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The influence of sperm competition and life-history traits on brain size evolution in male and female birds

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THE INFLUENCE OF SPERM COMPETITION AND LIFE-HISTORY TRAITS ON BRAIN SIZE EVOLUTION IN MALE AND FEMALE BIRDS

by

Melissa Leila Abdellah

A Thesis
Submitted to the Faculty of Graduate Studies through Biological Sciences in Partial Fulfillment of the Requirements for the Degree of Master of Science at the University of Windsor

Windsor, Ontario, Canada
2010

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I hereby declare that this thesis incorporates material that is the result of joint research, as follows: in all cases, the key ideas and data collection, analysis, and interpretations were primarily performed by the author. Chapters 2 and 3 were co-authored with my advisor, Dr. Stéphanie Doucet, who supported my research financially, provided feedback on ideas, assisted with statistical interpretation, and imparted editorial suggestions during the writing process of both manuscripts. Chapter 2 is being prepared for resubmission to *Proceedings of the Royal Society of London B*, and Chapter 3 is being prepared for submission to the *Journal of Evolutionary Biology*.

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ABSTRACT

Interspecific variation in brain size is described as being an adaptive consequence of development and behaviour. Despite growing interest in sex-specific influences on the development of conspicuous traits, little research has focused on investigating sex-specific determinants of brain size. The overall goal of my thesis was to investigate the sex-specific influences on brain size evolution in birds. By measuring endocranial volume from museum specimens, I collected a large sex-specific dataset spanning 106 avian species. In Chapter 2, I found that male brain size was negatively associated with testis size, suggesting that sperm competition may be critical in determining brain size in males. In Chapter 3, I determined that brain size in females is mostly influenced by pair-bond strength and the presence of a male partner, which both ultimately increase reproductive success. Overall, different selective factors appear to influence brain size evolution in male and female birds.
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# Table of Contents

DECLARATION OF CO-AUTHORSHIP ............................................. iii
ABSTRACT .................................................................................. v
ACKNOWLEDGEMENTS .............................................................. vi
LIST OF TABLES .......................................................................... x
LIST OF FIGURES ......................................................................... xi
LIST OF APPENDICES ................................................................... xii

CHAPTER 1 .......................................................... 1
   MODELS OF BRAIN EVOLUTION AND ORGANIZATION ................. 2
   EVOLUTIONARY BRAIN GROWTH ............................................. 5
   FACTORS INFLUENCING THE EVOLUTION OF LARGER BRAINS .... 6
       Natural selection .................................................................. 7
       Sexual selection .................................................................. 13
   METABOLIC COSTS OF BRAIN DEVELOPMENT ....................... 17
   SEX-SPECIFIC INFLUENCES ON BRAIN SIZE EVOLUTION ........ 19
   MEASURING BRAIN SIZE ....................................................... 20
   THESIS OBJECTIVES ............................................................. 21
   REFERENCES ........................................................................... 22

CHAPTER 2 .............................................................................. 30
   SUMMARY ............................................................................ 31
   INTRODUCTION ...................................................................... 31
   MATERIAL AND METHODS .................................................. 35
       Endocranial volume ......................................................... 35
       Testis mass ...................................................................... 36
       Mating system and migratory behaviour ......................... 37
       Comparative methods ................................................... 37
   RESULTS .............................................................................. 39
   DISCUSSION ......................................................................... 40
   CONCLUSIONS ...................................................................... 45
   ACKNOWLEDGEMENTS ....................................................... 45
   REFERENCES ........................................................................... 46

CHAPTER 3 .............................................................................. 55
   SUMMARY ............................................................................ 56
   INTRODUCTION ...................................................................... 57
   METHODS ............................................................................. 63
       Endocranial volume ......................................................... 63
       Life-history traits ............................................................ 64
       Comparative methods ................................................... 65
   RESULTS .............................................................................. 67
   DISCUSSION ......................................................................... 69
LIST OF TABLES

Table 2.1. Relationship between total brain mass, body mass, and testis mass in males and females across all birds, passerines, and non-passerines....................................................... 52

Table 2.2. Relationship between total brain mass, body mass, and mating system in males and females across all birds.................................................................53

Table 2.3. Relationship between total brain mass, body mass, and testis mass in males and female across long-distance migrants and all-other species........................................54

Table 3.1. Relationship between relative brain mass and life-history traits in males and females.................................................................81
LIST OF FIGURES

Figure 3.1. Comparisons of relative brain size in females and males according to pair-bond strength................................................................. 82
Figure C.1. Composite phylogeny.................................................................. 110
LIST OF APPENDICES

Appendix A. Measuring brain size................................................................. 94
Appendix B. Measuring testis size.............................................................. 100
Appendix C. Composite phylogeny............................................................. 104
CHAPTER 1

GENERAL INTRODUCTION
Why does brain size vary among species? The evolution of a larger relative brain size has long been studied in primates, emphasizing their unique intellectual capabilities in comparison to other non-primate animals (Dunbar 1998). Primatologists have often focused on the transition from an ecologically-centred environment to one involving complex social interactions when explaining selective pressures favouring highly flexible behaviours (Humphrey 1976; Bailey 1986; Delius et al. 2001). The ability to adapt and modify behaviours according to changes in the environment has now emerged as a hallmark of larger brains in not only primates but in a number of taxa sharing similar biological, ecological, and behavioural characteristics (Hodos 1986; Delius et al. 2001; Sol et al. 2005b). As a result, researchers have studied the impact of these influences on brain size in detail. Among a wide variety of taxa, larger relative brain size has been associated with altricial development (Bennett & Harvey 1985a; Iwaniuk & Nelson 2003), behavioural flexibility (Lefebvre et al. 1997; Lefebvre et al. 2004), habitat complexity (Kotschall et al. 1998; Pollen et al. 2007), migratory behaviour (Sol et al. 2005b, 2010), and social living (Dunbar 1998; Byrne & Bates 2007).

Models of brain evolution and organization

Models of brain evolution attempt to explain how and why species undergo developmental and structural changes leading to larger brains. Moreover, these models describe not only overall changes in brain size, but the structural organization and composition of the brain and how particular areas develop in relation to one another. Currently, there are two broad models proposed to explain brain size organization in vertebrates: concerted and mosaic evolution. Both of these models are centred on the idea
that the brain is composed of many areas interacting with one another to form functional units (Healy & Rowe 2007).

*Concerted evolution* proposes that selection for the enlargement of one brain region will be constrained by selection for simultaneous increases in the whole brain (Jerison 1973; Finlay & Darlington 1995; de Winter & Oxnard 2001). As such, the whole brain gets larger when one part increases because all other parts of the brain simultaneously become larger. This model is driven by the principle of developmental constraint, which postulates that functionally unrelated areas of the brain will change and evolve in coordination as a consequence of the way in which the brain develops (Finlay & Darlington 1995; Finlay et al. 2001). The coordination of these changes in brain parts can therefore vary among species depending on the timing and length of development (Finlay & Darlington 1995; Iwaniuk et al. 2004). Consequently, it has been suggested that larger brains are merely an enlarged version of smaller brains, where each brain component retains the same proportion of the whole brain (Iwaniuk et al. 2004). Furthermore, the developmental constraint hypothesis predicts that a single component of a functional unit cannot develop without enlarging the entire brain (Finlay & Darlington 1995). For example, in mammals, the portion of the cortex associated with forelimb control can only increase through concurrent increases in total cortex size (Nudo & Masterton 1990; Finlay & Darlington 1995).

*Mosaic evolution*, on the other hand, emphasizes the evolutionary link between brain components that form the same functional system and how these components demonstrate correlated evolution. This model proposes that only the structures within a functional unit will evolve and change together because they are interconnected, while
other components will evolve independently (Barton & Harvey 2000; Barton 2001; de Winter & Oxnard 2001). However, the independent expansion of specific brain components will still lead to overall increases in brain size as other structures of the brain are not reduced to compensate for this increase (Iwaniuk et al. 2004). The mosaic model of brain evolution has received empirical support in a number of taxa, including mammals (Barton & Harvey 2000) and birds (Iwaniuk et al. 2004). Moreover, these systems demonstrate order-specific brain compositions, where species occupying similar ecological and behavioural niches evolve comparable brain proportions (de Winter & Oxnard 2001; Iwaniuk & Hurd 2005). These unique clusters are termed ‘cerebrotypes’ and have been documented in mammals (Clark et al. 2001; de Winter & Oxnard 2001), amphibians (Doré et al. 2002), fish (Huber et al. 1997; Wagner 2001a, b), and birds (Iwaniuk & Hurd 2005).

Although concerted and mosaic evolution are two competing theories, they do not appear to be mutually exclusive (de Winter & Oxnard 2001; Iwaniuk et al. 2004). Although evidence of the mosaic model would suggest that developmental constraints are not influencing brain size evolution, the observation of some coordinate changes make this model difficult to eliminate as a possible driving force in brain composition (Iwaniuk et al. 2004). In fact, de Winter and Oxnard (2001) propose that organizational variation between cerebrotypes is due to mosaic evolution through selective adaptation to behavioural or ecological niches, while variation between orders is a result of an interaction between both selective adaptation (i.e. mosaic evolution) and developmental constraints (i.e. concerted evolution).
Evolutionary brain growth

Brain growth is often described as following two evolutionary forms: passive and active growth. Each of these can lead to the development of a larger brain, making them difficult to distinguish (Aboitiz 1996). Passive growth highlights body size as the overall somatic determinant of brain size: larger animals tend to have larger brains (Aboitiz 1996). This pattern is based on the principle of proper mass, first described by Jerison (1973), where a larger brain is a direct result of a larger body weight, and consequently greater information processing used to control daily bodily functions (Jerison 1973; Aboitiz 1996). Passive growth is said to occur because the genetic systems for cell growth in the body and the brain are coupled (Jerison 1973; Aboitiz 1996, 2001). Consequently, this process will produce an allometric relationship between brain size and body size (Jerison 1973). Moreover, all brain components will be affected equally: there will be no disproportional growth rate between various parts of the brain and there will be little rearrangement of neuronal connections (Aboitiz 1996).

The second modality of brain development is active growth. This pattern corresponds to ecological conditions and adaptations that relate to perceptual and behavioural skills (Aboitiz 1996). Ecological conditions are said to be the non-somatic determinants of brain size and structure, as brain size will deviate from the standard trend imposed by brain-body coupling (i.e. passive growth). Active growth tends to be limited to later periods of development, as species face various ecological demands. Compared to passive growth, active growth follows a mosaic model and tends to affect specific brain components rather than the brain as a whole (Aboitiz 1996, 2001; Barton 2001).
However, changes in overall brain size typically result from the development of particular brain components that make up a significant portion of the whole brain.

Although the concept of passive growth implies that brain enlargement is merely a secondary consequence of body growth, it has been suggested that the body may have actually increased in size secondarily to selection for increased brain size (Aboitiz 2001; Finlay et al. 2001). For example, in hominids there is a particularly strong brain-body coupling where increases in body size triggered disproportionate increases in brain size (Finlay et al. 2001). However, this coupling could have also evolved because of selection for increased brain power (i.e. larger brain size). This is a rather important example of passive and active growth overlapping with one another (Aboitiz 2001). Consequently, passive growth has also been termed adjunct growth in order to describe changes in brain size that are coupled to some other growth mechanism, regardless of whether changes in brain size were secondary (Finlay et al. 2001). In addition, Jerison (1973) proposed that increases in processing capacity (and consequently brain size) can be used to either control bodily functions or behaviours. As a result, both passive and active growth may be working simultaneously within an organism and both growths may end up being pooled together depending on the needs of the individual (Jerison 1973; Aboitiz 1996).

Factors influencing the evolution of larger brains

Brain evolution can be influenced by a number of selective pressures, including habitat (Sol et al. 2007; Sol & Price 2008), developmental patterns (Bennett & Harvey 1985a; Iwaniuk & Nelson 2003), social interactions (Dunbar 1998; Dunbar & Shultz 2007; Dunbar 2009), and reproductive strategies (Garamszegi et al. 2005; Pitnick et al. 2006; Lemaître et al. 2009). Over evolutionary time, these selective pressures have lead
to the development of larger-than-expected brains in a number of animal lineages (Hodos 1986). As both overall brain size and brain morphology have been shown to be heritable, researchers have been very interested in studying the evolutionary changes of brain size in response to selection (Cheverud et al. 1990). Although in the past brain size evolution was predominantly studied in primates, researchers have recently broadened the study to birds because they share many convergent traits with primates, including larger than expected brain-to-body ratios, long developmental periods, and involvement in complex social groups (Emery 2006).

**Natural selection**

The role of natural selection on brain evolution has been greatly emphasized in the literature and remains a critical driving force in determining whether a species develops a larger brain for their given body size (Lefebvre & Sol 2008). Moreover, natural selection is imperative for understanding interspecific variation in brain size. Some of the pioneering work in this field was conducted by Bennett and Harvey (1985a, b), and focused on the influence of behavioural and ecological factors in determining differences in the relative size of various parts of the avian and mammalian brain. Today, the study of natural selection and its role in brain size evolution has been extensively researched and studies have typically concentrated on three major areas (Lefebvre & Sol 2008): development and parental care (Iwaniuk & Nelson 2003) social interactions (Beauchamp & Fernández-Juricic 2004; Iwaniuk & Arnold 2004; Emery et al. 2007), and behavioural flexibility (Lefebvre et al. 1997; Sol et al. 2002, 2005a).
**Developmental and parental care** – Ontological differences are often highlighted as an important driving force in brain size variation across avian species. Differences in brain size are said to stem from the continuum of development from altricial to precocial (Bennett & Harvey 1985a), where species differ in a number of characteristics such as feeding behaviour, down plumage, motor activity, vision, and parent-offspring relationships (Starck 1993; Starck & Ricklefs 1998). Altricial species hatch relatively immobile, lacking down, and generally have under-developed organs except for their digestive organs (Bennett & Harvey 1985a, b; Starck 1993). Furthermore, these chicks have closed eyes and are immobile, making them blind and vulnerable to predators. As a result, parental care is critical to the survival and future development of these offspring (Bennett & Harvey 1985a; Starck 1993). In fact, it has been suggested that post-hatching investment by parents enables young altricial species to develop larger brains (Bennett & Harvey 1985a). Conversely, precocial species are immediately mobile and vigilant for predators. These hatchlings are fully downed, have open eyes, and in large part resemble adults of their species (Starck 1993; Starck & Ricklefs 1998). As these offspring leave the nest fairly early after hatching, parental care is less essential to their immediate survival. However, it has been suggested that the lack of continued investment in precocial species prevents post-natal brain development that would allow adults to develop relatively larger brains (Bennett & Harvey 1985a).

