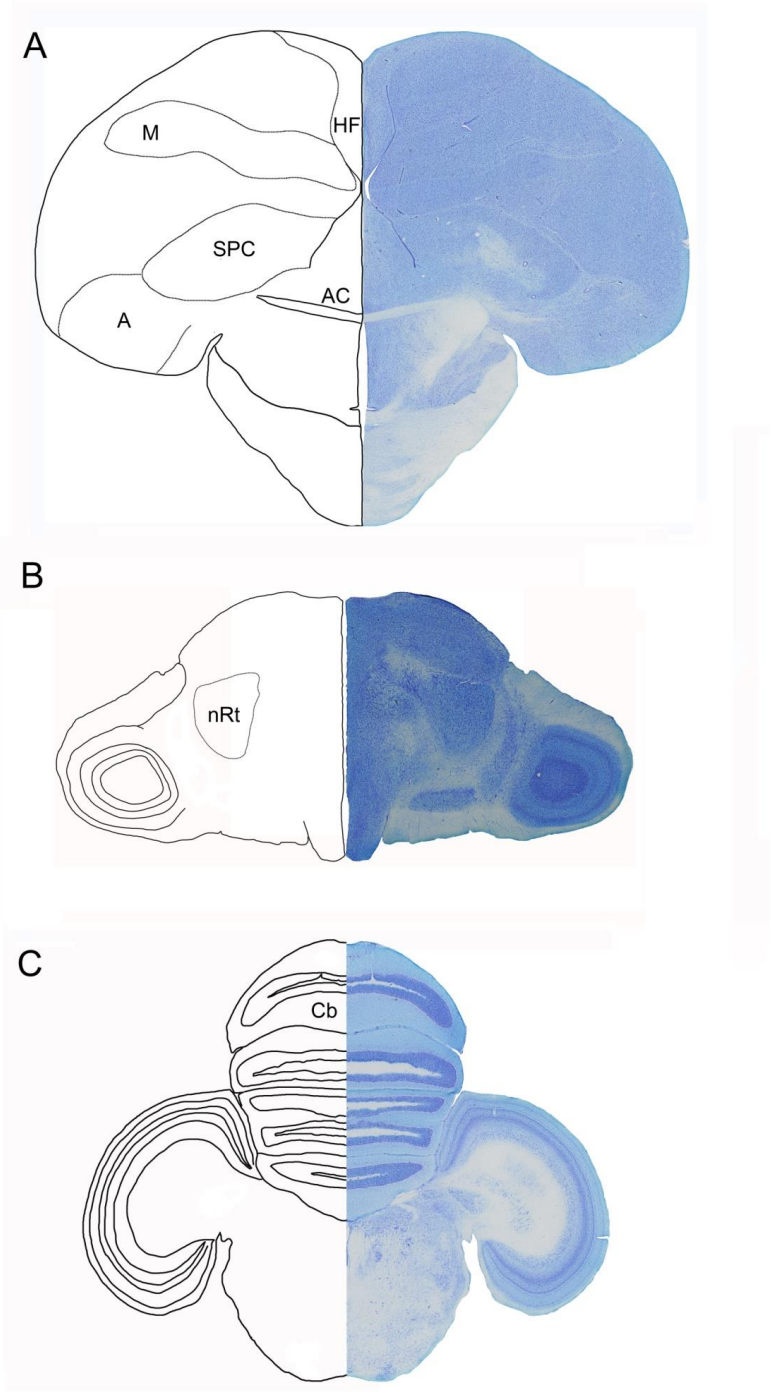

Figure 3.2. Schematic rostrocaudal representations of Nissl stained sections taken from the brain of a male Ruffed Grouse (*Bonasa umbellus*). A) Telencephalic regions: arcopallium (A), anterior commisure (AC), hippocampal formation (HF), mesopallium (M), striatopallidal complex (SPC); B) Brain stem: nucleus rotundus (nRt); C) Cerebellum (Cb).

