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The evolution of iridescent plumage in the Galliformes: Proximate mechanisms and ultimate functions

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by

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DECLARATION OF CO-AUTHORSHIP

I hereby declare that this thesis incorporates material that is result of joint research, as follows:

Chapter 1: I am the sole author.

Chapter 2: I am the primary author, and share authorship with Dr. Stéphanie M. Doucet and Pierre-Paul Bitton. Both Dr. Stéphanie M. Doucet and Pierre-Paul Bitton contributed in the writing of this chapter, as well as providing ideas to help develop this study. Dr. Stéphanie M. Doucet provided logistical and monetary support for this project.

Chapter 3: I am the primary author, and share authorship with Dr. Stéphanie M. Doucet. Dr. Stéphanie M. Doucet contributed in the writing of this chapter, as well as provided logistical and monetary support for this project.

Chapter 4: I am the sole author.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from each of the co-author(s) to include the above material(s) in my thesis.

I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work.

ABSTRACT

Iridescence is a specialized type of structural colouration that produces some of the most spectacular visual displays found in animals. However, the proximate mechanisms and ultimate functions that shape the evolution of iridescent colouration remain poorly studied. The Galliformes comprise a diverse order of birds with multiple sexually dimorphic traits thought to have evolved by sexual selection. Using a phylogenetic approach, I model the evolution of iridescent plumage and its corresponding barbule nanostructures in Galliformes. I show that nanostructural innovations have allowed iridescent colouration to evolve multiple times in Galliformes, allowing them to produce a much broader range of colours. I also show that visually modelled spectral dichromatism and size dimorphism are related to mating system and paternal care in this group. My research suggests that iridescence is a highly labile trait that is likely influenced by a complex combination of selective pressures.

DEDICATION

I dedicate this thesis to that other road. I will travel both.

And to Dave, who kept my wits about me for the last 2 years.

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CHAPTER 1

General introduction

Sexual Selection

Visual displays are found across most animal taxa, including reptiles, fish, insects, mammals, and birds, and often incorporate the erection of ornaments to show off large areas of bright colour or intricate patterning (Andersson, 1994). Darwin (1871) recognized that natural selection could not account for the elaborate and mesmerizing colours associated with these visual displays, such as the three-dimensionally coloured occelli of the Great Argus, *Argus argus*, and proposed that such traits must be under a different type of selective pressure: sexual selection. Sexual selection can lead to the evolution of extravagant secondary sexual traits that are useful in intrasexual competition or intersexual mate choice (Darwin, 1871; Andersson, 1994). In the majority of species, female gametes are larger and more energetically expensive than male gametes, limiting female reproductive success to the number of gametes they can afford to produce (Bateman, 1948; Andersson, 1994). Reproduction in males, on the other hand, is generally limited by the number of mating opportunities (Bateman, 1948). In most cases, this results in male-male competition for access to females, and females becoming choosy of male secondary sexual traits (Andersson, 1994). If specific traits provide competitive advantages in male-male agonistic interactions, such as large body size, or are preferred by females, such as complex vocalizations, those traits can become elaborated to extremes in males (Trivers, 1972; Kirkpatrick, 1982; Andersson, 1994).

Sexual dimorphism

Sexual dimorphism, or differences in male and female phenotypes, can include a combination of size dimorphism, colour dichromatism, or even the presence of traits in males that are absent in females (Andersson, 1994; Badyaev & Hill, 2003). Size dimorphism refers to differences in morphological measurements between males and females. Colour dichromatism specifies different colouration and patterning between males and females. In most cases, males will be the larger, more colourful sex, and females will be the smaller, more drab sex (Andersson, 1994; Badyaev & Hill, 2003). Sexual dimorphism in birds, especially plumage dichromatism, is often the result of both sexual and natural selection pressures (reviewed in Badyaev & Hill, 2003). For example, in many extremely polygynous species, males have exaggerated secondary sexual traits as a result of female choice, and females are cryptically coloured either from a lack of male choice or from natural selection to be less noticeable while incubating or caring for offspring (Shine, 1989; Andersson, 1994). In that case, the combination of natural and sexual selection would increase the total sexual dimorphism to an even greater extent. Darwin (1871) admitted that moderate cases of sexual dimorphism could be explained by natural selection, but more extreme cases, such as the bright iridescent blue male Peacock, *Pavo cristatus*, with his long tail coverts speckled with occeli, required a much stronger selective pressure. Sexual selection pressure is greatest in mating systems where males can maximize their reproductive success, when the reproductive sexual skew among males is highest, and female choice is strongest (Darwin, 1871; Andersson, 1994).

Mating systems

Several ecological factors influence how many females a male can have access to for mating opportunities, such as the availability of food and nesting

resources, or the social tendencies and reproductive synchrony of females (Emlen & Oring, 1977). Monogamous mating systems are identified by relatively permanent pair-bonds and similar sex roles between males and females (Emlen & Oring, 1977). In polygynous mating systems, ecological factors give males the opportunity to breed with multiple females, and no pair-bond is formed (Emlen & Oring, 1977). Male-male competition is greater in more polygynous mating systems, and females are choosier (Darwin, 1871; Andersson, 1994). As a result, males and females of more polygynous species are likely to exhibit greater sexual dimorphism compared males and females of monogamous species (Darwin, 1871; Andersson, 1994).

Paternal care

Parental investment can incorporate different activities, including nest building, incubation, feeding, and guarding offspring, and is under natural, rather than sexual, selection (Andersson, 1994; Owens & Bennett, 1994). In birds, males can potentially participate in all parental duties (Daly & Wilson, 1983). In monogamous mating systems, males generally remain with their female partners and raise offspring cooperatively (Clutton-Brock, 1991). In polygynous mating systems, males seek out a maximum number of females and generally contribute no paternal care (Clutton-Brock, 1991). However, there is an ecological trade-off: when males assist with parental care duties, a higher proportion of his offspring are likely to reach maturity (Emlen & Oring, 1977). With female-only parental care, more of the young are likely to die before maturity, but if a male secured multiple copulations, with more offspring, his net fitness could be higher (Emlen & Oring, 1977).

Colouration mechanisms

In birds, some of the most notable forms of sexual dimorphism are colourful or elaborate plumage patches in males that are more subtle, or absent, in females (Andersson, 1994). There are two main mechanisms of feather colouration in birds: pigment-based colours and structural colours. Pigment colouration can be achieved by the deposition of coloured molecules, such as carotenoids or melanins, into growing feathers (McGraw, 2006a; b). Pigments produce colours by absorbing certain wavelengths of light; the light wavelengths that are not absorbed are reflected by the coloured molecules and produce the perceived colour (Prum, 2006). Carotenoids tend to produce most of the red, orange and yellow colours we see in birds, whereas melanins produce blacks, browns, and greys, but also some rufous colours (McGraw, 2006a; b). Carotenoid precursors must be obtained from an organism's diet and subsequently modified, and as a result, are often believed to be honest indicators of an individual's quality or health (Hill, 2006; McGraw, 2006a; Mendes-Pinto *et al.*, 2012). On the other hand, melanin can be synthesized de novo from basic amino acid precursors, and may therefore be less costly to use as a colourant (McGraw, 2006b). Melanin is also used in many feathers to strengthen the structure to prevent fraying, inhibiting effects of wear and tear (Burtt, 1979).

In contrast to pigment-based colours, structural colouration is the result of differential refraction and reflection of light by keratin, melanin, and air by the feather barb or barbule (Appendix A; Dyck, 1976; Prum, 2006). This refraction and reflection of light, or scattering, can be coherent, where light waves are reflected in an organized, non-random, manner, which produces many of the ultraviolet, blue, green, violet and iridescent colours seen in bird plumage (Prum, 2006). Alternatively, incoherent scattering, where light waves are reflected randomly, produces white plumage (Prum, 2006). The two categories of structural colouration, non-iridescent and iridescent, are broadly separated by the nanostructural

organization of the refractive and reflective materials, which directly affects the coherent scattering of light (Dyck, 1976; Prum, 2006). There are three types of nanostructural organization that coherently scatter light within the keratin matrix of the feather barb or barbule: laminar arrays, hexagonal arrays, and quasi-ordered arrays (Prum & Torres, 2003). Unlike the first two types of arrays, quasi-ordered arrays are not organized beyond the uniform size and shape of nanostructural components, and thus are generally only capable of producing non-iridescent structural colours (Prum & Torres, 2003; Prum, 2006). Laminar and hexagonal arrays exhibit higher order organization and thus are capable of producing the more sophisticated type of structural colouration, iridescence (Prum & Torres, 2003).

Iridescence

Iridescence is perhaps the most specialized type of structural colouration, characterized by a dramatic change in colour when the angle between the observer and light source is altered (Doucet & Meadows, 2009). This type of structural colouration is almost always produced in the feather barbule (Appendix A; Doucet & Meadows, 2009), and is capable of producing a greater diversity of colours than any other mechanism of colouration (Stoddard & Prum, 2011). Iridescent colouration is widespread and has evolved numerous times in a diversity of animal taxa, including many avian families (Durrer, 1977; Doucet & Meadows, 2009). Iridescent plumage is commonly associated with sexual dichromatism and male-biased ornamentation, implying that sexual selection has likely played an important role in its evolution (Andersson, 1994; Doucet & Meadows, 2009). Iridescent colours are produced using a variety of nanostructural components and organizations (Durrer, 1977; Prum, 2006). The type of melanin-filled structure, called a melanosome, can be solid or hollow, and be spherical, rod-shaped or flattened into platelets (Durrer, 1977; Prum, 2006). Melanosomes can line the outside edge of a barbule in a single layer, or in

multiple, densely packed layers (Durrer, 1977; Prum, 2006). Alternatively, multiple layers of melanosomes can be separated by layers of keratin or air for an even higher level of organization (Durrer, 1977; Prum, 2006). As a result of the variety of combinations of melanosome type, fill, and organization, iridescent colours vary in quality from subtle colouration changes over a narrow range of angles, to very intense, saturated colours that can be seen from most angles (Auber, 1957; Durrer, 1977). Although researchers have made recent progress in characterizing the proximate mechanisms responsible for producing iridescent colours (Vukusic & Sambles, 2003; Prum, 2006; Kinoshita *et al.*, 2008; Seago *et al.*, 2009), their evolution and function remain poorly understood. In this thesis, I examine the proximate mechanisms and ultimate functions that may be implicated in the evolution of iridescent plumage, using Galliformes as a model system.

Study system

The order Galliformes is generally understood as the order containing the gamebirds – turkeys, quails, pheasants, and grouse (Carroll, 1994; de Juana, 1994; del Hoyo, 1994; Elliot, 1994; Martinez, 1994; McGowan, 1994; Porter, 1994). This order is generally divided into seven families: Megapodiidae, Cracidae, Meleagrididae, Tetraonidae, Odontophoridae, Phasianidae, and Numididae (Carroll, 1994; de Juana, 1994; del Hoyo, 1994; Elliot, 1994; Martinez, 1994; McGowan, 1994; Porter, 1994). Galliformes represent a highly diverse order. Birds within this order range from a few grams to several kilograms, and males and females can range from identical in size to males having measurements over twice the size of females (Dunning, 1993; Elliot, 1994; de Juana, 1994; del Hoyo, 1994; Martinez, 1994; McGowan, 1994; Porter, 1994; Madge & McGowan, 2002). This order also exhibits dramatic variation in plumage from very cryptic to conspicuous, and often highly iridescent plumage, and the sexes within a species range from perfectly

monomorphic to extremely sexually dimorphic (Elliot, 1994; de Juana, 1994; del Hoyo, 1994; Martinez, 1994; McGowan, 1994; Porter, 1994). Variation is even evident within sexually monomorphic species: in many species males and females bear cryptic brown and black feathers, such as in the Common Quail, *Coturnix coturnix*, whereas in others males and females are equally ornamented and dramatically coloured, such as in the Green Peafowl, *Pavo muticus* (McGowan, 1994). The Galliformes exhibit multiple nanostructural strategies for producing iridescence, which ultimately results in a broad variation in the qualities of iridescent plumage produced (Durrer, 1977). In addition to elaborate plumage ornaments, many galliform species have fleshy ornaments on the face, head or neck, which include snoods, wattles, lappets, and eye rings (Kimball & Braun, 2008). These fleshy ornaments also exhibit variation in dimorphism, from identical between males and females, to present in the male and absent in the female (Elliot, 1994; de Juana, 1994; del Hoyo, 1994; Martinez, 1994; McGowan, 1994; Porter, 1994; Madge & McGowan, 2002). Furthermore, tarsal spurs are present in many galliform species, and also exhibit variation in dimorphism (Davison, 1985; Sullivan & Hillgarth, 1993). Tarsal spurs can range from protruding nubs on the back of the tarsometatarsus, to long, pointed weapons, and can present as a single spur on each tarsus, or as multiple spurs (Davison, 1985).