Developmental and behavioural differences between altricial and precocial species are said to be a consequence of timing of neural growth (Iwaniuk & Nelson 2003). At hatching, altricial species have relatively smaller brains than precocial species; however, in adulthood this pattern is reversed with altricial species developing larger
brains than precocial species (Bennett & Harvey 1985a, b; Iwaniuk & Nelson 2003). Researchers have documented a number of interesting patterns relating brain size and development; for example, to grow a larger brain, species require both prolonged embryonic development (i.e. incubation) as well as prolonged post-embryonic development (i.e. parental care, Iwaniuk & Nelson 2003; Iwaniuk et al. 2004). These extended bouts of development lead to longer periods of neural growth, myelination, synaptogenesis, and neurogenesis (Iwaniuk & Nelson 2003). By comparing differences between developmental modes, Iwaniuk and Nelson (2003) demonstrated that each mode is influenced by different mechanisms; for example, altricial species appear to be affected by the extension of the developmental period (both pre-and post hatching), while semi-precocial species are most influenced by post-fledging parental care (Bennett & Harvey 1985a; Iwaniuk & Nelson 2003). Moreover, it appears as though brain volume and developmental rates most likely evolved independently of one another, suggesting that development is not the only force driving interspecific variation in brain size (Iwaniuk & Nelson 2003).

**Social interactions** – Traditional explanations for the evolution of larger brains have focused heavily on ecological problem solving (Dunbar 1998; Dunbar & Shultz 2007). The social brain hypothesis, first proposed in primates, suggests that the demands of group living can favour the enlargement of the brain to enable individuals to better process and integrate social information (Dunbar 1998). This hypothesis has received growing empirical support in primates, whereby a number of measures of social complexity, including social group size and number of females in the group, have been
found to be positively correlated with brain size (Dunbar 1992; Lindenfors 2005). The broader implication of this hypothesis, however, is that individuals in stable social groups are faced with increased daily challenges when attempting to maintain group cohesion and coordinate behaviours among individuals; these demands are not present in individuals living alone (Dunbar 1998; Dunbar & Shultz 2007).

The avian brain appears to have also adapted in part to solve socio-ecological problems (Emery 2006). As a result, researchers have attempted to correlate relative brain size with a number of measures of group living in birds (Beauchamp & Fernández-Juricic 2004; Emery & Clayton 2004; Iwaniuk & Arnold 2004; Emery et al. 2007). In fact, it has been suggested that cooperative breeding involves greater cognitive skill as species must be able to maintain a complex social network, e.g. identify kin, perform elaborate displays, and maintain group cohesion during foraging bouts (Iwaniuk & Arnold 2004; Emery et al. 2007). To date, support for the social brain hypothesis in birds has been limited. In a number of studies, no clear relationship has been established between brain size and flock size (Beauchamp & Fernández-Juricic 2004), cooperative breeding (Iwaniuk & Arnold 2004), or simple social structure (Emery & Clayton 2004) across various avian families.

Social organization in birds has been described as being very flexible and transitory (Emery et al. 2007). For example, species may vary in their social system based on different ecological or geographical pressures (Emery & Clayton 2004). A universal measure of sociality in birds has been difficult to determine; however, social network size (or average group size) and mating system have been named as contenders (Emery et al. 2007). Birds forming lifelong monogamous pair-bonds have been shown to
develop larger relative brain sizes in comparison with species engaging in other mating strategies (Dunbar & Shultz 2007; Emery et al. 2007; Shultz & Dunbar 2010). More recently, pair-bond strength has emerged as an important determinant of brain size in birds, even when considering the role of other traits associated with larger brains (Dunbar & Shultz 2007; Scheiber et al. 2008; Shultz & Dunbar 2010). It has been suggested that pair-bonded species may be better able to coordinate their behaviours in order to maximize their reproductive success, leading to mate fidelity rather than divorce (Shultz & Dunbar 2010). In fact, it has been argued that the social brain hypothesis be modified to reflect the quality of relationships rather than the number of relationships, as quality may impose a greater cognitive burden (Scheiber et al. 2008; Shultz & Dunbar 2010).

**Behavioural flexibility** – Behavioural flexibility has been defined as an individual's ability to detect and respond to changes within the environment (Lefebvre et al. 1998; Sol et al. 2005a). In birds, this has been characterized by a number of different behaviours, including innovation success, foraging ability, and migration (Lefebvre et al. 1997; Sol et al. 2002; Sol et al. 2005b). Feeding innovations, or the development of new and unique feeding behaviours, have been said to reflect learning ability, opportunism, and adaptability in an animal (Lefebvre et al. 1998). In birds, innovation frequencies are positively correlated with forebrain size, suggesting that birds with larger brains are more adaptable to a changing environment (Lefebvre et al. 1997, 1998, 2004). Furthermore, behavioural flexibility is also proposed to predict species richness (Nicolakakis et al. 2003), as the number of species varies significantly among avian orders. According to the behavioural drive hypothesis, behavioural adaptations to a changing environment and the
transmission of new skills within a population can lead to accelerated rates of evolution, which could account for the diversity of species richness in birds (Nicolakakis et al. 2003).

Migration is a particularly flexible behaviour in birds, which can range across a continuum from completely migratory to purely sedentary strategies (Sol et al. 2005b; Pravosudov et al. 2007). The specific factors driving the evolution of migration still remain unclear since species experiencing similar seasonal changes in environmental conditions can engage in drastically different strategies. One of the main challenges facing birds in seasonal environments is drastic variation in foraging conditions. According to the behavioural flexibility-migratory precursor hypothesis, species with larger brains are better able to modify their foraging behaviours and should be better able to survive stressful conditions throughout the winter (Sol et al. 2005b). This hypothesis suggests that resident species have more flexible behaviours than migratory species, and in turn have larger brains that allow them to respond to environmental challenges (Sol et al. 2005b). To date, evidence from several studies has supported this relationship, whereby migratory species have relatively smaller brain sizes than non-migratory or resident species (Winkler et al. 2004; Sol et al. 2005b, 2010). However, Pravosudov et al. (2007) found that sedentary subspecies of white-crowned sparrow (Zonotrichia leucophrys nuttalli) developed significantly larger brains than migratory subspecies (Z. l. gambelii and Z. l. oriantha). Considering that the white-crowned sparrow has a migratory ancestor, these results suggest that brain size might be an evolutionary consequence of changes in behavioural strategies in these birds rather than larger brains being a precursor for the development of sedentary strategies (Pravosudov et al. 2007). As a result, the
constraints of migration may have lead to the evolution of smaller brains. In fact, Sol et al. (2010) have recently shown that the most likely evolutionary pathway would have resulted in migration selecting for smaller brains.

Sexual selection

Sexual selection is a selective mechanism driven by competition over mates and mate choice, which influences the development of reproductive traits, whether these are physical or behavioural (Andersson 1994). The role of sexual selection in brain size evolution has only recently been investigated, especially in mammals and birds (Garamszegi et al. 2005; Pitnick et al. 2006; Lindenfors et al. 2007). Sexual selection was commonly thought to produce localized changes in specific specialized areas within the brain (e.g. song nuclei of passerines or the hippocampus in brood parasites; see Jacobs 1996 for a review). In general, however, sexual selection may lead to an overall reduction in whole brain size as species invest more energy into sexual traits that increase the success of mate attraction or rival competition (Jacobs 1996). In order to attract mates, for example, species may develop exaggerated or showy ornaments (Winquist & Lemon 1994), which can be coupled with complex displays (McDonald 1989). Rival competition on the other hand can lead to larger body sizes (Dubey et al. 2009), enlarged sexual organs (Pitnick et al. 2006), as well as the development of defensive structures (Emlen 2001). In some orders, selection on brain structures may also be sex-specific; for example, brain size evolution in male primates is said to be driven by physical interactions with competing males, whereas female brain size appears to be driven by social interactions within groups (Lindenfors et al. 2007).
The general negative association between brain size and sexual selection is by no means the rule. Exceptions to this trend are seen in bowerbirds (family Ptilonorhynchidae) and zebra finches (Taeniopygia guttata castanotis). Bowerbirds engage in extraordinarily complex courtship and mating behaviour, where males build a bower, a large display structure made of grass and bright objects, to attract mates (Marshall 1954). Researchers have shown that species building more complex bowers have larger brains (Madden 2001), but more specifically larger cerebella (Day et al. 2005), than species who build simpler bowers or no bowers at all. In zebra finches, individuals producing more complex songs develop larger high vocal centres (HVC) and telencephalon than individuals with less complex repertoires (Airey et al. 2000). Both these behaviours, building elaborate bowers and producing complex songs, are highly influenced by female preference, suggesting that female choice may be selecting for larger neural structures in these species (Madden 2001; Day et al. 2005; Lefebvre & Sol 2008). Furthermore, in polygynous meadow voles (Microtus pennsylvanicus), females choose males with better spatial ability, which is correlated with a larger hippocampus (Spritzer et al. 2005), suggesting that this trend is not exclusive to birds.

**Sperm competition** – Sperm-competition refers to a post-copulatory strategy used during the reproductive period, whereby ejaculates from multiple males will compete for access to and fertilization of female ova (Parker 1970; Birkhead 1998). This process is often considered an evolutionary consequence of sexual selection as the outcome can lead to differential reproductive success of individuals within a population (Birkhead 1998). Sperm competition theory can be applied to both internal fertilizers, where females
typically copulate with multiple male partners during a single reproductive cycle, and external fertilizers, where multiple males simultaneously release sperm near a spawning female (Birkhead 1998). In birds, who are internal fertilizers, females have sperm storage tubules where male spermatozoa can survive for up to a few weeks (Birkhead & Möller 1992). Consequently, the avian reproductive tract may facilitate the occurrence of sperm competition.

A large portion of monogamous birds were once considered to be both socially and genetically monogamous (Westneat et al. 1990). However, the advent of molecular paternity analysis has revealed that this assumption was erroneous, as less than 25% of socially monogamous species have been shown to practice true genetic monogamy (Griffith et al. 2002). Genetic monogamy refers to an exclusive copulatory relationship between a male and female (Neudorf 2004). Social monogamy, however, refers to a relationship between a male and female for reproductive purposes only; this relationship does not need to be exclusive (Neudorf 2004). In socially monogamous species, males and females form a pair-bond but will sneak extra-pair copulations from other conspecifics (Westneat et al. 1990). Extra-pair copulations typically take place during the female’s fertile period, as this is the best chance for a male to fertilize her eggs. Bird species vary drastically in their degree of extra-pair paternity whereby non-passerine species tend to have a rather low rate (0-5% of offspring are fathered by a male other than the female’s social partner; Birkhead & Möller 1992) and passerines have much higher rates (typically 10-20% of nests include extra-pair sired young and some are even as high as 70%; Birkhead & Möller 1992; Double et al. 1997).
As a result, sperm competition is one of the main forces in the diversification of male reproductive traits and behaviours, such as sperm morphology and mate-guarding strategies (Birkhead & Möller 1992; Lemaître et al. 2009). Birds facing high sperm competition develop larger testes containing more and longer spermatozoa (Birkhead & Möller 1995; Briskie et al. 1997) and typically follow their female partner more closely during her fertile period (Birkhead & Möller 1992; Birkhead 1998).

Larger testes allow males to release more sperm per ejaculate, thereby increasing the chance of fertilization (Lemaître et al. 2009). In birds where females mate multiply, males will invest a substantial amount of energy into developing relatively larger testes (Moller 1991; Möller & Briskie 1995; Pitcher et al. 2005). As costly sexually selected traits (including testis size and ejaculatory traits) have been shown to trade-off against other energetically expensive functions (e.g. immunity, Verhulst et al. 1999; weapons, Simmons & Emlen 2006), a similar trade-off between testis size and brain size could occur. The expensive sexual tissue hypothesis postulates that brain tissue will trade-off specifically with investment in costly sexually selected traits such as large testes favoured under sperm competition (Pitnick et al. 2006; Lemaître et al. 2009). Consequently, this hypothesis provides an ideal mechanism by which male birds with high sperm competition may develop larger testes while limiting the size of the brain (Pitnick et al. 2006; see Chapter 2).

Although the relationship between sperm competition and brain size has not been supported in mammals (Pitnick et al. 2006; Schillaci 2006; Lemaître et al. 2009), birds demonstrate an interesting pattern with regards to brain size dimorphism (Garamszegi et al. 2005). In species with high-rates of extra-pair paternity, males develop relatively
smaller brains than their female counterparts, whereas in species with low rates of extra-pair paternity, males develop larger brains than females (Garamszegi et al. 2005). These differences in overall brain size between the sexes are said to arise because of sexual differences in reproductive behaviours, leading to divergent selection pressures in males and females (Jacobs 1996; Garamszegi et al. 2005).

Sperm competition, therefore, is a great example of sexual selection arising from sex-dependent behaviours (Garamszegi et al. 2005). Moreover, sperm competition is an important driving force in the diversification of male traits, particularly those affecting fertilization success (Birkhead 1998; Garamszegi et al. 2005; Lemaître et al. 2009). The role of sperm competition in brain size evolution has been well-studied in mammals (Pitnick et al. 2006; Schillaci 2006; Lemaître et al. 2009); however, this relationship has received little attention in birds (but see Garamszegi et al. 2005), despite its widespread occurrence within this group.

**Metabolic costs of brain development**

Recent hypotheses concerning the evolution of larger brains focus heavily on the benefits or advantages associated with this process. However, there are also a number of hypotheses concerned with the costs of an enlarged brain. Moreover, it has been suggested that these costs may help explain variation in relative brain size across taxa (Isler & van Schaik 2006a, b). Many of these theories originally centred on mammals, but have recently been applied to birds.