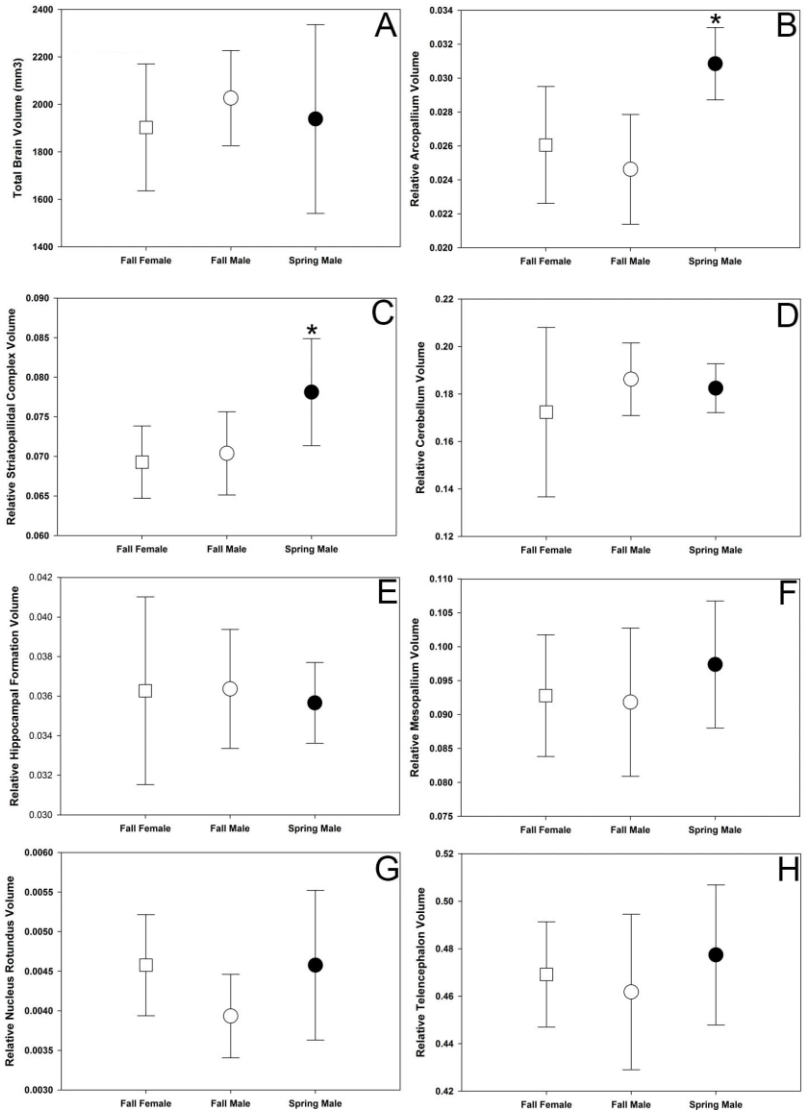

Figure 3.3. Plots of the volume of each region relative to whole brain size. A) total brain volume, B) relative arcopallium volume, C) relative striatopallidal complex volume, D) relative cerebellum volume, E) relative hippocampal volume, F) relative mesopallium volume, G) relative nuclues rotundus volume, H) relative telencephalon volume. Both the arcopallium ($p = 0.005$) and striatopallidal complex ($p = 0.02$) were significantly larger in spring males than fall males or females.