The Galliformes exhibit extensive variation in mating systems, from monogamy to extreme polygamy, as well as mixed mating systems with different proportions of those two strategies (Ali & Ripley, 1980; Cramp & Simmons, 1980; Elliot, 1994; de Juana, 1994; del Hoyo, 1994; Martinez, 1994; McGowan, 1994; Porter, 1994; Madge & McGowan, 2002). No evidence exists for polyandry, where females mate with multiple males (Emlen & Oring, 1977), and other specialized mating strategies that could produce unexpected selective pressures on male and female plumage are rare (one exception is cooperative breeding in Buff-throated

Partridge, *Tetraophaisis szechenyii*; Xu *et al.*, 2011). All galliform species have precocial young: hatchlings have open eyes, are fully feathered, are fully mobile and can even feed themselves within a few hours or days (McGowan, 1994). These characteristics could remove some existing constraints on mating system and parental care that would not be possible in other orders with less developed young (Emlen & Oring, 1977).

Thesis objectives

Although iridescence is found in a variety of taxa, including many avian species, how different production mechanisms affect iridescent colouration, how these colours evolve, and whether that colouration is under sexual selection pressure remains poorly understood. In this thesis, I use genetic data from a public database to generate a phylogenetic hypothesis for a subset of 70 galliform species from 6 families. In Chapter 2, I use a phylogenetic approach to estimate the pattern of gains and losses of iridescent plumage, and examine that pattern in relation to barbule nanostructure. I also examine how innovations to structure and organization influence the total extent of colours produced by different mechanisms. I then determine how structural innovations to colour producing mechanisms influence speciation, extinction, transition, and diversification rates. In Chapter 3, I investigate the influence of mating system and parental care on the evolution of multiple ornaments in Galliformes. I quantify six categories of sexual dimorphism and dichromatism in this group, and examine whether sexual dichromatism is related to the type of mating system or by the level of paternal care. Variation in plumage dichromatism, iridescent dichromatism, size dimorphism, mating system, and level of paternal care make the Galliformes a well-suited study system to examine the evolution of iridescent plumage from both a proximate and an ultimate perspective.

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CHAPTER 2

Feather nanostructure and the evolution of iridescence in the Galliformes

Chapter summary

Iridescence is produced by the nanostructural arrangement of materials that differ in refractive indices in an organism's integument. In birds, nanostructural components of melanosomes, keratin, and air vary in size, type and organization, ultimately producing different colours and qualities of iridescence. How these nanostructures evolve to produce iridescence remains virtually unstudied. The Galliformes produce some of the most spectacular iridescent displays in nature, and the quality or intensity of iridescent plumage varies extensively across species. Through ancestral state estimations, we determined that iridescence is a highly labile trait, and has independently evolved and been lost several times within the Galliformes. The evolution of iridescent colouration dramatically increased the range of colours produced in this order, and iridescent species had higher diversification rates than non-iridescent species. Iridescence appears to have evolved from a non-iridescent ancestor with unorganized melanosomes in a variety of different ways through innovations in melanosome type and organization. We determined that the organization and layering of solid melanosomes, which are the ancestral melanosome type, produced a more generalized set of iridescent colours and occupied a larger volume in tetrahedral colour space than more derived hollow melanosomes. Our results provide insight on how nanostructural innovations affect the evolution or iridescent colouration at a proximate level.

Introduction

Colour production in mammals is largely melanin-based, limiting fur and skin colour to browns, greys, blacks, and whites (McGraw, 2006b). However, colouration strategies in other animal groups have undergone multiple innovations, leading to spectacular visual displays such as those found in the elytra of scarab beetles (Seago *et al.*, 2009), the dewlaps of anoles (Macedonia *et al.*, 2000), and the plumage of hummingbirds (Schmidt & Ruska, 1962). Bird plumage colouration mechanisms have undergone multiple well-documented innovations (Stoddard & Prum, 2011). Although many birds still rely on the direct deposition of melanins, which can be synthesized de novo (McGraw, 2006b) to produce colour, they also commonly utilize the deposition of other pigments, especially diet-derived carotenoids, to produce bright reds, oranges, and yellows that cannot be achieved by melanin pigmentation (McGraw, 2006a). Avian plumage colours can also be produced by structural coloration (Prum, 2006). Although structural coloration relies on melanin-filled structures called melanosomes, unlike melanin-based colours, the melanosomes in structural plumage colors are highly organized to differentially refract and reflect light (Prum, 2006). As a result of this organizational flexibility, structural colouration is able to produce a more diverse set of colours than any other colour mechanism (Stoddard & Prum, 2011). Sexually selected traits such as plumage colouration are thought to promote reproductive isolation (West-Eberhard, 1983; Panhuis *et al.*, 2001), and have been implicated in speciation (Møller & Cuervo, 1998; Barraclough *et al.*, 1995; Owens *et al.*, 1999; but see Morrow *et al.*, 2003). However, speciation rates have never been examined in relation to different colouration strategies. Since structural colours have the ability to produce a broader diversity of colours compared to other mechanisms (Stoddard & Prum, 2011), they could potentially be more labile in response to selection pressures through the rearrangement of existing nanostructural components.

Perhaps the most specialized type of structural colouration mechanism, iridescent colouration, is characterized by a change in appearance when the angle between the observer and source of light changes (Doucet & Meadows, 2009). Iridescence is produced at the interface between air and the refractive materials found in the feather barbule (Appendix A), and requires at least some organization of nanostructural components (Doucet *et al.*, 2006; Shawkey *et al.*, 2006; Maia *et al.*, 2009). These nanostructural components include melanosomes, layers of keratin, and air, and exist in many variations (Prum, 2006; Maia *et al.*, 2009). The thickness of any of these layers can influence the colour produced (Prum, 2006; Shawkey *et al.*, 2006). Moreover, melanosomes can be organized in single or multiple layers (Durrer, 1977), and multiple layers can either be densely packed, so there is no space between individual melanosomes, or layered with keratin or air (Durrer, 1977). Adding yet another layer of complexity, melanosomes can be spherical, rod-shaped, or multiple rods can converge and be flattened into large platelets (Durrer, 1977). Rod-shaped melanosomes can be either solid, filled completely with melanin, or hollow, where melanin is found only around the edge of the melanosome, leaving an air-filled center (Durrer, 1977). As a result of these different nanostructural components and their organization, iridescent colours vary in quality from very intense, saturated colours visible at a wide range of angles, to weak, subtle colouration changing over a narrow range of angles (Auber, 1957; Durrer, 1977). Innovations to the mechanisms of iridescent colour production are hypothesized to occur in specific steps: for example, from solid melanosomes to hollow ones (Maia *et al.*, 2013b). Although different nanostructures often distinguish different qualities of iridescence, they are also capable of producing a similar intensity of iridescence (Eliason & Shawkey, 2012). For example, hollow melanosomes can produce brighter colours than solid melanosomes, but adding a keratin or air layer between layers of solid melanosomes can achieve a similar effect (Eliason & Shawkey, 2012).

In this study, we investigated the evolution of feather nanostructure and plumage iridescence in the Galliformes using ancestral state estimations, assessed the influence of nanostructural innovation on the range of colours produced, and evaluated the influence these traits on species diversification in this group. The galliform order includes the pheasants, quails, turkeys, guans, and currasows, with iridescent plumage occurring in many species throughout the phylogeny (de Juana, 1994; del Hoyo, 1994; Elliot, 1994; Martinez, 1994; McGowan, 1994; Porter, 1994). This group represents the full range of the different qualities of iridescence, from the subtle iridescence displayed by the male Black Grouse, *Tetrao tetrix*, to the intense iridescence displayed by the male Peacock, *Pavo cristatus* (de Juana, 1994; McGowan, 1994; Porter, 1994; Madge & McGowan, 2002). The Galliformes have been the focus of many recent molecular phylogenetic studies (Kimball *et al.*, 1997, 2011; Meng *et al.*, 2008; Huang *et al.*, 2009; Bao *et al.*, 2010; Ma *et al.*, 2010; Shen *et al.*, 2010), and sequence data for multiple genes are available in public databases. In this study, we collected plumage reflectance measurements from males of 209 museum specimens representing 70 galliform species, constructed a combined phylogeny based on published sequence data, and inferred barbule nanostructure from previously published nanostructural imaging (Schmidt & Ruska, 1962; Durrer & Villiger, 1975; Durrer, 1977). Based on nanostructural and measured reflectance data, we examined the evolutionary patterns of gains and losses of iridescence within the Galliformes, and the consequences of structural innovation on the range of iridescent colours produced. Finally, we examined the speciation and extinction rates of iridescent and non-iridescent states, as well as different melanosome states among iridescent species.

Methods and Analyses

Phylogeny

We reconstructed a phylogeny encompassing 70 of the approximately 280 species of Galliformes, including members of six of the seven families in the order (de Juana, 1994; del Hoyo, 1994; Elliot, 1994; Martinez, 1994; McGowan, 1994; Porter, 1994). We chose these species based on the phylogeny in Shen *et al.* (2010), substituting species at the genus level based on museum specimen availability. These 70 species represent almost all iridescent genera within this order, in addition to 37 non-iridescent species. We obtained nuclear and mitochondrial genes sequences from the public database GenBank (Appendix B), aligned each gene using ClustalW, and hand-edited the alignment where necessary (Thompson *et al.*, 2002). We generated phylogenetic trees from nuclear and mitochondrial genes separately, under a GTR + I + G (Generalized Time Reversible + Proportion Invariant + Gamma) substitution model in MrBayes 3.2.1 (Huelsenbeck & Ronquist, 2001), and with the complete mitochondrial genome for 34 species (Appendix B). We simulated the complete mitochondrial genome separately to avoid pseudoreplication of the mitochondrial gene segments in the same run. The nuclear gene analyses simulated 6,330,000 generations with the first 612,000 generations discarded as burn-in, and the mitochondrial genes simulated 7,800,000 generations with the first 630,000 generations discarded as burn-in, sampling every 1000 generations. The complete mitochondrial genome sequence simulated 60,000 generations, with 10% of those discarded as burn-in. The standard deviation of the frequencies of all three simulations were less than 0.01 by the final generation. We used Mesquite (Maddison & Maddison, 2007) to create a single consensus tree from all the trees produced from the three individual MrBayes runs combined (nuclear genes, mitochondrial genes, and complete mitochondrial genome). This single consensus tree was used for the ancestral state reconstructions and speciation and extinction

analyses. The final tree yielded very similar phylogenetic relationships to those found in recent studies (Kimball *et al.*, 1997, 2001; Meng *et al.*, 2008; Huang *et al.*, 2009; Bao *et al.*, 2010; Ma *et al.*, 2010; Shen *et al.*, 2010), with the exception of the genus *Perdix*. This genus is sister to *Lophophorus* in our constructed phylogeny; in other work *Perdix* is sister to the genus *Syrmaticus* (Huang *et al.*, 2009; Shen *et al.*, 2010; Kimball *et al.*, 2011).