The *direct metabolic constraints hypothesis* was the first theory proposed to explain the costs of a larger brain and focused on a direct link between basal metabolic rate and brain size (Armstrong 1983; Hofman 1983). This hypothesis suggested that the
total daily energy budget of a species, including skeletal muscles and visceral organs, may constrain the size of the brain. Although Martin (1981) found a positive correlation between brain mass and basal metabolic rate in mammals, where species expending more daily energy had larger brains, this same relationship has not been supported in birds (Isler & van Schaik 2006a). In birds, basal metabolic rate is not correlated to brain mass, nor is it related to reproductive effort, pectoral muscle mass, or heart mass (Isler & van Schaik 2006a). These findings allowed researchers to reject the idea that basal metabolic rate was influencing brain size evolution in birds.

A decade later, Aiello and Wheeler (1995) proposed the *expensive tissue hypothesis*. This theory postulates that an animal has the ability to sustain the high metabolic costs of a larger brain without associated increases in basal metabolic rate by decreasing the amount of energy utilized by other highly metabolically expensive tissues (Aiello & Wheeler 1995). In anthropoid primates, a negative relationship was found between brain mass and gut mass, providing support for this hypothesis (Aiello & Wheeler 1995). However, other studies investigating the negative relationship between brain size and gastro-intestinal tract size have been rare: there has been some support in fishes (Kaufman et al. 2003), a positive relationship in bats (Jones & MacLarnon 2004), and no correlation in birds (Isler & van Schaik 2006a). Moreover, in birds, other tissues such as the liver, kidneys, heart, and lungs also show no correlation with brain mass (Isler & van Schaik 2006a). Consequently, this hypothesis has received little support in non-primate species. In 2006, Pitnick et al. proposed an extension of this hypothesis, the *expensive sexual tissue hypothesis*, to include potential trade-offs between the
development of the brain and the development of costly sexual traits, such as organs, ornaments, and armaments (see Chapter 2).

In order to generalize their original hypothesis, Aiello and Wheeler (1995) proposed the energy trade-off hypothesis. With everything else being equal, they suggested that increases in brain size relative to body size could be compensated for by a decrease in energy consumption by other functions that were more plastic (i.e. digestion, locomotion, or reproduction; Aiello & Wheeler 1995; Isler & van Schaik 2006a). This hypothesis consisted of three predictions: brain size may be negatively associated with 1) the gut or other expensive organs (the expensive [sexual] tissue hypothesis); 2) the maintenance of larger muscles (muscle mass) or locomotion (distance travelled); or 3) the costs of reproduction (reduced fecundity or longer developmental period) (Aiello & Wheeler 1995). In birds, all three predictions have been investigated (Isler & van Schaik 2006a, b). First, as mentioned previously, visceral organs do not correlate with brain size. Second, pectoral muscle mass, used for long-distance flight, was negatively correlated with brain size. Finally, incubation length and egg mass per clutch were positively correlated with brain size, while clutch size and annual fecundity were negatively correlated with brain size (Isler & van Schaik 2006a). These results provide partial support for the energy trade-off hypothesis, and suggest that trade-offs between particularly systems may be taxon-specific and rely more heavily on the group’s life-history traits and evolutionary adaptations.

**Sex-specific influences on brain size evolution**

Previous studies on the influence of sexual selection on brain size evolution have largely focused on trait variation within a species or within closely-related species (e.g.
bowerbirds, Madden 2001; Day et al. 2005). Additionally, sexual dimorphism in brain size has been studied with regards to song production (Arnold et al. 1986), extra-pair paternity (Garamszegi et al. 2005), and body size (Schillaci 2006). Comparative studies are now more commonly being used to ask relevant larger-scale biological questions within a single sex (Lindenfors et al. 2007; Gonzalez-Voyer et al. 2009). Despite the growing interest of sex-specific influences on the development of sexual traits (Jawor et al. 2004; Murphy 2007), little research has focused on investigating sex-specific determinants of brain size.

**Measuring brain size**

Over the past 30 years, researchers have debated whether or not to measure whole brain size or specific brain structures when investigating evolutionary questions. Typically, specialized parts of the brain do not evolve in isolation from one another (Iwaniuk et al. 2004), as many behaviours activate a number of different brain regions, often collectively termed functional systems (Barton & Harvey 2000; Lefebvre & Sol 2008). Consequently, within and between functional systems, brain structures are tightly interconnected and their development can be strongly coupled (Iwaniuk et al. 2004). Although whole brain size is most commonly measured, many researchers have attempted to correlate complex behaviours to isolated structures within the brain (Sherry et al. 1993; Lefebvre et al. 1997; Beauchamp & Fernández-Juricic 2004; Day et al. 2005). For example, Day et al. (2005) investigated the relationship between bower complexity by bowerbirds and the size of the cerebellum. Although a positive correlation was found between bower complexity and cerebellum size (Day et al. 2005), it is difficult to determine a cause-and-effect relationship as the cerebellum plays a key role in motor skills and coordination.
(Jerison 1973; Healy & Rowe 2007). Consequently, assigning a single function to a brain region can be misleading, especially when different behaviours can also activate a number of structures within the brain.

As connected brain structures are often correlated, evolving and changing in concert in response to environmental or behavioural challenges (Clark et al. 2001; Iwaniuk et al. 2004; Gonzalez-Voyer et al. 2009), measuring the entire brain can provide valuable, although still incomplete, information concerning the expansion of neuronal connections and organization of the brain. Studies concerning these structural changes in response to selective pressures often highlight the effect on total brain size rather than isolated components (Hutcheon et al. 2002; Iwaniuk & Hurd 2005; Pollen et al. 2007). Over the past few decades, researchers have devised a number of techniques for measuring whole brain size (see Appendix A) and these measures are indispensable when asking questions concerning behavioural changes that are accompanied by changing neuronal connections. These preliminary correlations can provide a platform for the study of more specific questions concerning isolated brain structures.

**Thesis objectives**

The overall goal of my thesis was to explore the sexually selected mechanisms affecting brain size evolution in birds. In Chapter 2, I investigated the potential evolutionary trade-off between developing a large brain and developing large testes by testing the expensive sexual tissue hypothesis in birds (Pitnick et al. 2006). In Chapter 3, I investigated the influence of developmental mode, parental care, and pair-bond strength on the evolution of female brain size and its association with lifetime reproductive output. Together, these studies provide a more comprehensive view of the sex-specific selection
pressures faced by males and females when developing larger brains. Both data chapters were written in preparation for submission to scientific journals: Chapter 2 is currently in preparation for resubmission in *Proceedings of the Royal Society of London B*, and Chapter 3 is currently in preparation for submission to the *Journal of Evolutionary Biology*.

References


CHAPTER 2

EVOLUTIONARY CONFLICT BETWEEN BRAIN SIZE AND TESTIS SIZE IN BIRDS
Summary

The high energetic demands of the brain may limit the allocation of energy to other essential organs or behaviours necessary for survival and reproduction. The expensive sexual tissue hypothesis postulates that investment in costly sexually selected traits will limit the development of the brain. Testicular tissue has been proposed as an ideal trait in which to investigate this potential trade-off. Despite a number of recent studies on the topic, empirical support for a trade-off between testis size and brain size has only been documented in echolocating bats. We used a large, sex-specific dataset to test the predictions of the expensive sexual tissue hypothesis in birds. We show that male passerines exhibit a significant negative relationship between testis mass and brain mass, whereas females and non-passerines do not. To explore the possibility that birds are more likely to experience this trade-off due to energetic and size constraints imposed by long-distance flight, we compared these patterns in long-distance migrants and other species. Accordingly, long-distance migrants demonstrated a negative relationship between testis mass and brain mass, whereas other species did not. Our findings identify a potential conflict between selection for increased brain size and selection for enhanced sperm competition in birds.

Keywords: brain size; testes; migration; birds; comparative analysis; sexual tissue

Introduction

Adaptation to environmental pressures has lead to interspecific variation in brain size across many diverse taxa (rodents, Mann & Towe 2003; dolphins, Marino 1997; fish, Kotrschal et al. 1998; birds, Iwaniuk & Nelson 2001; bats, Hutcheon et al. 2002;
primates, Reader & Laland 2002). In general, increases in brain size have been associated with a number of evolutionary advantages such as greater behavioural flexibility (Lefebvre et al. 1997), reduced adult mortality (Sol et al. 2007), and group cohesion and cooperation (Dunbar 1998; Shultz & Dunbar 2007). Although selection favours evolutionary changes that produce an overall net benefit, costs are often associated with new adaptations.

Brain development and maintenance is metabolically costly (Aiello & Wheeler 1995; Isler & van Schaik 2006b). Moreover, neural tissue expends significantly more energy at rest than most other somatic tissues (Mink et al. 1981), and the brain is unable to temporarily divert its energetic needs (Karasov et al. 2004). As a result, the high energetic demands of the brain may limit the allocation of resources (i.e. energy) to other essential organs or behaviours necessary for survival and reproduction (Pitnick et al. 2006; Lemaître et al. 2009). Aiello & Wheeler (1995) first proposed the ‘expensive tissue hypothesis’ to explain this trade-off in anthropoid primates, where the increased energetic demands of a larger brain could be compensated for by a reduction in the metabolic rate of other tissues (e.g. the gut). However, further tests of this hypothesis have produced mixed results in various comparative studies (Kaufman et al. 2003; Jones & MacLarnon 2004; Isler & van Schaik 2006a). In African freshwater fish with larger brains, gastrointestinal tract length was significantly reduced (Kaufman et al. 2003). By contrast, the evolution of a larger brain in bats has been coupled with an increase in relative intestine length (Jones & MacLarnon 2004). Moreover, encephalization was not correlated with a number of somatic tissues in birds, including heart, liver, lung, and kidney; however, it was negatively associated with pectoral muscle mass (Isler & van Schaik 2006a).
Recently, the role of sexual selection in encephalization has been of great interest, particularly in birds and mammals (Pitnick et al. 2006; Lindenfors et al. 2007; Guay & Iwaniuk 2008; Lemaître et al. 2009). As a result of selective pressures to maximize reproductive success, Pitnick et al. (2006) proposed the 'expensive sexual tissue hypothesis', which postulates that investment in costly sexually selected traits (i.e. ornaments, armaments, or organs) will limit the proliferation of brain tissue, leading to smaller overall brain size. Reproductive investment, the proportion of energy allocated to sexual tissue rather than somatic tissue (MacLeod & MacLeod 2009), can vary considerably between species (Møller & Briskie 1995; Byrne et al. 2002). Across various taxa, however, testis size relative to body size is considered an accurate estimate of male reproductive investment (MacLeod & MacLeod 2009).

Sperm competition, a post-copulatory strategy used by males to compete for access to and fertilization of female eggs, is a leading driving force explaining testis size variation across species (Parker 1970; Birkhead 1998). According to sperm competition theory, males in systems with high sperm competition should develop larger testes relative to their body mass (Møller 1991; Møller & Briskie 1995). Investment in the maintenance of these large testes can be very expensive as it can utilize 5-10% of an individual’s basal metabolic rate (Kenagy & Trombulak 1986). Moreover, increases in testis size during the breeding season are mediated through increases in circulating androgens, and researchers have demonstrated that increases in testosterone can lead to reduced survivability through decreased immune function (Muehlenbein & Bribiescas 2005) and reduced lifespan (van Voorhies 1992). Because of these costs, we might expect to see an evolutionary conflict between the development of larger brains and larger testes.
in these systems. This relationship has not been supported in a number of mammals, however, including primates, carnivores, and ungulates (Lemaître et al. 2009). Indeed, only echolocating bats exhibit a significant negative relationship between brain size and testis size (Pitnick et al. 2006; Lemaître et al. 2009; but see Dechmann & Safi 2009). In mammals, mating system appears to be a better predictor of brain size, where monogamous species develop larger brains than species with other mating systems (Schillaci 2006; Shultz & Dunbar 2007). In birds, pair-bonded species also appear to develop larger brains than other species (Dunbar & Shultz 2007; Emery et al. 2007; Shultz & Dunbar 2010). However, the relationship between reproductive organs and mating strategy has only been studied in waterfowl, where relative brain size dimorphism did not correlate with proxies of sperm competition, namely testis size and phallus length, or pair-bond duration (Guay & Iwaniuk 2008).

In addition to the high energetic demands of developing and maintaining sexual organs and ornaments, birds experience a number of ecological constraints related to seasonal behaviour, particularly migration (McNab 1994; Winkler et al. 2004). Recently, birds were shown to exhibit a significant negative correlation between pectoral muscle mass and brain mass, suggesting that the energetic demands of flight may constrain the development of larger brains (Isler & van Schaik 2006a; Sol et al. 2010). In addition, flight may impose size constraints on increases in brain size, as concomitant increases in weight would need to be balanced by increases in wingspan or flight speed (Norberg 1995). Indeed, long-distance migrants appear to have relatively smaller brains compared to short-distance migrants and sedentary species (Sol et al. 2005b; Sol et al. 2010). We therefore hypothesize that as a result of both energetic and size constraints imposed by
long-distance flight, migrating birds may be more likely to experience a trade-off between neural and testicular tissue development.

Here, we test the expensive sexual tissue hypothesis in birds, as it relates to testicular tissue and long-distance flight, using a large sex-specific dataset spanning several orders. We predict that the cost of developing larger testes in males will result in a compensatory decrease in the development of the brain, leading to a negative relationship between brain size and testis size. Conversely, as females do not need to develop this costly sexual organ, we expect to find no relationship between male testis size and female brain size. Due to metabolic and size constraints imposed by long-distance flight, we also predict that long-distance migrants will be more likely to experience a trade-off between testis size and brain size compared to other groups.

Material and methods

We collected data on body mass, testis mass, mating system, migratory behaviour, and total brain mass (approximated by endocranial volume). Although previous studies have used combined measurements from both males and females (Pitnick et al. 2006; Lemaître et al. 2009), our interests relied upon sex-specific measurements (Gonzalez-Voyer et al. 2009). As a result, brain and body masses were determined for each sex independently.

Endocranial volume

We measured endocranial volume from 2043 museum specimens, representing 106 species, housed at the University of Michigan Museum of Zoology. We measured sexed and intact adult skulls (i.e. lacking visible fractures or dents). When available, we
collected body masses from specimen vouchers. If this information was missing, we averaged sex-specific weights of all other specimens sampled or utilized body mass values from the CRC Handbook of avian weights (Dunning 2008). Finally, we selected specimens collected within a limited geographic range whenever possible to account for potential geographic variation in brain size due to environmental variation (Schuck-Paim et al. 2008).