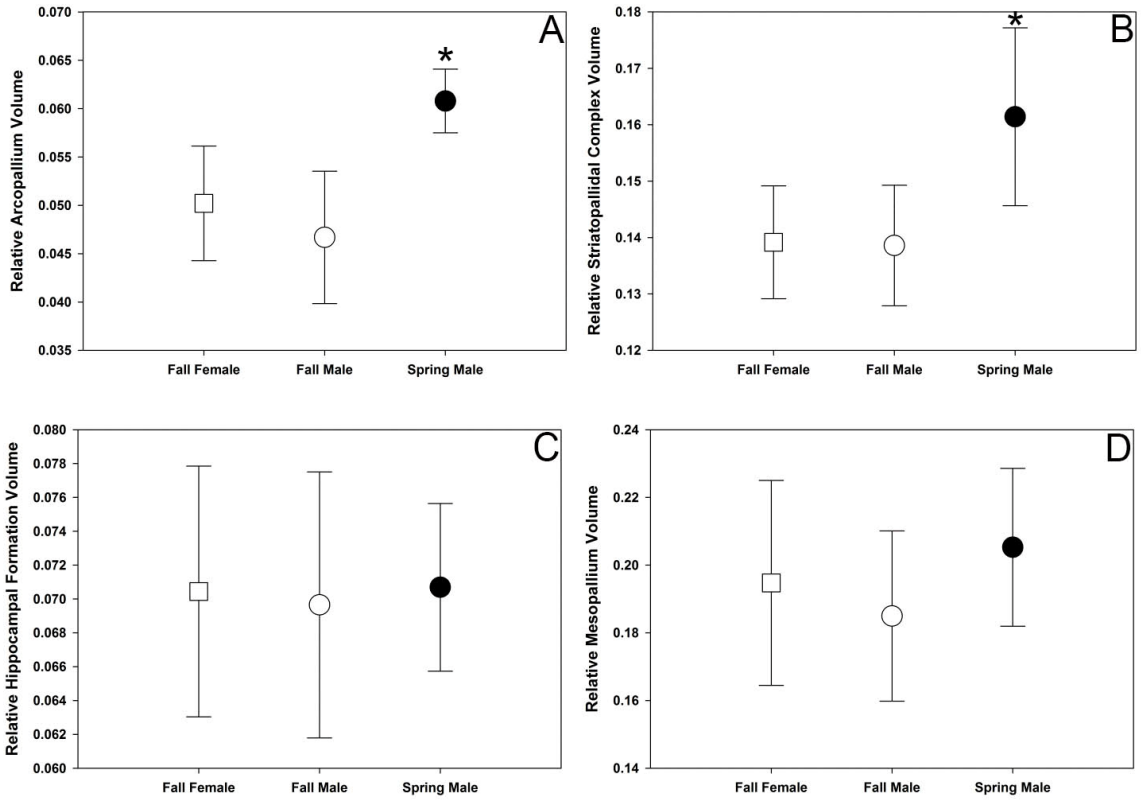

Figure 3.4. Plots of the volume of each region relative to telencephalon size. A) relative arcopallium volume, B) relative striatopallidal complex volume, C) relative hippocampal volume, D) relative mesopallium. Both the arcopallium ($p = 0.001$) and striatopallidal complex ($p = 0.006$) were significantly larger in spring males than fall males or females.

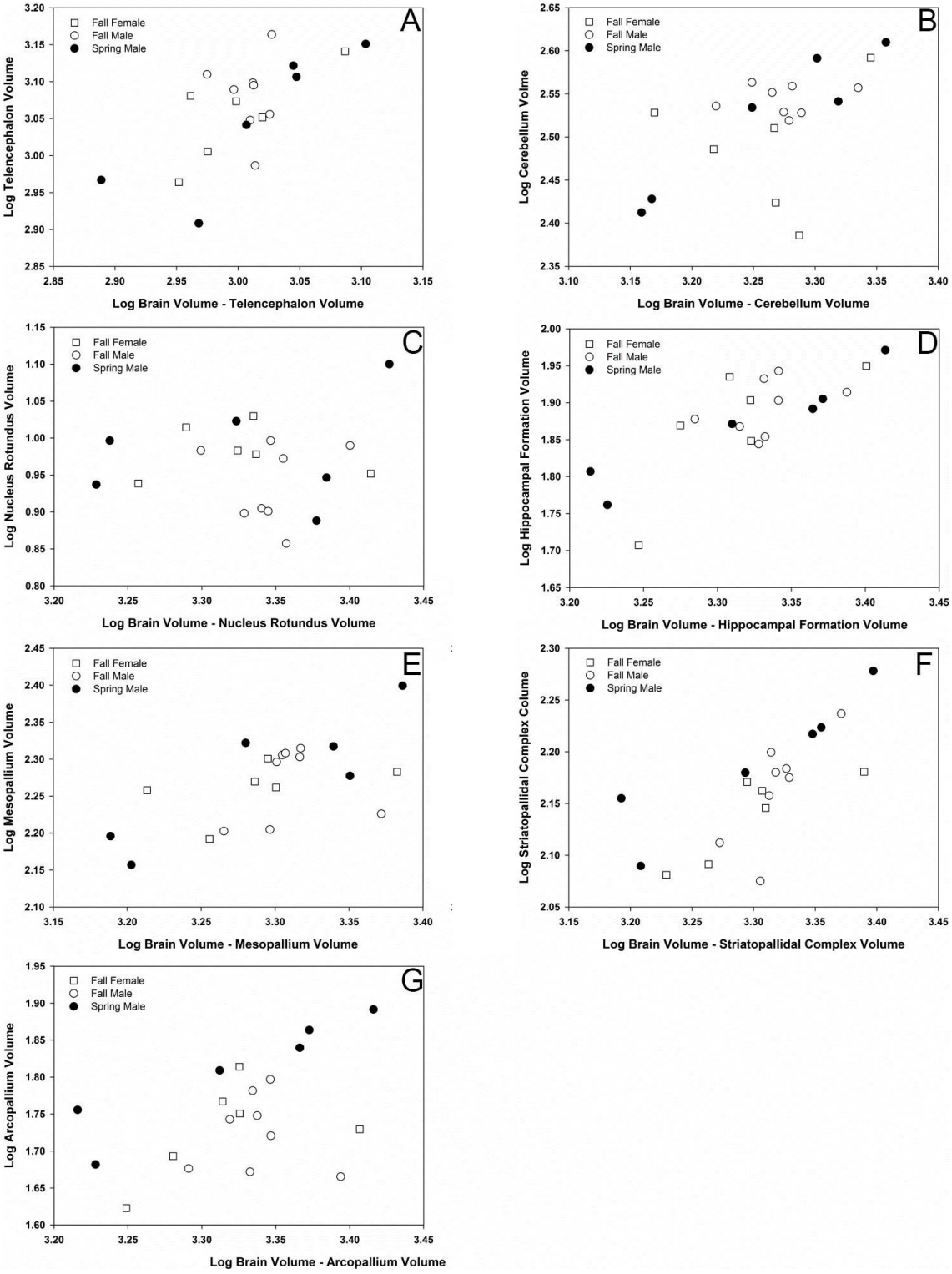
A
Fall Female
Fall Male
Spring Male
Log Telencephalon Volume
Log Brain Volume - Telencephalon Volume
B
Log Cerebellum Volme
Log Brain Volume - Cerebellum Volume
C
Log Nucleus Rotundus Volume
Log Brain Volume - Nucleus Rotundus Volume
D
Log Hippocampal Formation Volume
Log Brain Volume - Hippocampal Formation Volume
E
Log Mesopallium Volume
Log Brain Volume - Mesopallium Volume
F
Log Striatopallidal Complex Colume
Log Brain Volume - Striatopallidal Complex Volume
G
Log Arcopallium Volume
Log Brain Volume - Arcopallium Volume