Plumage reflectance measurements

We collected plumage reflectance data from 208 museum bird specimens representing males of all 70 species included in our phylogeny, at four natural history museums: the University of Michigan Museum of Zoology, the Field Museum, the Royal Ontario Museum, and the American Museum of Natural History. Previous work demonstrates that museum specimen colouration accurately reflects the colouration of wild birds (Armenta *et al.*, 2008; Doucet & Hill, 2009). We chose specimens that were labelled as adult birds and where the feathers appeared in good condition, and we sampled within subspecies to minimize plumage variation. We collected plumage reflectance data from 15 plumage regions on each specimen (Appendix C). Spectral reflectance measurements were obtained using a USB 4000 spectrophotometer combined with a PX-2 Xenon light source (Ocean Optics, Dunedin, FL). We used a bifurcated probe to collect reflectance measurements, and we used a rubber stopper on the probe to block out all ambient light and to ensure that all measurements were consistently taken perpendicular to and 3 mm above the feather surface. We measured each region 5 times, relocating the probe each time, for a total of 75 measurements per specimen. We measured three males for each species wherever possible; only two out of 70 species are not represented by three individuals due to the unavailability specimens at the four museums sampled (*Lophura edwardsi* and *Polyplectron malacense* are both missing one specimen).

Species were identified as iridescent or non-iridescent based on a visual assessment of any colour change with changes in viewing angle for any plumage patch (Appendix C).

Avian visual modelling

We determined how the various plumage patch colours would be perceived by the birds by modelling the plumage reflectance curves in a tetrahedral colour space (Endler & Mielke, 2005; Stoddard & Prum, 2008) in order to compare the colour space volume occupied by specific plumage mechanisms. Colour space is a representation of all the colours that a species or group is able to perceive (Burkhardt, 1989; Goldsmith, 1990). Birds possess four distinct types of colour-sensitive cones (Burkhardt, 1989; Hart *et al.*, 1999); these cones are represented in colour space by four vertices, resulting in a tetrahedral colour space (Burkhardt, 1989; Goldsmith, 1990). Each vertex denotes the stimulation limits of violet or ultraviolet, short wavelength, medium wavelength, and long wavelength colour-sensitive cones (Burkhardt, 1989; Goldsmith, 1990). The vast majority of galliform species possess a violet-sensitive cone (Hart *et al.*, 1999); therefore, we modelled the perceived colours using the visual system of a violet-sensitive average bird. However, we used an average ultraviolet cone stimulation model for genera *Tetrao*, *Melagris*, and *Agriocharis*, since species-specific experiments suggest these species have ultraviolet sensitivity (Siitari *et al.*, 2002; Barber *et al.*, 2006). In the models, we used an ideal, or wavelength independent, ambient illumination and background since the Galliformes are found in a variety of habitats with very different ambient light conditions and coloured backgrounds (de Juana, 1994; del Hoyo, 1994; Elliot, 1994; Martinez, 1994; McGowan, 1994; Porter, 1994).

Plumage nanostructure characterization

The nanostructures of iridescent feathers of many species of Galliformes have been described in a variety of sources (Schmidt & Ruska, 1962; Durrer & Villiger, 1975; Durrer, 1977). We were able to find information regarding the type and structural arrangement of melanosomes in 16 species from 16 genera. Since feather nanostructures are conserved at the genus level among iridescent species (M. Shawkey pers. comm.; genera *Onychognathus* and *Lamprotornis*, Durrer & Villiger, 1970; genera *Columba*, *Anas*, *Aix*, *Pilloris*, *Nectarina*, Durrer, 1977; family Trogonidae, Quintero & Espinosa de los Monteros, 2011; family Sturnidae, Craig & Hartley, 1985; also Maia *et al.*, 2013b; order Anseriformes, Eliason & Shawkey, 2012), we assumed that iridescent galliform species within the same genus had the same nanostructural organization. To increase our confidence in this assumption, we compared the iridescent spectral curves of species of unknown structural organization to the spectral curves of species of the same genus for which the structures were known. If those curves were very similar in shape, we assigned the same nanostructure across all iridescent species in a genus. We classified nanostructural organization into one of seven categories based on melanosome type and distribution within the barbule (Table 2.1; Fig 2.1).

Analyses

To evaluate the evolution of iridescent plumage and barbule nanostructures, we used ancestral state estimations (Paradis *et al.*, 2004). Ancestral state estimation is a useful statistical tool for reconstructing the likelihoods of ancestral states at phylogenetic nodes based on the states of extant species, especially extremely labile traits, such as plumage colouration, that can be under multiple selective pressures (Omland & Hofmann, 2006). To examine the evolutionary patterns of gains and losses of iridescence, we reconstructed the ancestral states of iridescent and non-

iridescent plumage. To examine that overall pattern in finer detail, we reconstructed ancestral states of the different nanostructure types (Table 2.1; Fig 2.1). To determine how often nanostructural innovations occur we divided nanostructural type into melanosome rod type and melanosome layering and reconstructed those state changes. To make sure the discrepancy in placements of low resolution nodes did not affect the sensitivity of our analyses, we re-ran all ancestral state estimations using 5 different trees from Kimball *et al.* (2011) and Wang *et al.* (2013); these analyses yielded the same patterns (data not shown). We used the ace function within the `ape` package (Paradis *et al.*, 2004) for R statistical software v.2.15.1 (R Development Core Team, 2008) to carry out ancestral state estimations.

We plotted our spectral measurements in tetrahedral colour space as single points, and used the function `voloverlap` in the R package `pavo` (Maia *et al.*, 2013a) to compare the colour volume occupied by iridescent and non-iridescent colours. We ran the same analysis on colours produced by the two different melanosome rod types and two layering strategies. Because the volumes were produced by different sample sizes of spectral curves, based on the number of species within particular categories, we also calculated adjusted volumes by dividing volume by sample size.

To determine whether iridescent plumage influences species diversification within the Galliformes, we calculated the speciation, extinction, transition, and diversification rates of species with and without iridescent plumage using the `make.bisse` function of the `diversitree` R package (FitzJohn, 2012). We compared an unconstrained maximum likelihood binary state speciation and extinction (BiSSE) model against 9 different parameter constrained models for both non-iridescent and iridescent states (Appendix D). Best fitting models were identified using Akaike's Information Criterion (AIC) values (Burnham & Anderson, 2002). The lowest AIC values corresponded to the models with the transition rate from non-iridescent to iridescent species is constrained to zero, speciation rates of non-iridescent and

iridescent species constrained to equal, and no constraints (Appendix D). The AIC values for these 3 models differed by exactly 2.00, which is the maximum value for equal plausibility (Burnham & Anderson, 2002). ANOVAs confirmed there was no statistical difference between those models (data not shown). We considered the transition rate constraint not biologically relevant with regards to the question we are examining in this study, so chose to use the unconstrained model as the starting point for the Markov chain Monte Carlo (MCMC) analyses. We followed the same methods to examine the speciation, extinction, transition, and diversification rates between solid and hollow melanosome states within iridescent species only. We found no statistical difference between any of the ten maximum likelihood BiSSE models using ANOVAs (data not shown), so we used the unconstrained parameter model as the starting point for the MCMC analyses (Appendix E).

Results

Ancestral state estimations

The majority of iridescent Galliformes (families Tetraonidae, Meleagridae, and Phasianidae) can be divided into two broad clades; these two clades share a non-iridescent ancestor (Fig. 2.2). Reconstructing iridescent and non-iridescent plumage states at ancestral nodes shows that iridescence likely evolved independently at least five times, once at a very basal node, and the four others relatively recently (Fig. 2.2). In this scenario, iridescence was lost six times. Four of those losses appear to have affected entire genera: the common ancestor to *Tragopan*, *Perdix*, *Tetraophasis* and *Lophophorus*; *Bonasa*; *Bambusicola*; and the most recent ancestor to *Rheinardia* and *Argusianus* (Fig. 2.2). The remaining two losses of iridescence affected single species within otherwise iridescent genera: *Syrmaticus reevesii* and *Lophura nycthemera* (Fig. 2.2).

By reconstructing the ancestral state of the seven different types of nanostructural organization found within the Galliformes (Table 2.1; Fig. 2.1), we identified at least nine instances of structural innovation (Fig. 2.3). That is, there were at least nine transitions from an unorganized arrangement of solid melanosomes that does not produce iridescence to changes in melanosome morphology, organization, or spacing that produced either glossy or iridescent colouration. These different nanostructural types (Type 3 through Type 7) produce different qualities of iridescence using different nanostructual components and organization (Durrer, 1977). Type 2 nanostructure produces glossy black plumage (Durrer, 1977). In this reconstruction, structural organization was lost four times, resulting in the loss of iridescence in the common ancestor to *Argusianus* and *Rheinardia*, the genus *Bonasia*, *S. reevesi* and *L. nycthemera* (Fig. 2.3). Surprisingly, the ancestral state estimation implies that some of the most spectacular displays of iridescence, associated with the most complex structural organization, such as the colouration found in the genus *Lophophorus*, evolved from a non-iridescent ancestor with an unorganized nanostructure (Fig. 2.3). The ancestor to *Meleagris* and *Agriocharis* is the only instance where a seemingly more complex state evolved from less complex iridescent nanostructure (from a densely packed hexagonal array of solid melanosomes to a densely packed hexagonal array of hollow melanosomes) (Fig. 2.3). *Francolinus francolinus*, *Alectura lathami*, and *Acryllium vulturinum* have evolved a slightly organized nanostructure arrangement (Type 2; Table 2.1; Fig. 2.1) that produces glossy black, but not iridescent, plumage from completely unorganized barbule nanostructure (Fig. 2.3).

The melanosomes in the barbules of galliformes have changed from a solid to hollow state three times, but have never reversed from a hollow to a solid state (Fig. 2.4). Ancestors to the genera *Lophophorus* and *Gallus*, and sister taxa *Meleagris* and *Agriocharis* independently evolved hollow melanosomes from solid melanosomes

(Fig. 2.4). In contrast, melanosome layering among iridescent species has only undergone one change, from the ancestral state of multi-layer organization, producing iridescence, to a single layer of melanosomes producing iridescence. Iridescence in the junglefowl (genus *Gallus*) is produced by single layers of melanosomes instead of the multiple layer mechanisms used in all other iridescent galliform nanostructures. We found no evidence of highly organized single layers of solid melanosomes producing iridescence in this order.

Extent of colours produced by different mechanisms

Iridescent colours (n = 203 spectral measurements) occupied 4.5 times more volume in tetrahedral colour space than non-iridescent colours (n = 607), and non-iridescent colours produced only 15.9% of the same colours produced by iridescent plumage (Fig. 2.5A). When adjusting for sample size, this pattern became even more pronounced, with iridescent colors occupying on average 13.4 times more volume in tetrahedral colour space. Colours produced using solid melanosomes (n = 59) occupied 1.5 more times more colour volume than hollow melanosomes (n = 144), and solid melanosomes produced 78.7% of the same colours as hollow melanosomes (Fig. 2.5B). Adjusting for sample size, this pattern became even more pronounced, with solid melanosome nanostructures occupying 4.02 times more volume in tetrahedral colour space. Colours produced using multiple layers of melanosomes (n = 190) occupied 14.9 times more colour volume than colours produced by single layers (n = 13), and multiple layers of melanosomes produced 91.5% of the same colours as single layers of melanosomes (Fig. 2.5C). However, very few species produced iridescence using a single layer, and adjusting for sample size, colours produced single and multiple layers occupied a similar volume (ratio of multiple layers to single layer of 1.02). Densely packed solid melanosomes (n = 112) occupied 6.5 times greater colour volume than densely packed hollow melanosomes (n = 17),

and densely packed solid melanosomes produced 93.7% of the same colours as densely packed hollow melanosomes (Fig. 2.5D). However, few species produced iridescence using densely packed hollow melanosomes, and adjusting for sample size, colours produced using densely packed solid and hollow melanosomes occupied a similar volume (ratio of densely packed solid to hollow of 0.99).

Colouration strategies and speciation

Our analysis comparing the speciation rates of non-iridescent and iridescent states within the entire galliform order revealed that species with iridescent plumage did not have higher rates of speciation (Fig. 2.6A) or extinction (Fig. 2.6B) than species without iridescent plumage. Nevertheless, the diversification rate of iridescent species was estimated to be significantly higher than the diversification rate of non-iridescent species (Fig. 2.6D). The transition rate for gains of iridescent plumage is almost zero; the loss of iridescent plumage is much higher (Fig. 2.6C).