We measured endocranial volume following the procedure outlined by Iwaniuk and Nelson (2002). Briefly, we blocked orbital fissures and external acoustic meatuses to delineate the approximate cranial space previously occupied by fresh brain tissue (Jue 1990). We then filled each skull via the foramen magnum with dust lead shot (Iwaniuk & Nelson 2002). To minimize interstitial space between each shot, we repeatedly tapped the skull and probed through the foramen as the shot was being poured in (Iwaniuk & Nelson 2002, 2003). Once the endocranial cavity was filled, we decanted the lead shots into 10 and 20 ml volumetric cylinders (Ashwell 2008), and measured the volume to the nearest 0.25 ml. Volumetric endocranial measurements were converted into brain masses by multiplying by the density of fresh brain tissue (1.036 g ml⁻¹; Stephan 1960; Iwaniuk & Nelson 2002).

**Testis mass**

For male testis mass, we obtained data from published reviews (Møller 1991; Dunn et al. 2001; Calhim & Birkhead 2006), which included both natural testis mass (fresh weight) and mass calculated from linear measurements in museum collections (see Appendix B). For each species, testis mass comprised an average of the left and right testis. Testis mass values for each species were averaged across several adult males sampled during the
breeding season (Calhim & Birkhead 2006). Although we recognize that females do not develop testes, we wanted to investigate the relationship between female brain size and male testis size. A negative relationship between male testis mass and both male and female brain size could indicate that female brain size changes merely as a by-product of selection on males through genetic correlation (Lande 1980). On the other hand, the lack of a negative relationship between female brain size and male testis size could result if females are shielded from the proposed energetic constraint since they do not invest in testes. Female brain size may also be influenced in other directions by a different suite of sex-specific selective factors.

*Mating system and migratory behaviour*

We collected data on mating system and migratory behaviour from published species accounts, including the Handbook of Birds of the World, the Birds of North America, and the Cornell Lab of Ornithology. We transformed qualitative descriptions of mating systems into qualitative continuous variables that reflected the degree of polygyny for each species (Emery et al. 2007): long-term monogamy was coded as 1; serial monogamy as 2; seasonal monogamy as 3; social monogamy as 4; lekking as 5; and polygyny and promiscuity as 6. For migratory behaviour, we separated species into two groups: long-distance migrants, travelling between temperate and tropical environments, and all other groups, including a range of strategies from residents to short-distance migrants.

*Comparative methods*

A key assumption in any statistical analysis is the concept of independence of data points (Freckleton et al. 2002). Empirical evidence and theoretical models have suggested that
more closely related species are more likely to be similar to one another (Garland & Ives 2000), as they may share similar genetic markers or ecological niches (Freckleton et al. 2002). In large datasets, particularly those concerned with many species of varying degrees of relatedness, controlling for phylogenetic non-independence becomes increasingly important. In order to account for these effects, we used phylogenetic generalized least squares analyses (PGLS, Grafen 1989; Ihaka & Gentleman 1996; Paradis et al. 2004), implemented in R 2.10.1 (Ihaka & Gentleman 1996) using the APE (Analysis of Phylogenetics and Evolution) and ‘nlme’ packages (Paradis et al. 2004).

PGLS analyses allow the comparison of multiple models of evolution, such as Brownian motion (Felsenstein 1985), Pagel’s λ (Pagel 1999; Freckleton et al. 2002), and the Ornstein-Uhlenbeck (OU) process (Hansen 1997; Martins & Hansen 1997).

We used information from published supertrees and phylogenies to create a composite phylogeny of the species in our dataset (Appendix C) using Mesquite 2.72 (Maddison & Maddison 2009). As we did not have comparable absolute branch lengths and were unable to set branch lengths proportional to the number of species, we set all branch lengths equal to one (Garland et al. 1993). In all cases, we log-transformed brain mass and testis mass prior to analysis in order to compare proportional changes in size between different lineages (Felsenstein 1985; Garland et al. 1992). We then included log-transformed body mass as a covariate in all analyses (Freckleton 2002, 2009). In each PGLS analysis we entered brain mass as the dependent variable, with body mass plus testis mass or mating system as the independent variables. Multiple regression models with Pagel’s λ or the Ornstein-Uhlenbeck process (α parameter) described trait evolution better than a Brownian motion model. Pagel’s λ was a better predictor of trait evolution
when investigating the relationships between brain mass, mating system, and testicular mass, whereas the Ornstein-Uhlenbeck process (α) better predicted trait evolution with regards to migratory behaviour. We used the best fit model for each analysis.

Results

In all analyses, brain mass was positively correlated with body mass in both sexes (Tables 2.1-2.3). Across all species, we found a significant negative relationship between overall brain size and testis mass in males, but not in females (Table 2.1A). In passerines, the negative relationship between overall brain mass and testis mass was significant in males and was not present in females (Table 2.1B). In non-passerines, we found no relationship between overall brain mass and testis mass in both males and females (Table 2.1C).

Mating system has been suggested to affect brain size evolution in a number of taxa; we therefore evaluated its possible influence within our dataset. Contrary to our expectation, we found no effect of mating system on brain mass variation in males or females (Table 2.2).

Finally, we sought to determine whether the energetic and size constraints of long-distance flight might play a role in the relationship between overall brain mass and testis mass. In long-distance migrants, we found a significant negative relationship between overall brain mass and testis mass in males but not in females (Table 2.3A). There was no relationship between brain mass and testis mass in non-migrants and short-distance migrants in either males or females (Table 2.3B).
Discussion

The expensive sexual tissue hypothesis proposes an energetic trade-off between the development of a larger brain and larger testes. Our findings provide the first support for this hypothesis in birds. In males, there was a significant negative relationship between overall brain mass and testis mass across all species in our dataset. Male passerine birds also exhibited a similar negative relationship, but this pattern was not present in non-passerines. In addition, we found a negative relationship between testis mass and brain mass among male long-distant migrants, but not in other birds. Females did not exhibit a relationship between brain size and male testis size.

Sexually selected traits can be very costly (Andersson & Iwasa 1996). The expensive sexual tissue hypothesis was proposed to explain how intense sexual selection could constrain brain size evolution, leading to a trade-off with costly sexual traits (Pitnick et al. 2006). According to sperm competition theory, males in mating systems with intense sperm competition should opt for the development of traits that will maximize their reproductive success (Birkhead 1998). As a result, it has been proposed that an evolutionary constraint may arise between the development of a large brain and the development of large testes. Our findings are the first to provide supportive evidence of this relationship in birds, as shown by a significant negative correlation between brain size and testis size in males. However, we did not see a corresponding relationship with female brain size and male testis size, suggesting that the evolution of female brain size is not merely an indirect consequence of selection on males. Moreover, these data provide additional support for the idea that it is investment in the testes themselves that is constraining brain size evolution. Our findings also highlight the importance of collecting
sex-specific data, as neither relationship may have been apparent had we pooled information from males and females.

Many researchers have focused on the role of sociality in brain size evolution. In primates, this is often measured in terms of group size, where species living in larger groups develop larger brains (Dunbar 1998). In birds, however, an appropriate measure of sociality has been difficult to establish (Emery et al. 2007). To date, studies have used a number of approaches to describe sociality, including cooperative breeding (Iwaniuk & Arnold 2004), group size (Beauchamp & Fernández-Juricic 2004), and simple social structure (Emery & Clayton 2004). However, none of these measures have been correlated with brain size variation. More recently, studies have shown that birds establishing long-term pair-bonds develop larger brains (Dunbar & Shultz 2007; Emery et al. 2007; Shultz & Dunbar 2010). Although we found no relationship between brain size and mating system, this could perhaps be because mating system reflects such a large suite of life-history traits, including sperm competition, pair-bond formation, and parental care. Although both mating system and testis size likely reflect differences in sperm competition (Birkhead 1998), testis size may provide a more appropriate test of the expensive sexual tissue hypothesis because it focuses on a tissue that is likely to be directly involved in a possible trade-off.

We also wanted to determine whether the expensive sexual tissue hypothesis might apply to particular groups, rather than just across birds in general. We found that although male passerine birds exhibited a significant negative relationship between brain size and testis size, non-passerines did not. Although there exists tremendous diversity within these two groups, particularly among non-passerines, which comprise several
orders, they also differ in a number of characteristics that could influence the relationship between brain size and sexual tissue.

One notable difference between passerines and non-passerines is the development and production of song. Most passerines learn their song, whereas the songs of most non-passerines are innate, with some exceptions (Catchpole & Slater 2008). Among passerine birds, variation in the size and complexity of the song repertoire is reflected in divergent patterns of brain development (Nottebohm et al. 1981; Brenowitz 2004), where species learning more songs develop larger brains (Brenowitz & Kroodsma 1996). Due to limited resources, developing a more attractive song may conflict with developing the larger testes necessary for successful sperm competition (Gil & Gahr 2002). On the other hand, male non-passerines do not seem to face these same selective pressures because they produce innate songs that are less complex (Catchpole & Slater 2008) and may therefore have the opportunity to allocate resources to both traits. In fact, rejection of the expensive sexual tissue hypothesis has been established among a large group of non-passerines (waterfowl; order Anseriformes), whereby testis and phallus size were not correlated with brain size dimorphism between the sexes (Guay & Iwaniuk 2008). This may explain why only male passerines exhibited a negative relationship between brain size and testis size.

Another trait that differentiates passerines from non-passerines is developmental mode. Our dataset mirrored this differentiation, as all passerines were altricial species, while most non-passerines were precocial species. It has been well established that precocial species have larger relative brain sizes than altricial species at hatching and that this trend is reversed in adulthood (Starck 1993). Precocial species undergo most of their development embryonically, whereas altricial species tend to undergo the majority of
their development post-hatching (Starck and Ricklefs 1998). Altricial species continue
developing their brains into adulthood, when they are also faced with sexual
development. As a result, males must continue to develop larger brains while
simultaneously coping with the consequences of the breeding season, when organs and
ornaments undergo dramatic developmental changes (Calhim & Birkhead 2006; Guay &
Iwaniuk 2008).

Flight and body size may represent other key factors linking testis size and brain
size in birds. To date, the expensive sexual tissue hypothesis has only been demonstrated
in echolocating bats (Pitnick et al. 2006; Lemaître et al. 2009; but see Dechmann & Safi
2009). It has been suggested that their small size, greater energy budget, and foraging
strategy constrain the development of other tissues, particularly the brain (Hutcheon et al.
2002). Catching prey on the wing, for example, may be more costly than foraging
strategies used by non-echolocating bats (Hosken & Withers 1997). As such, selective
pressure for energetically efficient flight may impose constraints on increases in body
size, which may increase the likelihood of trade-offs between increases in brain size and
testis size. Passerine birds, which tend to be smaller than non-passerines, may experience
similar energetic constraints, since small birds have higher metabolic rates than large
birds (Walsberg 1983), and neural tissue is more energetically expensive at rest than
somatic tissue (Mink et al. 1981).

Interestingly, we found a negative relationship between testis size and brain size
in long-distance migrants, but not in other birds. A number of studies have shown that
migratory species develop smaller brains than resident species (e.g. Burish et al. 2004;
Winkler et al. 2004; Sol et al. 2005b; Sol et al. 2010). These differences in brain size
have long been associated with the ability of temperate resident species to survive and forage during harsh winter seasons (Lefebvre et al. 1997; Sol et al. 2005b). Although migratory birds do not have to endure a stressful winter, they are faced with the energetic demands of a long flight to their wintering grounds. Moreover, trade-offs between the size of different organs may be more likely in long-distance migrants because of an overall constraint on increases in body size to maintain aerodynamic efficiency (Norberg 1995; Winkler et al. 2004). Indeed, the mass of flight muscles in birds (Isler & van Schaik 2006a) and migration distance (Sol et al. 2010) are both negatively associated with brain mass. Migrants and non-migrants also differ in degree of sperm competition, with migratory species having larger testes (Pitcher et al. 2005) and greater extra-pair paternity (Spottiswoode & Møller 2004). In fact, testicular development during migration seems to be essential for reproduction (e.g. territory establishment) soon after arrival on the breeding grounds (Bauchinger et al. 2007). Such high levels of sperm competition may limit the development of neural tissue, in addition to the previously established costs of flight. Consequently, long-distance migrants appear to face energetic constraints from the maintenance of neural tissue, time constraints of reproduction, and migration, leading to trade-offs in resource allocation (Bauchinger et al. 2007). Our findings suggest that constraints experienced by migratory birds may play an equally important role in explaining brain size differences among residents and migrants. In fact, Sol et al. (2010) have recently shown that the most likely evolutionary pathway would have resulted in migration selecting for smaller brains.
Conclusions

Our findings identify a novel conflict between selection for increased brain size and selection for enhanced sperm competition in birds, providing support for the expensive sexual tissue hypothesis. As brain mass and testis mass were only negatively related in males, our findings emphasize the importance of using sex-specific data when investigating sex-specific influences on brain size evolution. In addition, the fact that this relationship persisted only in passerine birds stresses the importance of investigating biological questions in a number of subdivisions within taxa, as taxonomic differences in major life-history traits may obscure patterns within particular taxonomic groups. Our findings also highlight the energetic challenges faced by long-distance migrants, where the costs associated with migration may constrain the development of both a larger brain and larger testes. Although our findings emphasize a change in overall brain mass, they also allude to the importance of investigating specific regions of the brain, particularly those associated with song production and migratory behaviour. The study of these areas may provide better insight into which mechanisms are at work in maintaining competition between developing tissues.

Acknowledgements

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Engineering Research Council of Canada in the form of a Canada Graduate Scholarship to M.L.A. and Discovery and Research Tools and Instrumentation Grants to S.M.D.

References


Table 2.1. Phylogenetic generalized least-square regression (PGLS) analysis of the relationship between total brain mass, body mass, and testis mass. Results are presented for males and females in a) all birds ($n=80$), b) passerines ($n=44$), and c) non-passerines ($n=36$).