Figure 3.5. Scatter plots of the scaling relationship between whole brain size and the volume of each region measured here. A) telencephalon, B) cerebellum, C) nucleus rotundus, D) hippocampal formation, E) mesopallium, F) striatopallidal comples, G) arcopallium.

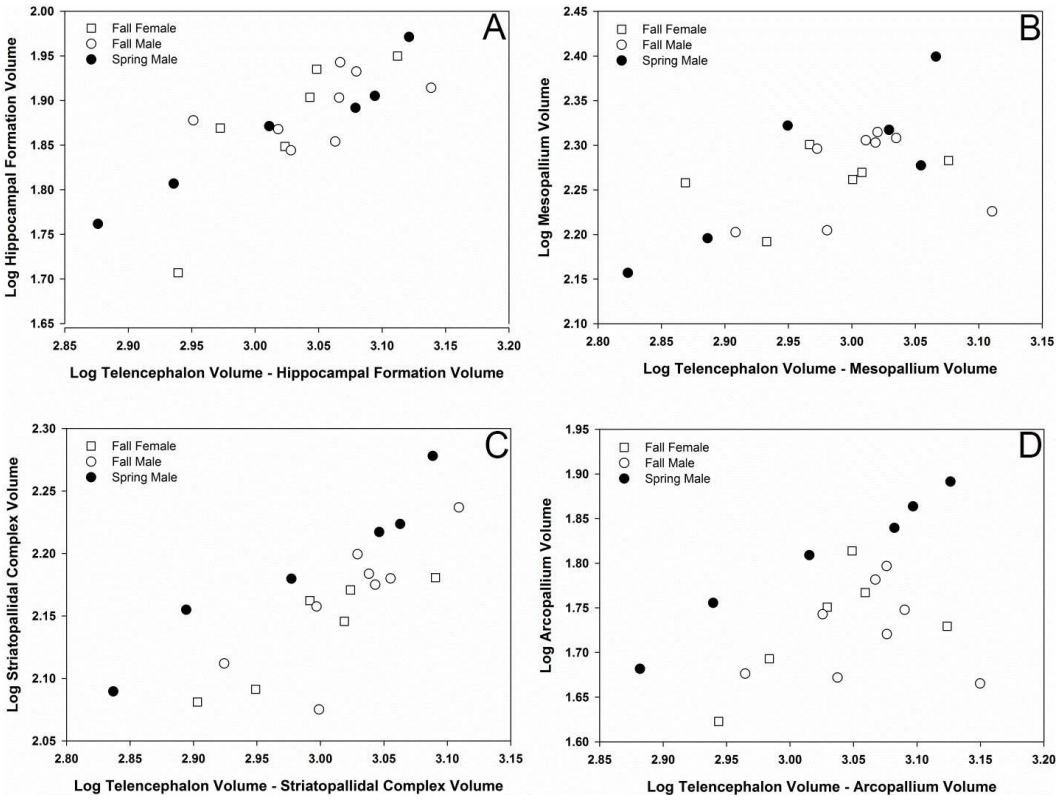

Figure 3.6. Scatter plots of the scaling relationship between telencephalon size and the volume of each region measured here. A) hippocampal formation, B) mesopallium, C) striatopallidal complex, D) arcopallium.