Our analysis within iridescent galliform species revealed that species producing iridescence with solid melanosomes did not have higher rates of speciation (Fig. 2.7A) or extinction (Fig. 2.7B) than species producing iridescence with hollow melanosomes. Likewise, there was no difference in the diversification rate between these two iridescent production strategies (Fig. 2.7D). The transition rates between solid and hollow melanosomes were estimated to be similar (Fig. 2.7C).

Discussion

In this study, we demonstrate that iridescence is a derived trait in the Galliformes, and has been gained and lost numerous times. We also show that

iridescent plumage allows species to exploit a larger range of colours compared to non-iridescent species, and our findings suggest species with iridescent plumage could experience higher diversification rates, although we found no difference in speciation rates between iridescent and non-iridescent states. The Galliformes produce iridescence through several distinct nanostructure types, including variation in the placement, spacing, and morphology of melanosomes. Nanostructural innovations producing iridescence have evolved from an unorganized state at least nine times in this group, and we found no evidence of reversals of nanostructural innovation within iridescent species to a more primitive state aside from the complete loss of iridescence. Innovations to melanosome morphology, changing from solid to hollow, allowed iridescent species to exploit an even larger range of colours. Our findings suggest that small changes in feather nanostructure can lead to large changes in the type and extent of iridescent colour produced, and that iridescence can be easily lost through loss of nanostructural organization, resulting in a complex and highly labile trait.

We estimate that iridescent plumage has evolved independently in the Galliformes at least five times, representing convergent evolution of iridescence between two large clades in this order. Iridescent plumage appears to be a relatively recent innovation from an ancestral non-iridescent state in this group. Our analyses show that within the Galliformes, iridescent colours occupy a much larger volume in tetrachromatic colour space than non-iridescent colours, and thus that iridescent colouration has enabled the production of a much larger diversity of colours, a finding that is paralleled across birds as a whole (Stoddard & Prum, 2011). Unusually, carotenoid-based plumage colours are either very rare or completely absent in this group (pers. obs.; pers. comm. R.T. Kimball). As a result, structural colouration represents the only innovation to plumage colouration mechanisms within this order beyond ancestral melanin-based coloration, and the evolution of

iridescent colouration has expanded the range of producible colours by an even greater extent. This lack of alternative plumage colouration mechanisms may be one of the reasons iridescence has evolved independently so many times within this order. Despite these advantages of iridescence, however, this specialized plumage has also been lost several times, either by random evolutionary events, or through direct selection against this trait in some species (Wiens, 2001). Interestingly, in many clades where iridescence has been lost, other exaggerated secondary sexual traits are present that are unique to the non-iridescent species, such as the elongated secondary flight feathers with shaded occelli of Great Argus, *Argus argus*, the two elongated tail feathers of Reeve's Pheasant, *Syrmaticus reevesi*, and the erectile lappets of male Tragopans.

Our analyses show that iridescent species had higher diversification rates than non-iridescent species (Fig. 2.6D). Although speciation and extinction rates did not differ between the two groups, diversification is calculated as the difference between speciation rate, which was slightly higher in iridescent species, and extinction rate, which was much lower in iridescent species, resulting in significantly higher diversification rates among iridescent species. Our findings suggest that iridescent colouration, perhaps through the diversity of colours that can be produced, has had an impact on species richness in the Galliformes. However, it is unclear whether this pattern is driven by sexual selection or some other combination of selective factors (Panhuis *et al.*, 2001; Morrow *et al.*, 2003). We also determined that the transition rate from iridescent to non-iridescent plumage, or the rate of loss, was much higher than the transition rate from non-iridescent to iridescent plumage, or the rate of gain (Fig. 2.6C). This implies that nanostructural innovations producing iridescence are more easily lost than gained. Because iridescence is a recently derived trait, we did not expect the rate of loss to be so much higher than the rate of gain, since the trait is being lost over a relatively short

period of evolutionary time. This could imply that iridescent plumage has natural selection costs associated with it, such as predation or physiological costs, and that this trait was not lost solely by chance (Wiens, 2001; Badyaev & Hill, 2003). If iridescence is costly, and can be lost relatively easily, there is likely to be selection to maintain it in species that continue to exhibit the trait (Wiens, 2001). We have recently shown that sexually dimorphic plumage colouration is related to mating system in the Galliformes, suggesting that sexual selection may play an important role in maintaining plumage elaboration in this group (Chapter 3).

There are five different nanostructural types that produce iridescent plumage in the Galliformes (Table 2.1; Fig. 2.1), but our analyses show that almost all iridescent nanostructures evolved directly from a non-iridescent ancestor. Given the nanostructural modifications required to change from non-iridescent to iridescent, it is reasonable to expect that different types of iridescence would evolve through a series of transitional states. In the blackbirds (family Icteridae), for example, iridescence appears to have evolved from unorganized solid melanosomes to a single layer of solid melanosomes producing glossy black colouration, a possible transitional state to the multiple layers of solid melanosomes that produce iridescence in this group (Shawkey *et al.*, 2006; Maia *et al.*, 2011). We did not find evidence that single solid melanosome layers preceded iridescent producing nanostructures. Instead, Type 2 nanostructures producing glossy black plumage (Table 2.1; Fig. 2.1) apparently evolved in the same way as Type 3 through 7 nanostructures: directly from Type 1 unorganized nanostructures with no further innovation (Fig. 2.3). In fact, the most complex nanostructures, like the highly organized square array of solid melanosome rods found in peacocks, and the evenly spaced layers of hollow melanosome rods found in monals, appear to have evolved directly from a non-iridescent ancestor, though it is possible that transitional states existed in now extinct species. Iridescent nanostructures in the turkeys (*Meleagris*

and *Agriocharis* spp.) represent the only evidence for cumulative changes in barbule nanostructure from an ancestral unorganized nanostructure, from a hexagonal array of densely packed solid melanosomes, to a hexagonal array of densely packed hollow melanosomes that could increase the breadth of potential colours produced (Fig. 2.3; Fig. 2.5D). We could not examine the transitional patterns between single and multiple layers of melanosomes due to limited sample sizes of specific nanostructures. Certain nanostructural types were specific to a single genus, such as Type 5, a single layer of hollow melanosomes (Fig. 2.1), found only in the junglefowl (genus *Gallus*). These results are unlikely to be an effect of sample size, since our phylogeny represents over 80% of iridescent species within this order, but only 15% of non-iridescent species. Adding in more non-iridescent species would likely create even more definitive "islands" of iridescent nanostructure on the phylogeny. Form innovations to nanostructure that broadened colour production beyond the constraints of melanin-based plumage may have experienced intense selective pressure in this group, especially considering there is no evidence for alternative colouration mechanisms in this order (see above). Such intense selection pressure could fix an innovation in a relatively short period of evolutionary time.

We did not find any evidence of reversal to innovations of melanosome morphology or organization. Specifically, once melanosomes changed in form from solid to hollow, this was never reversed. Increased organization of melanosome layers to incorporate a layer of keratin or air was also never reversed (Type 3 to Type 4, and Type 6 to Type 7 innovations; Table 2.1; Fig. 2.1). Similarly, recent studies show that evolutionary innovations to iridescent nanostructures were not reversed in African starlings (Maia *et al.*, 2013b) and trogons (Quintero & Espinosa de los Monteros, 2011).

After adjusting for sample size, we found that the more basal iridescent nanostructure using solid melanosomes occupied a much larger volume in

tetrahedral colour space compared to nanostructures using hollow melanosomes (Fig. 2.5B). Hollow melanosomes add an additional refractive layer for light waves, and generally increase the overall brightness of colours produced (Eliason & Shawkey, 2012). Our findings imply that the more derived hollow melanosomes are capable of producing a more intense, higher quality palette of iridescent colours, but at the cost of being limited in the range of those colours. Once we adjusted to account for differences in sample sizes, we found iridescent producing nanostructures using single and multiple layers of melanosomes occupied almost identical volumes. Similarly, iridescent producing nanostructures using densely packed solid and hollow melanosomes occupied an equivalent volume in tetrahedral colour space. Future studies should consider expanding data on iridescent nanostructures and the spectral reflectance patterns they produce to better determine which forms have the potential to produce a wider range of colours. The expansion of nanostructural data to other families would also provide an enhanced ability to examine the speciation, extinction, transition, or diversification rates between iridescent plumage traits produced by different nanostructures.

Although iridescent plumage in Galliformes is produced by a diversity of melanosome types and organizations, we found no evidence of the melanin platelets that are present in other families (Schmidt & Ruska, 1962; Durrer, 1977; Craig & Hartley, 1985; Quintero & Espinosa de los Monteros, 2011). As a result, the pathways and strategies available for producing iridescent plumage in the Galliformes could be more restricted compared to orders with platelet nanostructure. This restriction may explain why there is convergent evolution of certain nanostructural types, such as densely packed solid melanosomes (Type 3, Fig. 2.1; Fig. 2.3). However, changing melanosome morphology from solid to hollow, and changing the spacing and layering of melanosomes could provide more than enough opportunity for producing different colours and qualities of iridescence.

Structural colouration has allowed for substantial diversification of plumage colouration in the Galliformes, and the evolution of iridescent colouration in particular has increased the potential for colour production to an even larger degree. Our analyses show that iridescent plumage is a highly labile trait in this order. This character has been gained and lost multiple times throughout the order, and is associated with higher species diversification rates than non-iridescent plumage. Surprisingly, the mechanisms of iridescent colour production do not seem to follow a graded order of evolution: highly organized nanostructures appear to have evolved directly from non-iridescent ancestors in many cases, and we found no evidence of a transitional state between non-iridescent and iridescent nanostructures. The Galliformes produce iridescence using multiple mechanisms, providing the opportunity to study the evolution of iridescent plumage among a relatively similar group of species. Expanding these analyses across multiple orders may provide more insight into how different mechanisms evolve within iridescent species, and help confirm developing hypotheses about the transitional steps and form innovations that affect iridescent colouration.

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Tables and Figures

Table 2.1. Nanostructural organization in the barbules of galliform birds. See Fig. 2.1 for illustrations. Organizational types 3 through 7 produce iridescence. Organizational type 2 produces glossy black plumage. Based on descriptions in Durrer (1977) and Craig and Hartley (1985).

Organization	Description
Type 1	Melanosomes are solid and not organized in any way. No structural colour is present.
Type 2	Melanosomes are solid and arranged in a single layer inside the outer edge of the barbule. Below this layer melanosomes are randomly spread throughout the barbule interior.
Type 3	Melanosome rods are solid and arranged in multiple layers. These layers are densely packed so that any one melanosome touches the sides of neighbouring melanosomes in a hexagonal array.
Type 4	Melanosome rods are solid and arranged in mulitple layers. Between each layer of melosome is a layer of keratin and/or air, so that a square array is seen in a cross-section.
Type 5	Melanosome rods are hollow and arranged in a single layer inside the outer edge of the barbule. Below this layer melanosomes are randomly spread throughout the barbule interior.
Type 6	Melanosome rods are hollow and arranged in multiple layers. These layers are densely packed so that any one melanosome touches the sides of neighbouring melanosomes in a hexagonal arrary.
Type 7	Melanosome rods are hollow and arranged in mulitple layers. Between each layer of melosome is a layer of keratin and/or air, so that a layered array is seen in a cross-section.

Figure 2.1. Idealized depictions of nanostructural arrangements found within the barbules of Galliformes, viewed in cross section. Type 1, which is not depicted, represents an unorganized, non-iridescent structure with solid melanosomes. Type 2 nanostructure produces glossy black plumage that does not change colour with angle of observation.Types 3 through 7 produce iridescent plumage. Circles represent melanosomes, either solid (filled) or hollow (empty). The keratin layer is synonymous with the outside barbule edge. See also Table 2.1 for descriptions of each nanostructure type. Based on descriptions and images in Durrer (1977).