<table>
<thead>
<tr>
<th>sex</th>
<th>ML $\lambda^a$</th>
<th>variable</th>
<th>slope ± s.e.</th>
<th>t-value</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) all birds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1.06</td>
<td>Body mass</td>
<td>0.28 ± 0.004</td>
<td>63.04</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Testis mass</td>
<td>-0.01 ± 0.002</td>
<td>-5.19</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Females</td>
<td>0.96</td>
<td>Body mass</td>
<td>0.62 ± 0.04</td>
<td>17.56</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Testis mass</td>
<td>0.01 ± 0.02</td>
<td>0.58</td>
<td>0.56</td>
</tr>
<tr>
<td>b) passerines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1.02</td>
<td>Body mass</td>
<td>0.31 ± 0.01</td>
<td>35.42</td>
<td>&lt;0.0001</td>
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<tr>
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<td>0.0152</td>
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<td>Females</td>
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<td>Body mass</td>
<td>0.61 ± 0.05</td>
<td>12.25</td>
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<tr>
<td></td>
<td></td>
<td>Testis mass</td>
<td>0.04 ± 0.04</td>
<td>1.09</td>
<td>0.28</td>
</tr>
<tr>
<td>c) non- passerines</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>-1.20</td>
<td>Body mass</td>
<td>0.19 ± 0.005</td>
<td>39.46</td>
<td>&lt;0.0001</td>
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<tr>
<td></td>
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<td>Testis mass</td>
<td>0.002 ± 0.003</td>
<td>0.87</td>
<td>0.40</td>
</tr>
<tr>
<td>Females</td>
<td>1.23</td>
<td>Body mass</td>
<td>0.71 ± 0.01</td>
<td>54.9</td>
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</tr>
<tr>
<td></td>
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<td>Testis mass</td>
<td>-0.04 ± 0.02</td>
<td>-1.42</td>
<td>0.17</td>
</tr>
</tbody>
</table>

$^a$Maximum-likelihood lambda (ML $\lambda$) values indicate the degree of phylogenetic dependence of the data according to a Brownian motion model. If $\lambda=0$ traits are evolving independently of phylogeny and if $\lambda=1$ trait evolution is highly dependent on phylogeny (Pagel 1999). Lambda values can be greater than one when traits are more similar than predicted by Brownian motion and negative when closely related species have negatively correlated phenotypes (Freckleton et al. 2002).
Table 2.2. Phylogenetic generalized least-square regression (PGLS) analysis using Pagel’s lambda of the relationship between total brain mass, body mass, and mating system. Results are presented for a) males and b) females (n=102).

<table>
<thead>
<tr>
<th>sex</th>
<th>ML $\lambda^a$</th>
<th>variable</th>
<th>slope ± s.e.</th>
<th>t-value</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) males</td>
<td>0.83</td>
<td>Body mass</td>
<td>0.23 ± 0.01</td>
<td>33.72</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mating system</td>
<td>-0.002 ± 0.002</td>
<td>-1.30</td>
<td>0.20</td>
</tr>
<tr>
<td>b) females</td>
<td>0.96</td>
<td>Body mass</td>
<td>0.60 ± 0.02</td>
<td>24.65</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mating system</td>
<td>0.005 ± 0.01</td>
<td>0.81</td>
<td>0.42</td>
</tr>
</tbody>
</table>

*Maximum-likelihood lambda (ML $\lambda$) values indicate the degree of phylogenetic dependence of the data according to a Brownian motion model. If $\lambda=0$ traits are evolving independently of phylogeny and if $\lambda=1$ trait evolution is highly dependent on phylogeny (Pagel 1999). Lambda values can be greater than one when traits are more similar than predicted by Brownian motion and negative when closely related species have negatively correlated phenotypes (Freckleton et al. 2002).
Table 2.3. Phylogenetic generalized least-square regression (PGLS) analysis of the relationship between total brain mass, body mass, and testis mass. Results are presented for males and females in a) long-distance migrants \((n=20)\) and c) all other species \((n=59)\).

<table>
<thead>
<tr>
<th>sex</th>
<th>ML (\alpha^*)</th>
<th>variable</th>
<th>slope ± s.e.</th>
<th>t-value</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) long-distance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>migrants</td>
<td>Males</td>
<td>0.56</td>
<td>Body mass</td>
<td>0.30 ± 0.01</td>
<td>32.7</td>
</tr>
<tr>
<td></td>
<td>Testis mass</td>
<td>-0.02 ± 0.007</td>
<td>-2.38</td>
<td>0.0285</td>
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</tr>
<tr>
<td></td>
<td>Females</td>
<td>0.15</td>
<td>Body mass</td>
<td>0.53 ± 0.69</td>
<td>7.61</td>
</tr>
<tr>
<td></td>
<td>Testis mass</td>
<td>0.03 ± 0.05</td>
<td>0.55</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>b) all other</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>species</td>
<td>Males</td>
<td>0.40</td>
<td>Body mass</td>
<td>0.23 ± 0.01</td>
<td>27.89</td>
</tr>
<tr>
<td></td>
<td>Testis mass</td>
<td>-0.01 ± 0.01</td>
<td>-0.88</td>
<td>0.38</td>
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<tr>
<td></td>
<td>Females</td>
<td>0.04</td>
<td>Body mass</td>
<td>0.64 ± 0.04</td>
<td>15.28</td>
</tr>
<tr>
<td></td>
<td>Testis mass</td>
<td>0.01 ± 0.03</td>
<td>0.47</td>
<td>0.64</td>
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</tr>
</tbody>
</table>

*Maximum-likelihood alpha (ML \(\alpha\)) values indicate the strength of evolutionary constraint. Values close to zero represent results identical to those using Felsenstein’s independent contrasts, while larger values indicate little evolutionary constraint, whereby phylogeny has no effect on the data (Martins & Hansen 1997).
CHAPTER 3

THE INFLUENCE OF LIFE-HISTORY TRAITS AND LIFETIME REPRODUCTIVE OUTPUT ON FEMALE BRAIN SIZE EVOLUTION IN BIRDS
Summary

Brain size evolution in animals is associated with a number of behavioural and social characteristics. Furthermore, these socio-ecological determinants can often be attributed to differences in life-history strategies. In the past, it was frequently assumed that these selective pressures act similarly in shaping male and female relative brain size. However, intersexual conflict can affect the evolution of male and female reproductive behaviours, leading to differences in overall brain size. Using a large sex-specific dataset, we show that brain size evolution is differentially influenced by behavioural and ecological pressures in males and females. We found the female brain size is significantly influenced by development, where altricial species develop larger brains than precocial species, and pair-bond strength, where species forming long-term pair-bonds develop larger brains than species forming short-term or no pair-bonds. However, these same life-history traits had no effect on variation in male brain size. Although we did not find an effect of parental care on brain size variation in males or females, we suggest that parental investment may have an indirect effect on the evolution of larger brains in females through pair-bond strength and altricial development. Finally, we provide support for a positive relationship between female brain size and increased lifetime reproductive output. We suggest that this relationship could occur through one of two key mechanisms: allomaternal care and social buffering. Our study emphasizes the importance of the pair-bond strength in shaping female brain size and highlights the significant impact of male parental care on increased female lifetime reproductive output.
Introduction

Variation in ontological, behavioural, and morphological characteristics between species can often be attributed to differences in life-history strategies (Partridge & Harvey 1988). Differences in development tend to reflect a “slow-fast continuum” in which particular strategies will be associated with a unique combination of life-history traits (Fisher et al. 2001). In birds and mammals, altricial species typically represent the ‘slow’ end of the continuum which is coupled with longer gestation periods, delayed sexual maturation, and greater longevity. In contrast, precocial species represent the ‘fast’ end of the continuum which is coupled with rapid development and independence from parents (Bennett & Harvey 1985; Promislow & Harvey 1990; Starck 1993). In addition, researchers have postulated that variation in life-history strategies within these groups might be correlated with ecological factors such as the complexity of the habitat or degree of intraspecific competition (Bennett & Harvey 1985; Owens & Bennett 1995; Fisher et al. 2001).

Interspecific variation in brain size is often described as being an adaptive consequence of variation in life-history strategies (Shultz & Dunbar 2010). Variation in brain size is thought to stem from developmental adaptations, such as variation in gestation and incubation periods or parental care (Bennett & Harvey 1985; Iwaniuk & Nelson 2003), and behavioural adaptations, such as foraging ability and migration (Lefebvre et al. 1997; Sol et al. 2002; Lefebvre et al. 2004; Sol et al. 2005a). Moreover, socio-ecological factors, or the interaction between the physical and social environment, has been shown to significantly impact brain size evolution in a number of taxa. For example, brain size in cichlid fish is positively correlated with both habitat complexity
and the number of adult conspecifics in the habitat (Kotrschal et al. 1998; Pollen et al. 2007). Additionally, social living in primates has proven to be a critical determinant of brain size, where species in larger social groups develop larger brains to maintain group cohesion and cooperation (Dunbar 1998; Byrne & Bates 2007).

Although sex-specific variation in brain size has been poorly studied, empirical evidence of differential selection on male and female brain size has been documented in a few taxonomically divergent groups (Jacobs 1996; Garamszegi et al. 2005; Lindenfors et al. 2007). In primates, brain size evolution in males is said to be driven by physical interactions with competing males, whereas female brain size appears to be driven by social interactions within groups (Lindenfors et al. 2007). In birds, species with high rates of sperm competition demonstrate female-biased brain size dimorphism, where females develop larger brains than males; however, the reverse is shown in species with low rates of sperm competition (Garamszegi et al. 2005).

Parental investment can vary dramatically between males and females, leading to the evolution of sex-specific reproductive strategies (Trivers 1972; Clutton-Brock & Vincent 1991; Andersson 1994). In monogamous species, intersexual conflict can continue after mating due to differential investment in parental duties (Lessells 1999; Morales et al. 2010). Studies investigating the role of parental investment in determining relative brain size have found that females providing uniparental care for offspring develop larger brains than females providing biparental care with the help of a male partner in carnivores (Gittleman 1994) and fish (Gonzalez-Voyer et al. 2009). These studies have emphasized the increased investment of uniparental females in terms of increased energy allocated towards searching for food and guarding offspring from
predators. These behaviours could select for a larger brain as they require greater processing of environmental conditions (Gittleman 1994). As uniparental females must invest a greater amount of energy towards offspring survival, it has also been proposed that they develop larger brains because they need be very selective when choosing a mate in order to gain indirect benefits (Kokko et al. 2006; Gonzalez-Voyer et al. 2009).

Birds are an ideal system for studying the relationship between relative brain size and parental care, as males and females invest differentially in reproductive behaviours (Clutton-Brock & Vincent 1991; Garamszegi et al. 2005). In many species, males invest much of their energy into acquiring a mate and successfully fertilizing female eggs, often using elaborate or complex traits and behaviours (Clutton-Brock & Vincent 1991; Jacobs 1996). Conversely, females allocate their energy towards selecting a high quality male and producing and developing eggs (Clutton-Brock & Vincent 1991; Jacobs 1996). Beyond that, male and female birds often differ in the amount of parental care they provide. Accordingly, female reproductive investment is considered to be greater than male investment in most monogamous bird species (Trivers 1972). The incidence of biparental care in birds has been overestimated in the past (reported as about 90%, Kendeigh 1952; Silver et al. 1985), which likely reflects the occurrence of pair-bonds rather than biparental care itself (Lack 1968; Cockburn 2006). In a recent review, other forms of parental care, particularly female only care, are acknowledged as having a much wider distribution than previously reported (Cockburn 2006). While biparental care still remains fairly high (i.e. 80.8%, Cockburn 2006), male and female parental investment within this group is not always comparable. For example, in a number of species
exhibiting biparental care, males invest considerably less than females in offspring care (Verner & Willson 1969; Trivers 1972; Clutton-Brock & Vincent 1991).

As parental care is closely tied to the pair-bond, researchers have recently shifted their focus on the significance of pair-bond strength in brain size evolution (Emery et al. 2007; Shultz & Dunbar 2010). Some birds form lifelong pair-bonds and these species tend to develop the largest brains (Dunbar & Shultz 2007; Emery et al. 2007; Shultz & Dunbar 2010). Moreover, the type and quality of the bonded relationship have also proven to be critical in determining relative brain size (Emery 2006; Emery et al. 2007; Shultz & Dunbar 2010). According to the relationship intelligence hypothesis, species may require larger brains to accurately read and interpret their partner’s social signals, respond to them appropriately, and predict their behaviour in an attempt to deflect tension and conflict between the pair (Emery 2006). Similarly, larger brains may also be required to coordinate allocation efforts and make long-term decisions about mate fidelity (Shultz & Dunbar 2010). Consequently, it has been suggested that an association may be created between pair-bonding, altriciality, and relative brain size in birds (Shultz & Dunbar 2010). As species with long-term pair-bonds must be willing to provide extended parental care to offspring, this allows a prolonged developmental period, greater opportunity for behavioural flexibility, and lower mortality, ultimately providing increased benefits for stable relationships (Emery et al. 2007; Shultz & Dunbar 2010). Consequently, birds are an ideal system in which to study the interaction between relative brain size, altriciality, parental care, and pair-bond strength.

The expensive brain hypothesis predicts that the development of the brain can only be achieved through trade-offs with other metabolically expensive functions (Isler &
van Schaik 2009). Accordingly, the energy allocated towards developing a larger brain can only be compensated for to a certain viable brain size, termed the ‘grey ceiling’ (Isler & van Schaik 2009). Beyond this point, species may not be able to survive their environment and could eventually become extinct. More specifically, it has been suggested that if a negative relationship exist between relative brain size and maximum rate of population increase ($r_{max}$), this would provide evidence of a grey ceiling (Isler & van Schaik 2009). For any given species, $r_{max}$ represents the combined influence of maximum reproductive lifespan and annual offspring production per female (Cole 1954). It has been postulated that this trade-off between $r_{max}$ and brain size should be weakened or disappear when females receive help from conspecifics in provisioning offspring (i.e. allomaternal care, Isler & van Schaik 2009). Allomaternal care, which occurs commonly in cooperatively breeding mammals and altricial birds, allows females to increase growth and reproduction as their energetic burden during the breeding season is alleviated through helpers (Isler & van Schaik 2009; Shultz & Dunbar 2010). As a result, these females can overcome the trade-off between $r_{max}$ and brain size, subsequently increasing their reproductive lifespan.

In birds, the relationship between $r_{max}$ and relative brain size differs between altricial and precocial species, whereby only precocial species demonstrate a trade-off (Isler & van Schaik 2009). After hatching, precocial birds are immediately able to feed and forage independently (Starck 1993; Starck & Ricklefs 1998); consequently, parents must invest the energy necessary to sustain such activity into the developing egg. Altricial species, on the other hand, are completely dependent on parents for food, warmth, and protection from predators (Starck 1993; Starck & Ricklefs 1998). Moreover,
it has been well established that altricial species develop relatively larger brains than precocial species in adulthood (Bennett & Harvey 1985). Consequently, it has been suggested that the absence of a trade-off between $r_{\text{max}}$ and brain size in altricial birds results from significant allomaternal input (Isler & van Schaik 2009). However, little support has been provided for this hypothesis as brain size does not appear to vary according to parental care in birds (Shultz & Dunbar 2010).

Few studies incorporate the potential energetic trade-offs associated with developing a larger brain when studying the relationship between brain size and life-history traits and ecology (Deaner et al. 2003; Barrickman et al. 2008; Isler & van Schaik 2009). Moreover, even fewer studies investigate these differences separately within males and females (Gittleman 1994; Gonzalez-Voyer et al. 2009). In order to study sex-specific selection pressures on brain size, researchers must include measurements taken from both mature males and females (Healy & Rowe 2007). As these data are not always easily obtainable, only a small number of studies have investigated sex-specific influences on brain size evolution.

Here, we collected sex-specific data to investigate the influence of life-history traits on brain size evolution in male and female birds. Specifically, we investigated the possible influence of developmental mode, parental investment, pair-bond strength, and lifetime reproductive output on relative brain size in male and female birds. First, we predicted that altricial and semi-altricial species would develop larger brains than precocial and semi-precocial species, as this has been extensively shown in birds. Second, we predicted that females providing uniparental care would develop larger brains than females receiving help from a male partner, as has been shown in fish and mammals.
Third, we predicted that species forming long-term pair-bonds would develop larger brains than species with short-term or no pair-bonds, as long-term pair bonds require greater coordination and synchronization of behaviour. Finally, we predicted that female brain size would be positively correlated with lifetime reproductive output in biparental but not uniparental species, as it has been suggested that allomaternal care allows females to increase their reproductive lifespan (Isler & van Schaik 2009). More specifically, we predicted that this relationship would be present in altricial but not precocial species, particularly within biparental altricial species.

Methods

Endocranial volume

We measured endocranial volume from 2043 museum specimens, representing 106 species, housed at the University of Michigan Museum of Zoology. We measured sexed and intact adult skulls (i.e. lacking visible fractures or dents). When available, we collected body masses from specimen vouchers. If this information was missing, we average sex-specific weights of all other specimens sampled or utilized body mass values from the CRC Handbook of avian weights (Dunning 2008). Finally, we selected specimens collected within a limited geographic range whenever possible to account for potential geographic variation in brain size due to environmental variation (Schuck-Paim et al. 2008).

We measured endocranial volume following the procedure outlined by Iwaniuk and Nelson (2002). Briefly, we blocked orbital fissures and external acoustic meatuses to delineate the approximate cranial space previously occupied by fresh brain tissue (Jue
1990). We then filled each skull via the foramen magnum with dust lead shot (Iwaniuk & Nelson 2002). To minimize interstitial space between each shot, we repeatedly tapped the skull and probed through the foramen as the shot was being poured in (Iwaniuk & Nelson 2002, 2003). Once the endocranial cavity was filled, we decanted the lead shots into 10 and 20 ml volumetric cylinders (Ashwell 2008), and measured the volume to the nearest 0.25 ml. Volumetric endocranial measurements were converted into brain masses by multiplying by the density of fresh brain tissue (1.036 g ml$^{-1}$; Stephan 1960; Iwaniuk & Nelson 2002).

**Life-history traits**

We collected information on life-history traits from the literature and from published species accounts (Handbook of Birds of the World, Birds of North America, and the Cornell Lab of Ornithology). Ontology has been described as a key determinant in brain size evolution in birds, particularly concerning developmental state at hatching (Bennett & Harvey 1985; Iwaniuk & Nelson 2003). Species were identified as precocial (well developed, active locomotion, downy feathered), semi-precocial (intermediate, reduced locomotion), altricial (lack motor activity, eyes closed, naked), and semi-altricial (intermediate, downy feathered with limited motor activity) (Starck 1993).

Parental care was broadly defined based on Cockburn’s (2006) classification as male only care, female only care, or biparental care. However, as parental care consists of a number of different behaviours performed throughout the breeding season, which may be equally or unequally performed by males and females, we specifically defined parental care according to a number of behaviours associated with offspring care: incubation, brooding, and feeding. Each behaviour was defined by the sex performing the care: male
only, female only, or pair. In the case of feeding behaviour, two additional categories were included: offspring (where young are able to find resources and feed for themselves soon after hatching) and offspring lead by female (whereby young are able feed on their own but are shown food sources by the female, Starck 1993; Starck & Ricklefs 1998).

We chose pair-bond duration, rather than mating system, as our proxy for stability in a relationship within a pair, as this life-history trait has been shown to be important in brain size evolution (Dunbar & Shultz 2007; Emery et al. 2007; Shultz & Dunbar 2010). Pair-bond duration was categorized according to Shultz and Dunbar (2010) as either polygynous (non-pair), short-term (seasonal or annual pairs, likely to change between years), and long-term (stable pairs across multiple breeding seasons).

We also calculated female lifetime reproductive output based on the number of offspring produced by a female throughout her reproductive lifespan. In order to calculate this we determined the number of theoretical reproductive years for each female (i.e. longevity minus age at reproductive maturity) and multiplied this by the number of broods per breeding season and the number of eggs per brood/clutch. Female lifetime reproductive output values were then log_{10}-transformed to include in our analyses.

**Comparative methods**

Large, comparative datasets often result in phylogenetic non-independence, as some species are more closely related than others (Harvey & Pagel 1991; Garland & Ives 2000) and may share similar genetic markers or ecological niches (Freckleton et al. 2002). To control for similarities between closely related species, phylogenetic relationships should be taken into account. We used information from published supertrees and phylogenies to create a composite phylogeny of the species in our dataset (Appendix C). We constructed
our tree using Mesquite 2.72 (Maddison & Maddison 2009) and set the branch lengths equal to one (Garland et al. 1993).

We utilized phylogenetic generalized least squares (PGLS) for our analyses as this method incorporates phylogenetic information and allows the degree of phylogenetic influence on each trait of interest to be determined and controlled for within our dataset (Freckleton et al. 2002). PGLS also allows the inclusion of both continuous and categorical variables in our models. All PGLS analyses were implemented in R 2.10.1 (Ihaka & Gentleman 1996) using the APE (Analysis of Phylogenetics and Evolution) and ‘nlme’ packages (Paradis et al. 2004), assuming a Brownian motion model, Pagel’s $\lambda$ (Grafen 1989; Pagel 1999). According to the Brownian motion model, lambda ($\lambda$) indicates the degree to which relatedness explains the data, where $\lambda=0$ if there is no autocorrelation and traits are evolving independently of phylogeny, and $\lambda=1$ if the data follow Brownian motion and trait evolution is highly dependent on phylogeny (Pagel 1999; Freckleton et al. 2002). Additionally, we calculated brain size/body size residuals using a phylogenetically controlled PGLS of log$_{10}$-transformed brain mass and log$_{10}$-transformed body mass (Shultz & Dunbar 2010). We used this analysis to calculate brain size residuals while removing the effects of phylogenetic autocorrelation (Shultz & Dunbar 2010).

We conducted sex-specific analyses to determine which life-history traits influenced brain size variation in males and females. We analyzed each of our traits using PGLS analysis, followed by post-hoc pairwise comparisons for categorical variables. First, we looked at the relationship between relative brain size and 1) developmental mode (i.e. altricial vs. precocial); 2) parental care (i.e. incubation, brooding, and feeding);
and 3) pair-bond strength in males and females separately. We entered these categorical variables as factors in our analysis. Next, we investigated the relationship between female relative brain size and lifetime reproductive output. We looked at this relationship in 1) precocial vs. altricial species; 2) uniparental vs. biparental species; and 3) uniparental vs. biparental altricial species. We entered relative brain size (i.e. male or female) as the dependent variable with our life-history traits as the independent variables. For all significant predictors (α<0.05), we used post-hoc pairwise comparisons to identify life-history traits that were significantly different within a category.

**Results**

We found that the influence of developmental mode on relative brain size differed between males and females. In particular, we found that altricial and semi-altricial females developed significantly larger brains than precocial or semi-precocial females (Table 3.1A). Although male brain size varied with developmental mode in a similar direction, these differences were not significant (Table 3.1B).

We also wanted to determine whether variation in parental roles (i.e. male only, female only, or pair) influenced brain size variation within each sex. We found that relative brain size did not vary according to variation in incubation, brooding, and feeding roles in males or females (Table 3.1).

We also found that pair-bond strength differentially influenced brain size in females (Table 3.1A) and males (Table 3.1B). In females, species forming stronger pair-bonds developed larger brains than species with short-term or no pair-bonds (Figure 3.1A). Although there was no significant influence of pair-bond strength on male brain
size, the direction of the relationship was reversed, where increases in brain size were associated with decreases in pair-bond strength and increases in polygyny (Figure 3.1B).

Finally, we focused on females and sought to determine the potential role of allomaternal care and lifetime reproductive output on interspecific variation in relative brain size. As it has been suggested that reproductive lifespan is more likely to be influenced by allomaternal care in altricial females, we investigated the relationship between female brain size and lifetime reproductive output in altricial and precocial species. We found that altricial species exhibited a significant positive relationship between relative brain size and lifetime reproductive output ($t = 3.19$, $N = 63$, $P = 0.002$), whereas there was no significant relationship in precocial species ($t = -0.17$, $N = 33$, $P = 0.86$). Next, we wanted to determine whether, in general, females who receive help at the nest (i.e. biparental females) gain a reproductive advantage compared to uniparental females, allowing them to develop larger brains. We found that biparental females demonstrated a significant positive relationship between brain size and lifetime reproductive output ($t = 3.53$, $N = 40$, $P = 0.001$), whereas there was no significant relationship in uniparental females ($t = 1.48$, $N = 48$, $P = 0.147$). Finally, to determine whether allomaternal care is in fact driving this positive relationship in altricial species, we compared uniparental and biparental altricial females. We found that only biparental females demonstrated a significant positive relationship between relative brain size and lifetime reproductive output ($t = 3.68$, $N = 27$, $P = 0.001$).
Discussion

Our findings reveal that life-history traits may have a differential influence on brain size evolution in male and female birds. First, we found that altricial species had larger brains than precocial species, but this relationship was only significant in females. Moreover, we found that parental care had no effect on male or female relative brain size. We also found that pair-bond strength significantly influenced relative brain size in females, where species with stronger pair-bonds developed larger brains. This relationship was not significant in males; indeed, the trend was reversed in males, where species with stronger pair-bonds developed smaller brains. Although parental care strategies did not influence male or female brain size, our findings suggest that parental care may have an indirect influence on brain size through its association with other life-history traits. In particular, we found that female brain size was positively correlated with lifetime reproductive output in biparental altricial species. Our findings suggest that pair-bond strength, through its strong association with biparental care and altricial development, may facilitate the development of a larger brain in females by alleviating the energetic burdens associated with rearing offspring, leading to greater lifetime reproductive output. Our findings also highlight the importance of including sex-specific measurements when studying brain size, as the influence of life-history traits may differ between the sexes.

Ontological differences have been shown to shape brain size variation across species, where altricial species develop larger brains than precocial species in adulthood (Bennett & Harvey 1985; Iwaniuk & Nelson 2003). As we predicted altricial and semi-altricial species developed larger brains than precocial and semi-precocial species. However, we also demonstrate that the influence of development on brain size evolution
appears to be stronger in females, as the relationship in males was not significant. Our study is the first to suggest that development may affect male and female brain size differently in adulthood.

Parental investment often differs dramatically between the sexes, and this can lead to intersexual conflict and the evolution of sex-specific reproductive behaviours (Trivers 1972; West-Eberhard et al. 1987; Lindenfors et al. 2007). In fish and mammals, parental care has been shown to significantly influence brain size variation, particularly in females (Gittleman 1994; Gonzalez-Voyer et al. 2009). The only study to investigate this relationship in birds found no evidence of increased brain size in species with uniparental care (Shultz & Dunbar 2010), although that study used pooled data on male and female brain size. Our sex-specific dataset corroborated these findings, wherein there was no difference in brain size between uniparental females and biparental females.

Pair-bond strength has been suggested to influence brain size as it requires the coordination and synchronization of reproductive behaviours to prevent cuckoldry and minimize potential investment conflicts between males and females (Shultz & Dunbar 2010). Although no correlation has been found between relative brain size and parental care in birds (this study; Shultz & Dunbar 2010), more recent studies have shown that pair-bond strength is also an important indicator of brain size variation among many taxa, including birds (Dunbar & Shultz 2007; Emery et al. 2007; Shultz & Dunbar 2010). The suggested importance of the pair-bond stems from the social intelligence hypothesis, which highlights the importance of negotiating or coordinating decisions, particularly those affecting reproductive success (Dunbar 1998). Although in the past this hypothesis has focused heavily on the size of the social group in primates; in birds, it has become
evident that the quality of close relationships, particularly that of the reproductive pair, is much more influential than the total number of social relationships (Emery et al. 2007; Shultz & Dunbar 2010). Accordingly our data showed that relative female brain size varied significantly with pair-bond strength, where long-term pair-bonds were associated with larger brains (Figure 3.1A). Although there was no significant variation in males, they followed the opposite pattern, where polygynous species that do not form pair-bonds tended to develop larger brains (Figure 3.1B). Perhaps these patterns emerge because females are being selected for larger brains to be choosy in mate selection in order to gain direct benefits through increased paternal investment, whereas males may develop larger brains in order to better attract as many mates as possible, both strategies leading to greater reproductive success.

Recently, Shultz and Dunbar (2010) created a path model showing the interaction between parental care, pair-bond strength, and larger brains. Their model suggests that biparental care may indirectly influence the evolution of larger brains through its association with long-term pair-bonding (Shultz & Dunbar 2010). Our findings are consistent with this evolutionary model; we found that brain size did not vary according to parental roles related to incubating, brooding or feeding, but female brain size did vary according to pair-bond strength. Furthermore, Shultz and Dunbar’s (2010) path model also indicated that altricial development was significantly associated with both biparental care and the evolution of large brains (Shultz & Dunbar 2010), providing a second opportunity for biparental care to indirectly influence brain size.