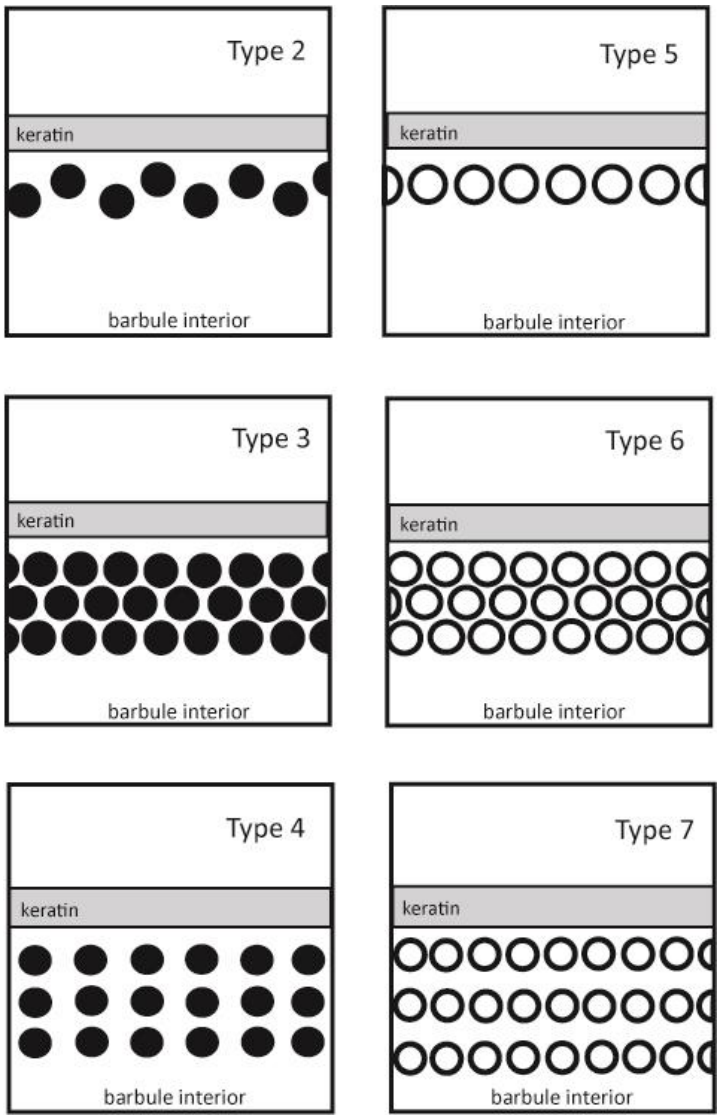


Figure 2.2. In male Galliformes, iridescence has evolved from an ancestral non-iridescent state at least five times, and has been lost several times. White boxes represent iridescent plumage in males for at least 1 of the 15 different plumage regions measured (Appendix C). Black boxes indicate non-iridescent species. Nodal pie charts represent the maximum likelihood probabilities for the ancestral state. Nodes where iridescence was estimated to have evolved (probability of iridescent state is greater than 50%) are identified by a star.

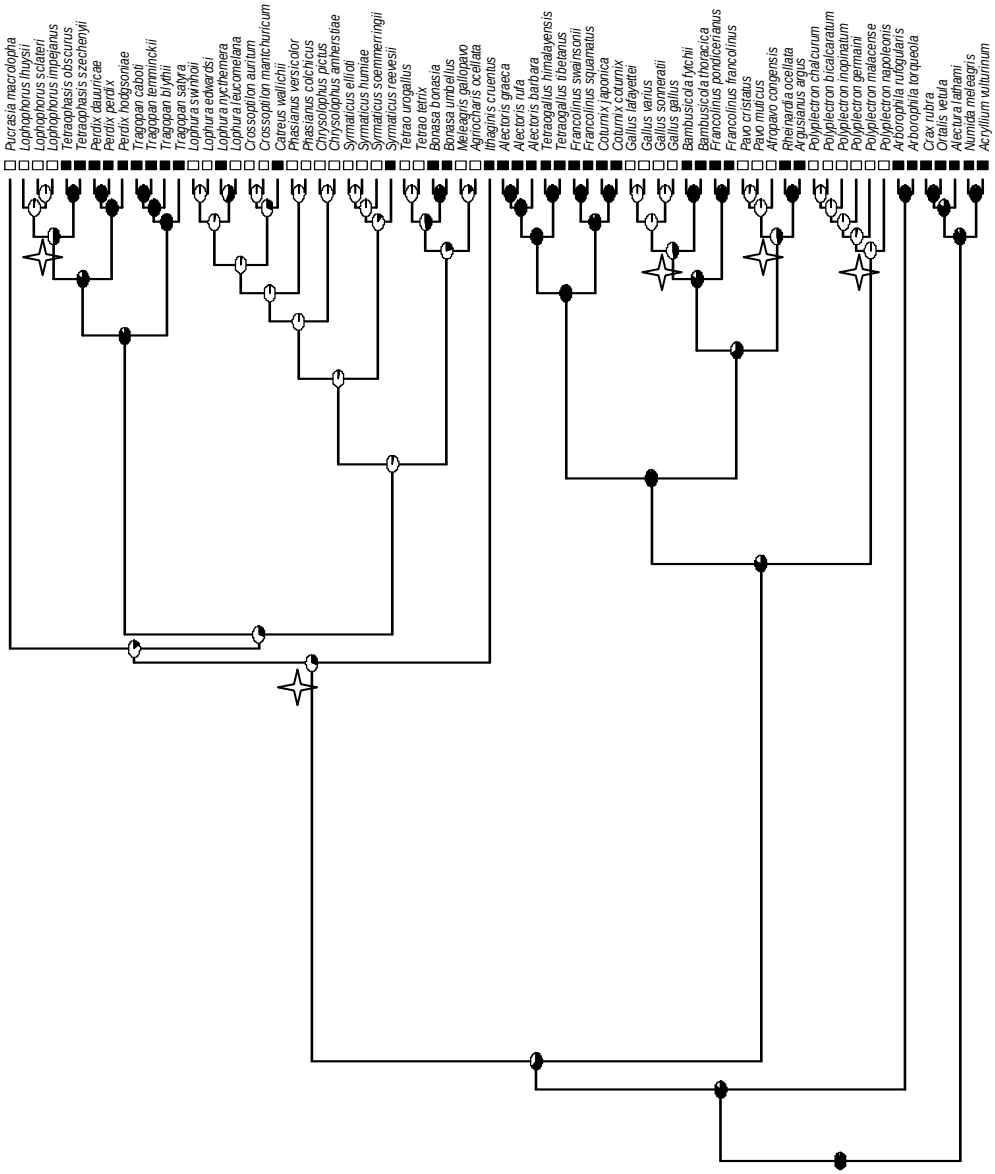


Figure 2.3. Solid melanosomes arranged in unorganized nanostructures that do not produce iridescence are the ancestral barbule state in the Galliformes. See Fig. 2.1 for illustrations and Table 2.1 for descriptions of nanostructural types. Nodal illustrations represent the maximum likelihood probabilities for each ancestral state. Nodes where nanostructural innovations were estimated to have evolved (probability of novel state has the greatest proportion) are identified by a star.

Figure 2.4. Hollow melanosome rods have evolved three times independently within iridescent galliform species, with no reversals to solid melanosomes. Black boxes indicate solid melanosomes (Type 3 or 4; Fig. 2.1). White boxes indicate hollow melanosomes (Type 5-7; Fig. 2.1). Nodal illustrations represent the maximum likelihood probabilities for each ancestral state. Nodes where hollow melanosomes were estimated to have evolved (probability of hollow state is greater than 50%) are identified by a star.

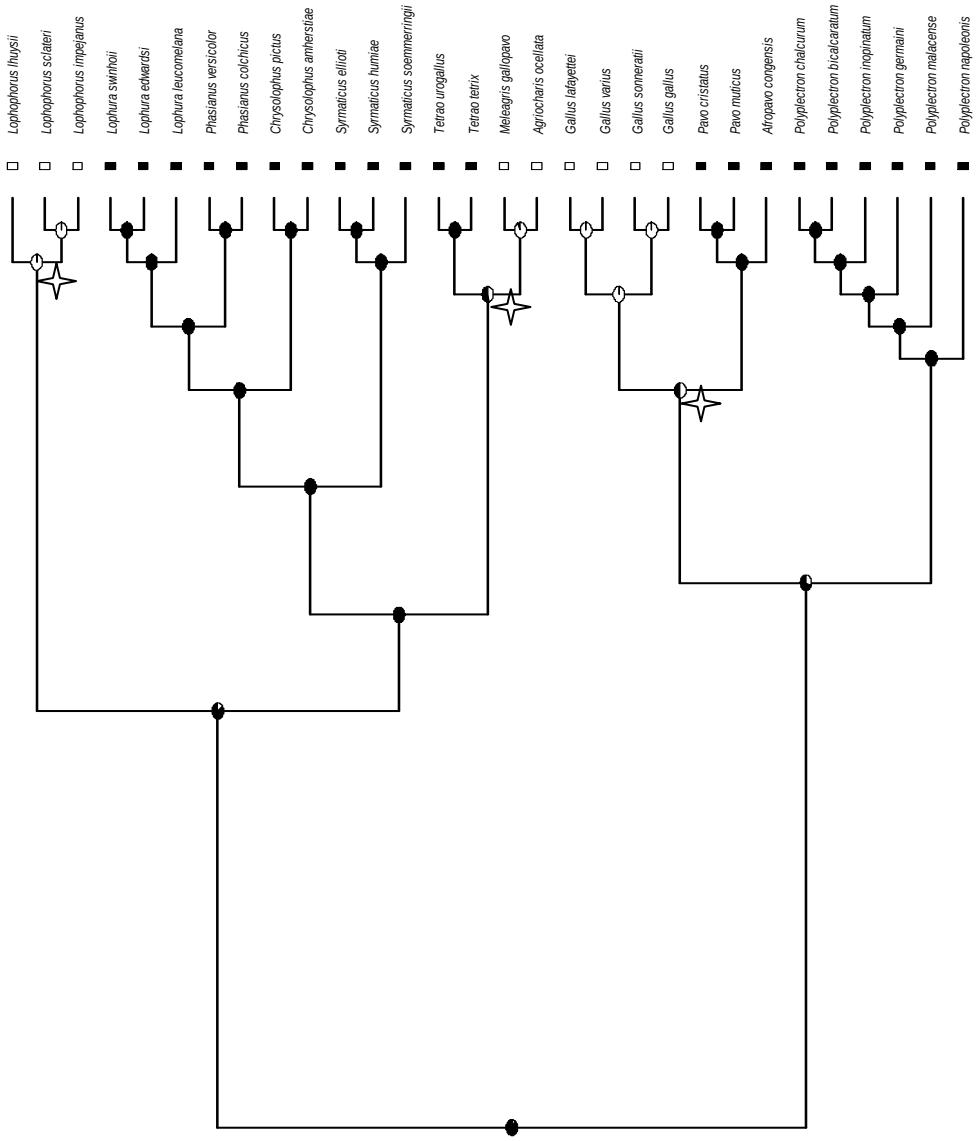


Figure 2.5. Tetrahedral colour space volumes occupied by different nanostructural types in the Galliformes. The labelled vertices of the tetrahedral colour space visualization correspond to the different cone types that are stimulated by short (s), medium (m), long (l), and ultraviolet or violet (v) wavelengths. The central grey dot represents the achromatic center in tetrahedral colour space (equal stimulation of all cones). Each spectral measurement is represented by a single point in the respectively coloured tetrahedral insets. The area in grey is the volume of overlap between the two nanostructural strategies. (A) Iridescent (blue) and non-iridescent nanostructures (red). (B) Solid (blue) and hollow melanosomes (red). (C) Multiple layers (blue) and single layers of melanosomes (red). (D) Densely packed solid (blue; Fig. 2.1, Type 3) and densely packed hollow melanosomes (red; Fig. 2.1, Type 6).

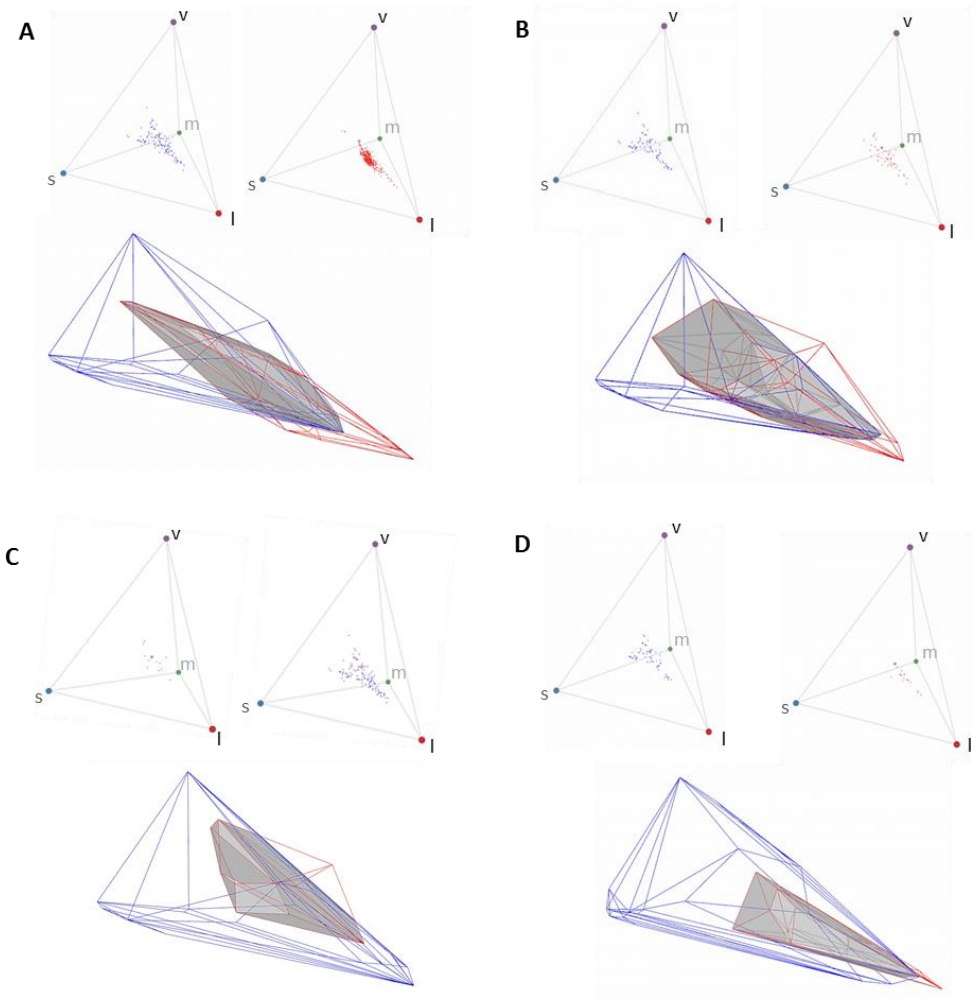


Figure 2.6. The posterior probability density distributions for non-iridescent (grey) and iridescent (blue) states within the Galliformes using a six parameter binary state speciation and extinction (BiSSE) model. The 95% credibility intervals are represented by bars above the x-axis. If the 95% credibility intervals overlap, the two states are not statistically different in rate. Parameter estimates are for: (**A**) state speciation rates (lambda); (**B**) extinction rates (mu); (**C**) transition rates for gains of iridescent plumage (q_{01}, grey) and losses of iridescent plumage (q_{10}, blue); and (**D**) the net diversification rates for non-iridescent states and iridescent states (difference between speciation [lambda] and extinction [mu] rates).

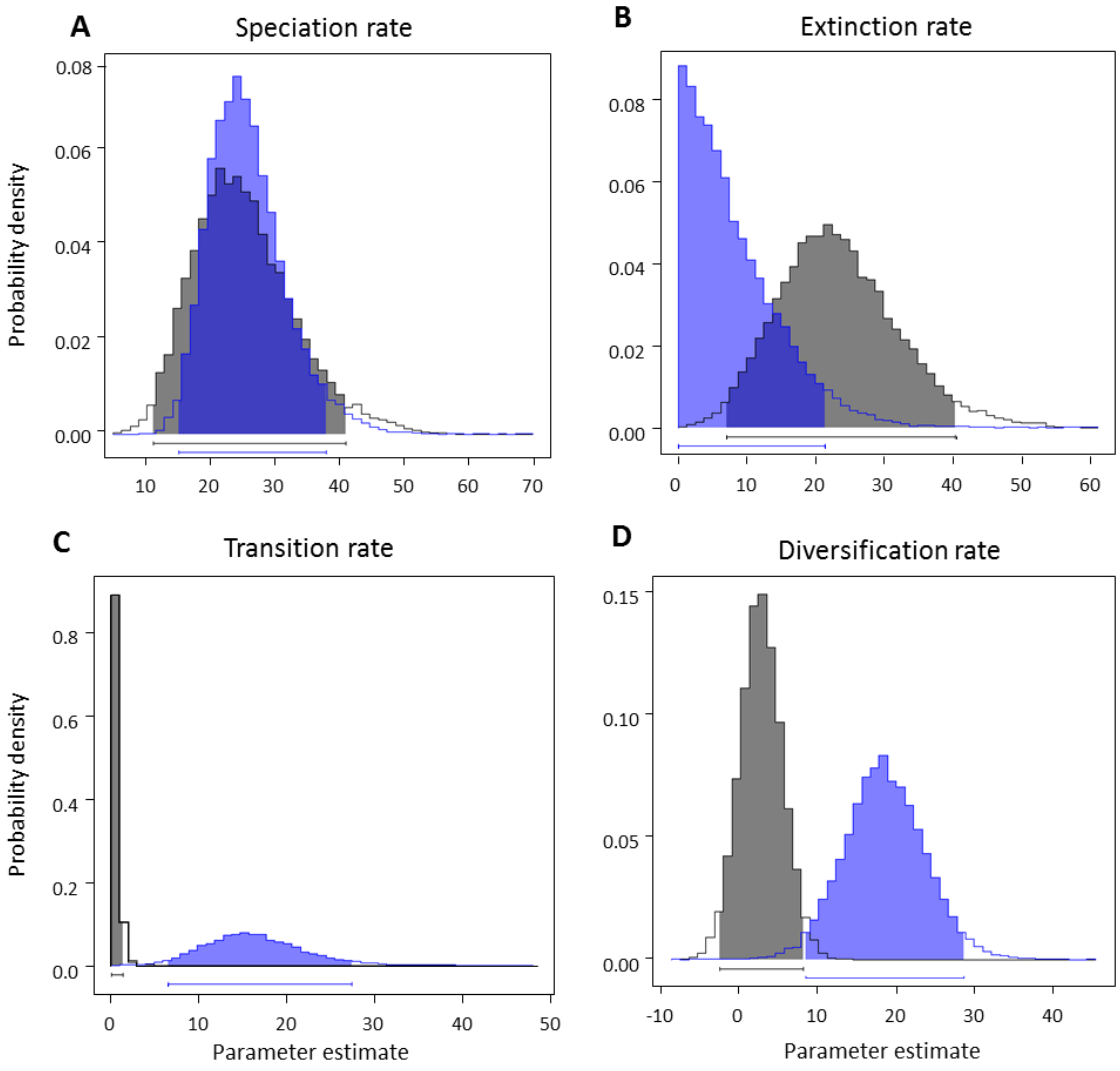
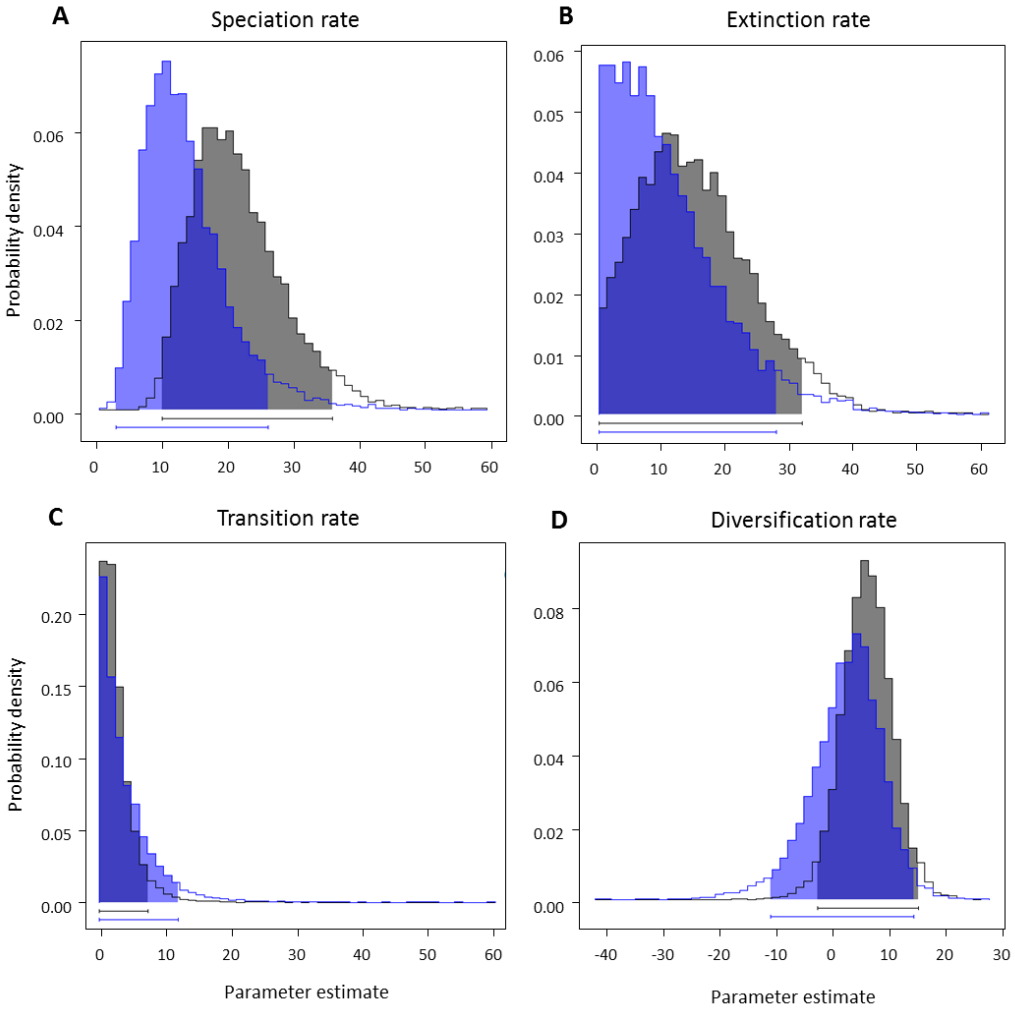


Figure 2.7. The posterior probability density distributions for solid (grey) and hollow (blue) melanosome states within iridescent galliform species using a six parameter BiSSE model. The 95% credibility intervals are represented by bars above the x-axis. If the 95% credibility intervals, the two states are not statistically different in rate. Parameter estimates are for: (**A**) state speciation rates (lambda); (**B**) state extinction rates (mu); (**C**) transition rates for gains of hollow melanosomes from solid melanosomes (q_{01}, grey) and losses of hollow melanosomes to ancestral solid melanosomes (q_{10}, blue); and (**D**) the net diversification rates for solid melanosome states and hollow melanosome states (difference between speciation [lambda] and extinction [mu] rates).



CHAPTER 3

Sexy dads and cryptic moms: the evolution of sexual dimorphism in the Galliformes

Chapter summary

Sexual selection often leads to the evolution of sexually dimorphic traits. In some species, sexual selection has led to the evolution of multiple secondary sexual ornaments, though the function of multiple ornaments remains poorly understood. In this study, we examined six measures of sexual dimorphism in relation to mating system and paternal care in the Galliformes. The Galliformes exhibit extreme variation in plumage dichromatism and size dimorphism, as well as additional specialized dimorphic traits, such as iridescent plumage, fleshy ornaments, and tarsal spurs. Mating systems range from monogamous to extremely polygynous, and variable paternal care strategies have been documented in this group. We found that modelled spectral dichromatism was predicted by both mating system and paternal care. In addition, all four measures of size dimorphism were predicted by mating system, but only two measures were predicted by paternal care. By contrast, there was no relationship between mating system or parental care and our other measures of dimorphism, including visually assessed dichromatism, visually assessed iridescence dichromatism, and dimorphism in facial fleshy ornaments and tarsal spurs. Our findings suggest that various selective pressures may have led to the evolution of multiple sexual ornaments in the Galliformes, but some patterns may be obscured by differences between historical and current selective pressures, or may be restricted to certain species or closely related groups of species. Our results also suggest that objective spectral measurements and visual modelling may yield important insights that could be overlooked by visual assessments of sexual

dichromatism. Our work emphases the importance of isolating multiple ornaments when making inferences concerning the evolution sexually selected traits.