Altricial development has long been linked to relatively larger brains in birds (Starck 1993). As these species typically exhibit slow life-history traits, such as
prolonged incubation, extended parental care, and delayed sexual maturation, they
typically require longer developmental periods (Partridge & Harvey 1988; Starck &
Ricklefs 1998). The cognitive buffer hypothesis postulates that the costs of developing a
large brain and having delayed reproduction in these species can be compensated for by
increased survival rates that favour a longer reproductive lifespan (Sol et al. 2007; Sol
2009). Our findings support this relationship as we found a significant positive
relationship between relative brain size and lifetime reproductive output in altricial but
not precocial female birds. Furthermore, we showed that within altricial species, only
biparental females exhibit this positive relationship, reinforcing the link between
biparental care and larger brains. We propose two alternative but not mutually exclusive
explanations for why we might see these relationships in females.

First, it has been proposed that because altricial species receive allomaternal care,
they are able to invest more in growth and reproduction since a portion of their energetic
burden is alleviated through help at the nest (Isler & van Schaik 2009). Consequently, it
has been suggested that these females can extend their reproductive lifespan. In a
comparative study in birds, the absence of a male partner during the feeding stage led to
reduced female reproductive success, measured as the number of fledged offspring
(Møller 2000). Furthermore, in California mice (Peromyscus californicus), mated females
were able to raise litters that were double or triple the size of unmated females, and
offspring survival varied drastically with only 21% of pups surviving when males were
absent and 81% surviving in the presence of a male (Cantoni & Brown 1997).
Additionally, the duration and strength of a pair-bond can also influence female
reproductive success, such as in Australian magpie-larks (Grallina cyanoleuca). These
birds form long-term monogamous pair-bonds, and it has been shown that established pairs have higher annual reproductive success than new pairs because they are able to breed earlier and successfully rear two broods (Hall 1999). Moreover, females gain additional benefits by staying with the same male for multiple breeding seasons, as they can afford to decrease their feeding rates, which are compensated for by their male partner (Hall 1999). These examples provide a mechanism whereby male parental care, and more specifically strong pair-bonds, can lead to increased female reproductive success. Furthermore, as females do not need to allocate as much energy towards offspring care, this could allow them to invest in other morphological traits or behaviours, such as brain size.

Although allomaternal care is one possible mechanism favouring the evolution of larger brain size in females, our current results do not provide direct evidence for this relationship as we do not see brain size variation among parental behaviours. An alternative hypothesis focuses more readily on social interactions and the formation of a strong, long-term pair-bond, which is significantly associated with the evolution of larger brains (this study; Shultz & Dunbar 2010). Social interactions, particularly between pair-bonded partners, are important for cooperation and protection from environmental stressors (Kikusui et al. 2006). It has been shown that the presence of a social partner reduces stress levels by diminishing the intensity of a perceived threat and down-regulating neuroendocrine responses (Rukstalis & French 2005; Kikusui et al. 2006). In a number of studies, the presence of a bonding partner in monogamous pairs induced what is termed “social buffering,” leading to subsequent decreases in corticosterone stress hormone levels (titi monkeys (Callicebus moloch), Hennessy et al. 1995; Siberian
hamsters (*Phodopus sungorus*), Castro & Matt 1997; prairie voles (*Microtus ochrogaster*), DeVries 2002; and black tufted-ear marmosets (*Callithrix kuhlii*), Rukstalis & French 2005). In fact, evidence of social buffering has been found in rodents, birds, non-humans primates, and even humans (Kikusui et al. 2006). Although the presence of a partner can decrease levels of stress hormones, the absence of a partner can produce the opposite effects by increasing corticosterone levels (Hennessy et al. 1995; DeVries 2002; DeVries et al. 2003). Moreover, in birds, these physiological and behavioural changes have negative effects on birth rate, leading to subsequent reductions in clutch size (Travers et al. 2010) as well as reduced offspring growth rate (Hayward & Wingfield 2004). Consequently, we propose that the relationship between female relative brain size and lifetime reproductive output may be due to long-term pair-bonding and the presence of a partner, rather than direct help with caring for offspring. Our data show that species with long-term pair-bonds develop larger brains than species with shorter or no pair-bonds. Perhaps the presence of a social partner reduces stress levels and other potentially detrimental physiological reactions, which may have enable females to develop larger brains while increasing their reproductive success.

**Conclusions**

Our study provides new insights on the evolutionary factors driving brain size evolution in birds. Importantly, our findings emphasize the differential selective pressures affecting males and females and stress the importance of using sex-specific data when investigating sex-specific influences. In addition, we also demonstrate that parental investment does not necessarily have a direct association with brain size but may be mediated by pair-bond strength and altricial development, which do appear to have a
direct influence on brain size. Furthermore, we suggest that allomaternal care may not be
the only mechanism allowing altricial species to develop larger brains, and propose social
buffering and strong-pair bonds as an alternative. Future studies should focus on
comparing reproductive success between species differing in pair-bond strength in order
to determine whether long-term pair-bonds generally lead to greater reproductive success
in females. Moreover, future research should also focus on how a diversity of male
contributions (e.g., whether he feeds the incubating female) could influence the evolution
of female brain size.

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Table 3.1. Relationship between relative brain size and life-history traits using univariate phylogenetically controlled generalized least squares analysis in (A) females and (B) males.

(A) females

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>P</th>
<th>AIC</th>
<th>Lambda</th>
<th>d.f.</th>
</tr>
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<tbody>
<tr>
<td>developmental mode</td>
<td>3.35</td>
<td>0.02</td>
<td>-228.94</td>
<td>0.93</td>
<td>3,102</td>
</tr>
<tr>
<td>pair-bond strength</td>
<td>4.24</td>
<td>0.02</td>
<td>-226.59</td>
<td>0.98</td>
<td>2,102</td>
</tr>
<tr>
<td>incubation</td>
<td>0.16</td>
<td>0.69</td>
<td>-220.17</td>
<td>0.98</td>
<td>1,101</td>
</tr>
<tr>
<td>brooding</td>
<td>0.18</td>
<td>0.67</td>
<td>-217.51</td>
<td>0.99</td>
<td>1,100</td>
</tr>
<tr>
<td>feeding</td>
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<td>0.50</td>
<td>-205.77</td>
<td>0.98</td>
<td>2,95</td>
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</table>

(B) males

<table>
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<td>pair-bond strength</td>
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<td>-457.37</td>
<td>0.83</td>
<td>2,95</td>
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</table>

AIC, Akaike information criteria.
Figure 3.1. The relationship between pair-bond strength (long-term, short-term, and polygynous) and phylogenetically controlled generalized least squares analysis of relative brain size in (A) females and (B) males.
CHAPTER 4

GENERAL DISCUSSION AND IMPLICATIONS
Thesis summary and implications

Why do some animals develop larger brains than others? Among vertebrates, relative brain size can vary considerably, and its relationship with perceptual or behavioural capacities has been under extensive study (Jerison 1973; Harvey & Krebs 1990). Although considerable variation can be attributed to body size allometry, substantial differences still remain when these variables are controlled for. What then is driving the remaining variation in brain size across species? Considering that the brain is an organ with the ability to process and integrate information from the environment, it has been proposed that developing a larger brain may incur adaptive benefits (Jerison 1973; Partridge & Harvey 1988; Lefebvre & Sol 2008). Over the past few decades, researchers have studied ecological, behavioural, and social influences on the evolution of the brain and its components. In a number of taxa, including mammals, birds, and fish, relative brain size is known to reflect differences in home-range size (Clutton-Brock & Harvey 1980), life-history (Bennett & Harvey 1985; Iwaniuk & Nelson 2003), habitat complexity (Kotrschal et al. 1998; Pollen et al. 2007), behavioural flexibility (Lefebvre et al. 1997; Lefebvre et al. 2004), migratory distance (Sol et al. 2005b; Sol et al. 2010), and social living (Dunbar 1992, 1998; Byrne & Bates 2007). Presumably, these factors should shape the evolution of male and female behaviours in the same way, leading natural selection to affect brain size in the two sexes similarly (Lande 1980). Then again, sex-specific behaviours may lead to sex-specific patterns of overall brain size evolution (Trivers 1972; Garamszegi et al. 2005). To date, sex-specific studies have been rather limited and have focused on the social environment, such as competitive demands in primates (Lindenfors et al. 2007) and parental care in carnivores (Gittleman 1994) and
fish (Gonzalez-Voyer et al. 2009). These studies demonstrate that sex-specific selection pressures could result in differential selection on brain structures in males and females.

The objective of my thesis was to investigate the role of sex-specific selective pressures on brain size evolution in male and female birds. We used a large, sex-specific comparative dataset to investigate multiple selective factors that might influence brain size evolution. In Chapter 2, we investigated the expensive sexual tissue hypothesis, or more specifically, the potential trade-off between developing larger testis size and brain size in male birds. This hypothesis postulates that the investment in costly sexually selected traits (i.e. ornaments, armaments, or organs) will incur energetic costs, limiting the development of the brain and leading to overall smaller brain size (Pitnick et al. 2006). We found that male passerines showed a negative correlation between testis mass and brain mass, whereas male non-passerines did not. Moreover, neither female passerines nor non-passerines exhibited this correlation with testis size. Furthermore, we found that long-distance migrants were more likely to experience this trade-off, presumably because of the additional energetic and size constrains imposed by flight. Our findings suggest a novel conflict between selection for increased brain size and selection for enhanced sperm competition in males, lending support for the expensive sexual tissue hypothesis in birds.

In Chapter 3, we investigated sex-specific determinants of brain size and focused on the influence of parental investment and reproductive strategies on female brain size. We found that males and females are differentially influenced by behavioural and ecological pressures, as brain size variation in females was significantly affected by development and pair-bond strength, whereas relative male brain size was not. Altricial
females and females with stronger pair-bonds developed larger brains. Neither male nor female relative brain size was influenced by variation in parental roles, but there may be an indirect influence of biparental care, as this trait correlates with pair-bond strength and altricial development, both of which significantly influence brain size evolution. Finally, this chapter also provides preliminary support for the hypothesis that the presence of a male partner may provide females with adaptive benefits that ultimately lead to greater female lifetime reproductive output.

Collectively, these two studies enhance our understanding of the selective factors influencing the evolution of brain size in males and females. Our findings in Chapter 2 suggest that sexual selection, through the development of costly traits that allow males to compete for access to females, is a critical driving force in brain size variation among males. Our findings in Chapter 3 highlight the importance of natural and social selection on interspecific variation in brain size among females. These findings also call into question the assumption that brain size evolution occurs in a unified manner in males and females. My research will make an important contribution to recently emerging research on sexual differences in brain size evolution. Investigating variation in brain size at a sex-specific level can provide insights into the selection pressures operating between the sexes as well as the pressures occurring within a single sex.

**Areas of future research**

The study of brain size evolution offers a number of potential avenues for future research, not only across birds but among all vertebrates. Although researchers have often measured and averaged male and female brain size as being equivalent, our findings suggest that this is clearly not the case. Future comparative analyses should include both
measures of male and female brain size, while controlling for sex-specific weights. Indeed, our findings suggest that pooling data together can obscure divergent patterns in males and females. For example, in Chapter 3 we saw that pair-bond strength exhibited opposite selection on male and female brain size, where female relative brain size increased with pair-bond strength while male relative brain size decreased with pair-bond strength. Similarly, other studies have shown that males and females can differ in relation to selective factors affecting brain development, such as parental behaviour (Gonzalez-Voyer et al. 2009), although such studies are relatively rare. Consequently, we encourage future studies to include sex-specific brain size measurements.

Recently, Möller (2010) proposed that external head volume can act as an accurate estimate of brain size. If this is the case, two main advantages can contribute to the field of brain size evolution. Firstly, with this technique, measurements could readily be taken in the field and would provide a non-destructive method of measuring brain volume in the wild. Furthermore, obscure species, which may not be well-represented in museum specimens, could be added to an ever growing dataset on brain size in birds. Second, measuring external head volume could allow studies to focus on intraspecific variation in brain size and to investigate changes in brain size within a lifetime. To date, it has been presumed that brain size is invariant within an individual’s lifetime (Healy & Rowe 2007); however, research has demonstrated that brain size can vary with experience and development (Rosenzweig & Bennett 1996; Moller 2010). Consequently, researchers could follow individuals within a population over their life, where behavioural challenges can be observed and their relationship with brain size can be
quantified. Furthermore, these changes in brain size could be coupled with investigations of sex-specific behaviours observable in the wild.

Although whole brain size can be very useful as an exploratory tool in order to investigate general patterns between species, brain composition may prove to be more informative as it can indicate which structures of the brain may differ between the species or even males and females of the same species. In primates, for example, males and females develop similar sized brains; however, this may be for different evolutionary reasons. In females, larger brains are associated with social living and in fact, females demonstrate a significant positive relationship between telencephalon size and female group size (Lindenfors et al. 2007). However, larger brains in males are associated with male competition and diencephalon size is positively correlated with male group size (Lindenfors et al. 2007). Consequently, males and females may have the same brain size, but vary in brain composition due to varying selective pressures. Researchers have suggested that species occupying similar physical and social environments evolve comparable brain proportions (de Winter & Oxnard 2001; Iwaniuk & Hurd 2005). The development of these cerebrotypes has been documented in a variety of taxa, including mammals (Clark et al. 2001) and birds (Iwaniuk & Hurd 2005). However, males and females within the same species could also be developing unique cerebrotypes because of the evolution of sex-specific behaviours. Future research should include measures of brain structures when investigating sex differences in order to determine whether males and females engaging in varying behaviours also differ in cerebrotypes.
Pair-bond strength has emerged as an important evolutionary determinant of brain size in birds (Dunbar & Shultz 2007; Emery et al. 2007; Shultz & Dunbar 2010). However, measures of pair-bond strength are often categorical, grouping birds with very different relationships into a single group. For example, species forming lifelong pair-bonds, such as the greylag goose (Anser anser) and the rook (Corvus frugilegus), can have very different ecologies and life-histories (Emert et al. 2007). Consequently, it is important to distinguish the differences when studying brain size evolution across species. As well, future studies investigating the importance of this bond should incorporate additional quantitative measures, such as the proportion of time spent with partner or the number of consecutive years of the pair-bond compared to total lifespan. These more quantifiable measures will allow researchers to better compare species within a single category and determine the role of pair-bond strength in brain size evolution.

Although the influence of evolutionary selective pressures on brain size has been well studied, other proximal factors have been overlooked (Healy and Rowe 2007). More recently, for example, Iwaniuk et al. (2006) have shown that pesticide (e.g. dichlorodiphenyltrichloroethane, DDT) exposure in American robins (Turdus migratorius) resulted in smaller brain and relative forebrain volume, reduction in two song nuclei, as well as reduction in the size of neurons. Furthermore, Brenowitz et al. (1991) found that activational hormone levels also negatively influenced the developing brain, leading to reduction in song nuclei. Consequently, these studies highlight the importance of investigating factors other than behavioural or social interactions, as these can lead to immediate changes in brain size.
Across species, males and females vary in a number of morphological and behavioural characteristics. Although these differences have been extensively studied in a variety of conspicuous physical traits (Andersson 1994), internal structures, which are less commonly studied, may be similarly affected (Martin & Atkinson 1977; Jacobs 1996; Murphy & James 2009). The study of sexual differences in brain size has received little attention until recently. Our research highlights the importance of investigating sex-specific behaviour and morphology in males and females, in order to determine whether these are differentially influencing the development and evolution of other traits, such as brain size.

References


APPENDIX A
MEASURING BRAIN SIZE

Studying brain size evolution is often accompanied by a challenging task: measuring brain size. In the past two decades, the amount of data available for a number of taxa, including bats (Hutcheon et al. 2002; Pitnick et al. 2006), cetaceans (Marino 2005; Marino et al. 2006), ungulates (Pérez-Barbería & Gordon 2005), primates (Reader & Laland 2002; Schillaci 2006), and birds (Iwaniuk & Nelson 2002; Garamszegi & Eens 2004), has increased significantly. Moreover, researchers have adapted and developed a number of techniques to measure or approximate brain size.

One of the oldest techniques is the measurement of dead specimens; however, this technique is far less common in more recent studies. When specimens are fresh, the brain can be perfused, fixed, and sliced, and then mounted and stained for visualization under a microscope (Day et al. 2005). However, specimens are not always fresh and are often preserved either by freeze-thaw techniques (Garamszegi et al. 2005; Møller et al. 2005) or rehydrated in formalin (Iwaniuk & Arnold 2004). For these specimens, post-mortem examinations are conducted where the brain is dissected out of the skull and weighed on a balance. However, these measurements have proven to be controversial as freezing or dissections change the water content, and hence the mass, of brain tissue (Yaskin 1984; Healy & Rowe 2007).

The avian brain, in particular, is a bulbous structure that lies in the back of the skull and completely fills the cranial cavity (Zusi 1993). Moreover, the meninges in the avian brain are much thinner than in mammals (Iwaniuk & Nelson 2002). Consequently, the brain conforms closely to the shape of the brain case and completely fills the cranium (Zusi 1993; Isler & van Schaik 2006a). Typically, researchers measure cranial capacity as
an estimate of brain size through two main methods: using a volumetric equation based on linear measurements of the skull (Madden 2001; Marino et al. 2006) or filling the skull with a substance that approaches the volume equivalent of a liquid (Iwaniuk & Nelson 2002). In order to determine linear measurements, researchers have utilized various imaging tools, including X-rays (Madden 2001) and computed tomography (Marino et al. 2003). With imaging, the length, height, and width of the skull are recorded and then brain volume can be estimated using a simple formula (i.e. length x height x width). When filling the skull, a liquid equivalent is poured into the hollow skull via the foramen magnum and decanted for volumetric measurement (Jue 1990; Iwaniuk & Nelson 2002; Ashwell 2008). Previously, researchers used mustard, millet, and flax seeds for this technique; however, today researchers more commonly use glass, plastic, or lead beads Marino 1997; Iwaniuk & Nelson 2002; Ashwell 2008). Based on these acquired volumetric measurements, brain mass can be determined by multiplying by the density of fresh brain tissue (1.036g/ml; Stephan 1960; Iwaniuk & Nelson 2002).

Previous studies have confirmed that endocranial volume is a reliable and accurate estimate of brain size in a variety of taxa (cetaceans, Marino 1999; birds, Iwaniuk & Nelson 2002; marsupials, Ashwell 2008; primates, Isler et al. 2008). In fact, it has recently been suggested that endocranial volume may be a more accurate representation of an individual’s brain size throughout their life (Isler et al. 2008), as preservation techniques may actually distort or alter the apparent mass of the specimen (formalin increases brain size while alcohol decreases brain size, Bauchot & Stephan 1969; Isler et al. 2008).
Finally, and very recently, external head volume has been suggested as an accurate estimate of brain size (Møller 2010). In barn swallows, Møller (2010) measured external head length, width, and height, and estimated head volume as an ellipsoidal function. These measurements were strongly correlated with estimates from previous studies using fresh brain tissue and endocranial volume (Møller 2010). This is the first study to investigate brain size in the field and provides an accurate, non-destructive method of measuring brain volume in the wild. Furthermore, this technique will allow researchers to follow individuals within a population over multiple years (or their entire lives), where behavioural tasks and challenges can be observed and their relationship with brain size can be quantified.

References


MEASURING TESTIS SIZE

Detailed studies of natural testis mass in the wild are scarce, and with good reason. As testes are internally located in birds, the only way to acquire these measurements is through animal sacrifice. As a result of this destructive sampling, most of the data available, to date, rely heavily on linear measurements taken from representative samples of males from museum collections (Møller 1991; Møller & Briskie 1995; Dunn et al. 2001; Pitcher et al. 2005). Generally, specimen vouchers provide testis length and width measurements. Based on these measures, testis mass can be estimated using the following equation: testes mass (g) = 2 x 1.087 g/cm3·1.33π·a^2 (cm^2)·b (cm), where a is the largest and b is the smallest radius of the testis (Møller 1991). Nevertheless, these measurements can prove to be erroneous when particular assumptions are ignored. Calhim & Birkhead (2006) proposed two key assumptions that must be met in order to appropriately utilize linear measurements of testis size.

Selecting representative samples of males

Seasonal variation in testis mass can be extensive in males, particularly between the breeding and non-breeding season. As a result, each species is often characterized by a unique “peak window” where testis size is maximal (Frith et al. 1976; Calhim & Birkhead 2006). To date, researchers have used two approaches in order to avoid this sampling error: the “means” and “polynomial” methods, which sample only males within their pre-determined peak window. The means method, most commonly applied in temperate species, averages testis mass across individual samples within a pre-defined breeding season (see Møller 1991; Dunn et al. 2001). However, as many tropical birds
breed year-long and do not have a distinct peak window, this method may not universally applicable (Ward 1969; Calhim & Birkhead 2006). Consequently, the polynomial method is preferred for these species (see Möller & Briskie 1995), as it fits testis size against calendar date within the breeding season (Calhim & Birkhead 2006).

In addition to acknowledging male breeding stage, geographic and age effects also need to be considered as they can influence testis mass between and within species (Pitcher et al. 2005; Calhim & Birkhead 2006). Accordingly, samples need to be taken from fully matured, adult males from the same geographic location.

**Obtaining testis mass from linear dimensions**

When making use of linear testis measurements, it is important to include length-to-width dimensions, as this ratio can differ considerably across species (Calhim & Birkhead 2006). Using a single measure (e.g. length) will result in incorrect values. In addition, testes tend to be asymmetric, so it is also important to include measures of both testes when calculating the combined testis mass in order to avoid overestimating testis size (Calhim & Birkhead 2006).

In our study (Chapter 2), both of these assumptions were met. Our measures of testis size were obtained from a large comparative dataset (Calhim & Birkhead 2006), where we only included combined testis mass from both the left and right testes. Furthermore, for each species we ensured that the measurements reflected a representative sample of mature males during the breeding season, found within the same geographic location as our measured endocranial volumes.
References


APPENDIX C
COMPOSITE PHYLOGENY

Our phylogenetic hypothesis for avian orders bests reflects a composite phylogeny (Figure C.1) based on Hackett et al. (2008) and Ericson et al. (2006). The avian tree developed by Hackett et al. (2008) was constructed based on a 32kb alignment of 19 different nuclear loci. Although this phylogeny seems to agree with many other classifications (namely Sibley & Ahlquist 1990; Livezey & Zusi 2007), there are some differences with regards to particular species of interest within our dataset. 1. Passeriformes have a sister relationship with Falconiformes; 2. Piciformes are found within Coraciiformes; and 3. Falconidae and Accipitridae form distinct clades rather than a monophyletic clade, Falconiformes. These are minor adjustments and do not affect major relationships within our dataset.

NON-PASSERIFORMES

Anseriformes: The relationships among genera in the order Anseriformes were resolved using Donne-Goussé et al.'s (2002) phylogeny, based on mitochondrial DNA control region (CR). All three trees computed using various methods (Neighbour-joining analysis (NJ); Maximum parsimony analysis (MP); and maximum likelihood estimation (ML)) generated phylogenetic trees with identical relationships. Additional trees were generated using mitochondrial protein-coding genes, ND2 and cytochrome b. However, the addition of these two genes did not improve the power of mitochondrial CR.

Galliformes: The relationship between Galliformes was based on the phylogeny by Crowe et al. (2006), comprising a number of character data: morphology and behaviour, ovomucid amino acids, and gene sequences in mitochondrial cytochrome b and D-loop.
Similar relationships between these species were found in Dyke et al.'s (2003) phylogeny, based solely on morphological data.

**Charadriiformes:** The relationship between genera within the order *Charadriiformes* was determined based on Baker et al.'s (2007) composite phylogeny using 5kb sequences of mitochondrial and nuclear DNA. In order to resolve the relationships between species of the genus *Charadrius*, we utilized Thomas et al.'s (2004) shorebird supertree. In addition, the relationship between the species in our dataset found using Baker et al.'s (2007) phylogeny were congruent with those present in Thomas et al.'s (2004) supertree.

**Strigiformes:** The relationship among *Strigiformes* was based on the phylogeny by Wink and Heidrick (2000), utilising cytochrome *b* sequences (1040bp). This analysis generated three phylogenetic trees according to three aforementioned techniques (NJ, MP, and ML). Although internal differences are noticeable among all three trees, the relationships among our four species of interest remained conserved.

**Piciformes:** The relationship among *Piciformes* was based upon the phylogenetic relationships modelled by Benz et al. (2006), based on a various genes: mitochondrial cytochrome *b* and NADH dehydrogenase subunit-2 (ND2), as well as the complete nuclear intro 7 of the β-fibrinogen gene. The relationships among the species within our data set were consistent with those presented by Webb & Moore (2005) using 12S, cyt* b*, and CO1, suggesting that these relationship are stable.

**Podicipediformes:** The relationship among *Podicipediformes* was found in Fjeldså 2004 (taken from Sibley & Ahlquist 1990), demonstrating the relationship between grebes,
loons, and flamingos. Sibley and Ahlquist’s (1990) phylogeny was based on morphological characters and DNA-DNA hybridization.

*All other non-passerine orders consisted of only a single or two species.

**Passeriformes (Oscines)**

The relationship between oscine passerines was determined based on an oscine supertree completed by Jønsson et al. (2006), using 99 published studies (comprising 1273 extent species and one extinct species). This supertree is estimated to be 83% resolved, which is similar to the resolution of many other supertrees in primates (79% - Purvis 1995); Chiroptera (46% - Jones et al. 2002) and Carnivora (78% - Bininda-Emonds et al. 1999). Although the majority of the oscine passerines in our data set were present in Jønsson et al.’s (2006) anaylsis, there were four species unaccounted for. Consequently, we supplemented this phylogeny with other published phylogenies and deduced relationships based on species of the same genus.

*Vireo griseus.* We confirmed that *Vireo griseus* can be classified as a sister-species to *V. olivaceus* and placed in the same group by the phylogeny of Murray et al. (1994) based on cytochrome b DNA sequences. However, it should be recognized that these are two distinct clades: *V. olivaceus* belonging to the eye-lined vireos and *V. griseus* belonging to the eye-ringed vireos.

*Lonchura cucullata.* We grouped this species with other species of the genus *Lonchura* that were present in Jønsson et al.’s (2006) supertree. We were unable to determine the
relatedness among species within this genus, however because we only have two species from this genus, reconciling this relationship was not a concern.

*Trichothraupis melanops.* Jønsson et al. (2006) do not include species from the Thraupidae family into their phylogenetic analysis of oscine passerines. However, based on Burns and Racicot’s (2009) phylogeny of lowland tanagers (using mitochondrial DNA – cytochrome *b* and ND2), we can place the Black-goggled tanager (*Trichothraupis melanops*) within the “crested” tanager clade containing their sister species from the genus *Eucometi*, which is included in Jønsson et al.’s (2006) supertree. In addition, to ensure that this species was placed correctly within the phylogeny, we compared this clade to a sister clade containing the genus *Ramphocephalus* and found that our species remains in the same location within the phylogeny, regardless of which clade it is grouped in.

*Dendroica petechia.* Based on the phylogeny by Lovette & Bermingham (1999), we concluded that the Yellow warbler (*Dendroica petechia*) could be grouped with other *Dendroica* species from Jønsson et al.’s (2006) supertree.

**Passerines (Suboscines)**

The suboscine passerines from our dataset are representative of two larger families: Pipridae (*Chiroxiphia caudata*) and Tyrannidae (*Tyrannus tyrannus* and *Pitangus sulphuratus*). Although manikins (*Pipridae*) were once thought to be part of the Tyrannidae family, recent molecular analyses have confirmed that this is group is in fact a separate, but sister-group to all Tyrannides (Tello et al. 2009). Therefore, in order to
determine the relationships between our subset of suboscines passerines we used Tello et al.'s (2009) phylogeny, which uses 4000bp nuclear DNA sequences (RAG-1 and RAG-2). We also compared our phylogeny to a composite phylogeny from the Tree of Life project, comparing Tello et al. (2009), Ohlson et al. (2008), Ericson et al. (2006), and others.
Figure C.1. Composite phylogeny of 106 avian species.
References


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