Introduction

Sexual selection can lead to the evolution of exaggerated traits and behaviours that are the result of competition over mating opportunities and differential reproductive success within a species (Darwin, 1871; Andersson, 1994). Sexual selection can result from both intersexual selection and intrasexual competition (Darwin, 1871; Andersson, 1994). In most species, females contribute larger, more energetically expensive gametes to sexual reproduction compared to males (Andersson, 1994). Consequently, female reproductive success is limited by the number of gametes they can produce, whereas male reproductive success is limited by the number of females they can access (Bateman, 1948). As a result, males are usually the competitive sex, whereas females are usually the choosy sex (Andersson, 1994). Both female mate choice and male-male competition can drive the evolution of exaggerated secondary sexual characters in males (Trivers, 1972; Kirkpatrick, 1982; Andersson, 1994). This elaboration of secondary sexual traits usually leads to strong sexual dimorphism: differences in size and colouration between males and females (Andersson, 1994). Intrasexual selection often leads to the evolution of traits that are useful in male-male competition, such as extreme body size, weapons, or status signals (reviewed in Andersson, 1994). In contrast, intersexual selection often leads to the evolution of traits that facilitate female choice, such as complex vocalizations, elaborate displays, or brilliant colouration (reviewed in Andersson, 1994). In some species, sexual selection has led to the evolution of multiple secondary sexual ornaments, though the function of multiple ornaments, and which ornaments are under current selective pressures, remains

poorly understood (Møller & Pomiankowski, 1993; Møller & Petrie, 2002; Papeschi & Dessì-Fulgheri, 2003; Kimball *et al.*, 2011; Husak & Swallow, 2011).

In monogamous mating systems, males and females form a pair-bond, breeding exclusively with one another (Emlen & Oring, 1977). In polygynous mating systems, males have the opportunity to mate with multiple females, which can be the result of female breeding asynchrony, male control of environmental resources, or skewed operational sex ratios (Emlen & Oring, 1977). Polygyny can exist in various proportions with monogamy depending on differences in the conditions that allow for multiple mating opportunities (Emlen & Oring, 1977). Lek breeding systems represent an extreme form of polygyny with no pair bonds, where males and females meet only to breed (Emlen & Oring, 1977). Previous work across multiple orders of birds has shown that sexual size dimorphism was related to mating system, with greater size dimorphism in more polygynous species (Owens & Hartley, 1998; Dunn *et al.*, 2001; Lislevand *et al.*, 2009). However, Figuerola and Green (2000) found no relationship between mating system and size dimorphism in Anseriformes. A number of studies have also found a relationship between visually assessed plumage dichromatism and mating system in birds (Figuerola & Green, 2000; Dunn *et al.*, 2001), though this pattern is not universal (Owens & Hartley 1998).

In birds, parental care duties can include nest building, incubation, feeding, as well as active and passive brood defense (Owens & Bennett, 1994). Passive brood defense is a general association with the offspring, protecting them from the environment under wings or tails (Cramp & Simmons, 1977). Active brood defense consists of actions intended to deter predators (Cramp & Simmons, 1977). Typically, females take on a greater share of parental care duties, although males can make substantial contributions in monogamous species (Emlen & Oring, 1977). In Galliformes and Anseriformes, decreasing parental care appears to be associated

with increased sexual size dimorphism, although this study did not consider the effect of phylogeny (Sigurjónsdóttir, 1988). By contrast, in a large comparative analysis controlling for phylogeny, there was no association between paternal care and visually-assessed dichromatism (Owens & Hartley, 1998). The authors then divided plumage dichromatism into carotenoid, melanin, and structural dichromatism, and determined that melanin-based plumage dichromatism was associated with one measure of parental care, the level of sex bias in passive brood defense (Owens & Hartley, 1998). In passerine birds, species where males and females share parental duties tend to be more monochromatic (Verner & Wilson, 1969; Soler *et al.*, 1998), but again, these studies differ in how sexual dichromatism is assessed and whether phylogenetic relatedness was taken into account. Overall, associations between measures of dimorphism and dichromatism with mating system and paternal care appear to vary greatly depending on the ranking criteria used and the species examined.

In this study, we investigated the evolution of multiple forms of sexual dimorphism in the Galliformes. This order includes pheasants, quails, turkeys, guans, and currasows, and exhibits dramatic variation in mating system, parental care, and sexual dichromatism and dimorphism. Mating systems range from monogamy to extreme polygyny (Ali & Ripley, 1980; Cramp & Simmons, 1980; de Juana, 1994; del Hoyo, 1994; Elliot, 1994; Martinez, 1994; McGowan, 1994; Madge & McGowan, 2002). Galliform species range from quail that are only a few grams to turkeys that weigh several kilograms, and males and females can range from identical in size to males having measurements over twice the size of females (Dunning, 1993; Elliot, 1994; de Juana, 1994; del Hoyo, 1994; Martinez, 1994; McGowan, 1994; Porter, 1994; Madge & McGowan, 2002). Most Galliformes have highly developed precocial young that require limited parental attention (McGowan, 1994). Nevertheless, paternal involvement varies among species (Ali & Ripley, 1980;

Cramp & Simmons, 1980; de Juana, 1994; del Hoyo, 1994; Elliot, 1994; Martinez, 1994; McGowan, 1994; Madge & McGowan, 2002).

The Galliformes exhibit a striking array of sexually dimorphic traits, with many species displaying multiple sexual ornaments. These birds exhibit a continuum of variation from complete monomorphism to extreme male-biased plumage dichromatism and size dimorphism (de Juana, 1994; del Hoyo, 1994; Elliot, 1994; Martinez, 1994; McGowan, 1994; Porter, 1994). Size dimorphism has previously been associated with mating system in this group (Lislevand *et al.*, 2009), but sexual dichromatism has never been investigated at a comparative scale. Sexual dichromatism appears particularly pronounced in species with striking iridescent plumage and elaborate feather ornaments such as those found in peacocks and other pheasants (de Juana, 1994; McGowan, 1994; Porter, 1994), but intraspecific studies provide equivocal evidence for an association between female choice and iridescent plumage (Mateos & Carranza, 1995; Ligon *et al.*, 1998; Loyau *et al.*, 2007). In addition to plumage dichromatism, many galliform birds exhibit dimorphic fleshy ornaments around the facial region (Kimball & Braun, 2008). These snoods, wattles, bibs, and eye rings appear to be important in female choice in a number of species (McGowan, 1994; Buchholz, 1995; Ligon *et al.*, 1998; Kimball & Braun, 2008). Furthermore, many galliform species have tarsal spurs in one or both sexes, which range from protruding nubs on the posterior side of the tarsometatarsus, to very long, sharp weapons (Davison, 1985). Tarsal spurs are generally used for intrasexual competition, although there is some evidence that this trait may also be used for female choice (Badyaev *et al.*, 1998). Although Badyaev *et al.* (1998) concluded that tarsal spurs in turkeys, *Meleagris gallopavo*, could indicate individual quality, most work has failed to find a relationship between mating system and the length or number of spurs within pheasants and peafowl (Sullivan & Hillgarth, 1993; Mateos & Carranza, 1996; Loyau *et al.*, 2005).

Our objective in this study was to investigate the evolution of multiple sexual ornaments in the Galliformes. We used publicly available sequence data to generate a phylogeny of the Galliformes, and quantified sexual dimorphism in six different traits to determine whether these traits were associated with mating systems or parental care in this order. In particular, we measured male and female plumage reflectance using spectrometry, and calculated sexual dichromatism as the distance between male and female reflectance data plotted in avian tetrachromatic colour space (Endler & Mielke, 2005; Stoddard & Prum, 2008). We also visually assessed overall sexual dichromatism and dichromatism in iridescence by examining museum specimens, to allow for comparisons between spectral data and visual assessments. We calculated four measures of sexual size dimorphism from published measurements of males and females. We assessed dimorphism in fleshy ornaments based on descriptions and images in species accounts. Finally, we quantified dimorphism in tarsal spurs by examining museum specimens.

Methods and Analyses

Phylogeny

We constructed a phylogeny of 70 galliform species using a Markov chain Monte Carlo (MCMC) Bayesian statistical approach in Mr. Bayes 3.2.1 (Huelsenbeck & Ronquist, 2001). We chose these species using a published phylogeny (Shen *et al.*, 2010) and substituting in available museum specimens. These 70 species represent approximately 25% of all galliform birds and 6 of the 7 families in the order, and include nearly all species with iridescent plumage (de Juana, 1994; del Hoyo, 1994; Elliot, 1994; Martinez, 1994; McGowan, 1994; Porter, 1994). We used the public database GenBank as a source for 11 nuclear and 4 mitochondrial gene sequences, in addition to the complete mitochondrial genome sequence for 34 of the 70 species

(Appendix B). After aligning and hand-editing each gene in ClustalW (Thompson *et al.*, 2002), we concatenated the nuclear and mitochondrial genes into 2 separate files and we generated phylogeny samples under a GTR + I + G model (Huelsenbeck & Ronquist, 2001). The nuclear gene analysis simulated 6,330,000 generations, the mitochondrial gene analysis simulated 7,800,000 generations, and the complete mitochondrial analysis simulated 60,000 generations. We ran all three simulations until the standard deviation of the frequencies between the posterior probabilities of species placement among the 4 chains was below 0.01. The first 8-10% of the generations in each run were discarded as burn-in. Trees were sampled every 1,000 generations in all three MrBayes runs. We created a single consensus tree from the collection of trees from all three analyses using Mesquite (Maddison & Maddison, 2007). We used this consensus tree in phylogenetically controlled analyses. We compared our tree to those in other recent phylogenetic studies (Kimball *et al.*, 1997, 2001; Meng *et al.*, 2008; Huang *et al.*, 2009; Bao *et al.*, 2010; Ma *et al.*, 2010; Shen *et al.*, 2010), and found similar genetic relationships, with the exception of the genus *Perdix*, which was sister to *Lophophorus* in our phylogeny, but sister to *Syrmaticus* in other studies (Huang *et al.*, 2009; Shen *et al.*, 2010; Kimball *et al.*, 2011).

Mating system and paternal care

We collected information on mating system and parental care for all 70 species from published species accounts (Ali & Ripley, 1980; Cramp & Simmons, 1980; de Juana, 1994; del Hoyo, 1994; Elliot, 1994; Martinez, 1994; McGowan, 1994; Porter, 1994; Madge & McGowan, 2002). We ranked mating system according to Table 3.1, using definitions in Emlen and Oring (1977). We ranked contributions to parental care activities by males and females according to Table 3.2. Parental care duties were divided into five categories and ranked from 0 to 2 based on male

participation in those activities (Table 3.2). The sum of those five parental care duties was the paternal care score. Species missing information in any of those categories were omitted from subsequent analyses (sample size after exclusions: mating system n=61; paternal care n=52).

Quantifying dimorphism

We collected plumage reflectance measurements and visually assessed other characters from 412 museum skin specimens at four natural history museums: the University of Michigan Museum of Zoology, the Field Museum, the Royal Ontario Museum, and the American Museum of Natural History. We measured three male and three female specimens for each species; only six out of 70 species were missing one or two specimens due to the unavailability of specimens when all four museum collections were combined (*Lophura edwardsi and Polyplectron malacense* were missing one male; *Crossoptilon auritum*, *Lophophorus sclateri*, *L. edwardsi*, *P. inopinatum*, *P. malacense*, and *Rheinardia ocellata* were missing one female). We identified 15 plumage regions on each skin to measure colour reflectance or to quantify dichromatism (Appendix C). We only measured birds that were labelled as adults, were in good condition, and, where applicable, identified species at the subspecies level to limit plumage variation. Previous work has determined that museum specimens accurately represent wild bird colouration (Armenta *et al.*, 2008a; Doucet & Hill, 2009). To quantify visually-assessed dichromatism, we scored dichromatism based on Owens and Hartley's (1998) methods (Table 3.3), except that we compared all 15 of our plumage regions (Appendix C) instead of their five grouped plumage regions. If any species had flesh covering an entire region, we omitted that region from scoring. To get a visual dichromatism score that was comparable among all species, we then divided the sum of the 15 rankings by the total number of regions we were able to score multiplied by the maximum score of

two. To quantify iridescence dichromatism, we classified each plumage region as iridescent or non-iridescent. For every plumage region where males had iridescent plumage and females had non-iridescent plumage, the species was given an iridescent dichromatism score of 1, up to a total potential score of 15. Again, we omitted fleshy regions, and to get a comparable iridescent dichromatism score, we divided the sum by the total number of regions we were able to score. Within the group of 70 study species, there were only two instances of female-biased iridescence. Including negative iridescent dichromatism scores had no influence on the significance of the results, so in those two cases this score was reduced to zero.

To quantify reflectance-based sexual dichromatism (hereafter spectral dichromatism), we collected objective plumage reflectance measurements using a USB 4000 spectrophotometer and a PX-2 xenon light source (Ocean Optics, Dunedin, FL), connected to a bifurcated probe. To maintain consistency between all measurements, a rubber stopper on the end of the probe ensured that all measurements were taken 3 mm from and perpendicular to the feather surface, as well as blocked out all ambient light. We repeated each measurement 5 times for each of the 15 plumage regions (Appendix C), for a total of 75 measurements per specimen. To more accurately determine the difference in colouration between male and female plumage, we modelled our reflectance curves in a tetrahedral colour space (Endler & Mielke, 2005; Stoddard & Prum, 2008). Different species perceive colours differently because of variation in the number and type of colour-sensitive cones; colour space is a representation of all of those colours a group of species is theoretically able to distinguish (Burkhardt, 1989; Goldsmith, 1990). Four distinct colour-sensitive cones have been identified in birds (Burkhardt, 1989; Hart *et al.*, 1999), and each cone type is represented by a vertex in colour space, resulting in a tetrahedral colour space (Burkhardt, 1989; Goldsmith, 1990). Those four vertices represent the maximum stimulation of violet or ultraviolet, short wavelength,

medium wavelength, and long wavelength colour-sensitive cones (Burkhardt, 1989; Goldsmith, 1990). The majority of galliform species have a violet, and not an ultraviolet, sensitive cone (Hart *et al.*, 1999) and live in a variety of habitats (de Juana, 1994; del Hoyo, 1994; Elliot, 1994; Martinez, 1994; McGowan, 1994; Porter, 1994). Therefore, we constructued our colour space model to use violet-sensitive cone stimulation of an average bird, and an ideal, or wavelength independent, ambient illumination and background. However, we used an average ultraviolet cone stimulation model for the genera *Tetrao*, *Meleagris,* and *Agriocharis*, since species-specific experiments suggest that these species exhibit ultraviolet sensitivity (Siitari *et al.*, 2002; Barber *et al.*, 2006). To quantify sexual dichromatism, we plotted male and female spectral measurements into tetrahedral colour space as two points for each body region within each species, and then calculated the Euclidean distance between those two points. We used the sum of those Euclidean distances across all 15 plumage regions as the spectral dichromatism score for each species (Stoddard & Prum, 2008).

To quantify facial flesh dimorphism, we used published species accounts as a source of facial fleshy ornament images (Ali & Ripley, 1980; Cramp & Simmons, 1980; de Juana, 1994; del Hoyo, 1994; Elliot, 1994; Martinez, 1994; McGowan, 1994; Porter, 1994; Madge & McGowan, 2002). We scored visual facial fleshy regions from species images and descriptions of size and colouration according to Table 3.3. To quantify tarsal spur dimorphism, we examined the museum specimens described above and scored tarsal spur dimorphism according to Table 3.3. To quantify sexual size dimorphism, we used published morphological measurements to calculate overall size dimorphism for four traits: tarsus length, wing length, body length, and mass (Ali & Ripley, 1980; Dunning, 1993; de Juana, 1994; del Hoyo, 1994; Elliot, 1994; Martinez, 1994; McGowan, 1994; Porter, 1994; Madge & McGowan, 2002). All measurements represent at least two individuals for each sex. Previous work has

detailed the importance of using multiple morphological measurements as proxies for body size dimorphism, since different morphological traits may be under different selective pressures (Björklund, 1990; Lislevand *et al.*, 2009). Since males are larger than females with only a few minor exceptions, the simple equation of dividing the male by female measurement to get a size dimorphism value is appropriate for the Galliformes (Lovich & Gibbons, 1992).

Analyses

Because our data were not normally distributed, we analyzed the phylogenetically controlled correlations between our different measures of dichromatism and dimorphism with mating system and paternal care using Markov chain Monte Carlo generalized linear mixed models (MCMC glmm). These analyses use a Bayesian framework to sample thousands of simulations from calculated distributions, and thus are not as sensitive to the non-normal distribution of the data as other statistical methods (Sorensen & Gianola, 2002; Hadfield, 2010). MCMC glmm incorporates phylogenetic relationships by using an inverse relationship matrix of branch lengths as a random effects variable (Hadfield, 2010). We ran univariate (fixed intercept) models for all measures of dichromatism and dimorphism with either mating system or paternal care as a fixed effect. We provided relatively weak informative priors by calculating the 95% confidence interval of the observed variation in the dimorphic or dichromatic measure being analyzed to the MCMC glmm. Each chain ran for 200,000 iterations, with the first 20,000 iterations discarded as burn-in, and a thinning interval of 50. To verify that the discrepancy in placements of low resolution nodes did not affect the sensitivity of our analyses, we re-ran a random subset of MCMC glmm analyses using 2 different trees from Kimball *et al.* (2011) and Wang *et al.* (2013); these analyses all yielded the same patterns. We carried out all analyses using R statistical software,

v.2.15.1 (R Development Core Team, 2008), in the R package `MCMCglmm` (Hadfield, 2010).

Results

Our analyses show that mating system and paternal care are negatively correlated in Galliformes; as mating systems become increasingly polygynous, paternal care decreases (parameter estimate = -0.3835; 95% confidence interval = -0.6001, -0.1573; $p < 0.001$). Spectral dichromatism was significantly positively related to mating system (Table 3.4). Males and females were more similar in colouration in more monogamous mating systems and diverged in colouration in more polygynous mating systems. All four measures of sexual size dimorphism were also significantly positively related to mating system (Table 3.4). Males and females were more similar in size in monogamous mating systems and diverged in size in more polygynous mating systems. In contrast, neither visually assessed dichromatism nor iridescent dichromatism was related to mating system (Table 3.4). Similarly, facial flesh and tarsal spur dimorphism were not related to mating system (Table 3.4).

We also found that spectral dichromatism was negatively related to degree of paternal care in Galliformes (Table 3.4). Males and females were more similar in colouration when they shared parental care duties, but diverged in colouration when male parental care decreased and female parental care increased. Two measures of sexual size dimorphism were also negatively related to degree of paternal care (Table 3.4). Males and females were more similar in size when they shared parental care, but diverged in mass and body length when male parental care decreased and female parental care increased. Visually assessed dichromatism and iridescent

dichromatism were not significantly related to the level of paternal care, nor were facial flesh or tarsal spur dimorphism.

The confidence interval for species as a random effect did not overlap zero in any of the analyses, implying a strong phylogenetic effect for all measures of dichromatism and dimorphism (data not shown).

Discussion

In this study, we demonstrate that spectral dichromatism and size dimorphism are related to both mating system and paternal care in the Galliformes. These patterns suggest that sexual selection, and perhaps natural selection, may be maintaining differences in size and colour between the sexes. In contrast, iridescent dichromatism, visually assessed dichromatism, facial flesh dimorphism, and tarsal spur dimorphism are not related to either mating system or paternal care strategies. Our findings suggest that different measures of dichromatism and dimorphism are currently under different selection pressures in this group, and highlight the importance of considering multiple measures of dichromatism and dimorphism in comparative studies.

Our findings revealed that spectral dichromatism was related to mating system in the Galliformes, where males and females were more similar in colouration in monogamous species, and diverged in colouration in polygynous species. More polygynous mating systems allow greater opportunity for female choice to affect male phenotype, so we would expect sexual selection to favour more elaborate plumage in males in mating systems with more pronounced male reproductive skew and choosier females (Darwin, 1871; Andersson, 1994). Our results parallel those of Dunn *et al.* (2001), but not Owens and Hartley (1998), who failed to find a relationship between plumage dichromatism and mating system.

Dunn *et al.* (2001) noted that they used different mating system and dichromatism scoring compared to Owens and Hartley (1998), but their study also examined 14 times as many species (Table 1 in Dunn *et al.* 2001). We used a mating system classification based on a combination of two studies, since we did not find evidence of cooperative breeding or polyandry within our study order (Table 3.1; but see Xu *et al.*, 2011 for an exception). Although our sample size is closer to that in Owens and Hartley (1998), our study focused on a single order, and our data include a larger proportion of species in each mating system rank. By focusing on a single order, we removed many potentially confounding ecological factors, such as habitat, sources of food, foraging and nesting habits, and development of young at hatching (Badyaev & Hill, 2003).

We also found that spectral dichromatism was related to the level of paternal care, where males and females were more similar in colouration when they shared parental care, but diverged in colouration when male parental care decreased and female parental care increased. Although we expect an association between mating system and parental care, as indeed there was in our study, there is not always a direct trade-off between these two traits, such that paternal care and mating system are highly correlated (Stiver & Alonzo, 2009). Whereas associations between sexual dichromatism and mating system should be driven primarily by changes in the intensity of sexual selection on male traits, associations with parental care likely result from a combination of sexual and natural selection (Andersson, 1994; Owens & Bennett, 1994). Parental care places parents at a higher predation risk (Ghalambor & Martin, 2001), and natural selection should favour more cryptic plumage to protect parents and the offspring they care for (Wallace, 1889; Martin & Badyaev, 1996). Thus, in species where females are the primary caregivers, natural selection may be promoting cryptic plumage in female Galliformes (Götmark *et al.*, 1997; Burns, 1998; Badyaev & Hill, 2003; Hofmann *et al.*, 2008), which may act to enhance

sexual dichromatism. By the same token, a reduction in male parental care could release males from some of this natural selection pressure to remain cryptic, allowing sexual selection to have a greater potential impact on male plumage. Very little research has examined this relationship, though Owens and Hartley (1998) found that only passive brood defense, was related to visually assessed plumage dichromatism, and only for melanin-based plumage.

One key outcome of our study was that our assessment of sexual dichromatism based on spectral reflectance measurements and visual modeling in avian tetrachromatic colour space were associated with mating system and parental care, but our visual assessment of sexual dichromatism, based on the same 15 body regions, were not related. This was despite these two measures of dichromatism being highly correlated with one another ($r = 0.83$, $n = 70$, $p < 0.0001$). This finding has important implications since the majority of comparative studies based on plumage colouration have focused on human visual assessments (e.g., Sigurjónsdóttir, 1981; Owens & Hartley, 1998; Dunn *et al.*, 2001; Morrow *et al.*, 2003; Martin *et al.*, 2009; but see McNaught & Owens, 2002; Doucet *et al.*, 2007). Three studies have explicitly compared human and spectral assessments of sexual dichromatism in birds. One study suggested that human assessments of sexual dichromatism vastly underestimate sexual dichromatism among species thought to be monochromatic (Eaton, 2005). Another study based on a large and diverse sample of birds suggested that in most cases, human visual assessments of dichromatism are similar to spectrally measured dichromatism (Armenta *et al.*, 2008b). The third study used visual modelling of spectral measurements and visual assessments to compare different dichromatism measures in antbirds, and found, as we did, that both measures were highly correlated (Seddon *et al.*, 2010). All studies caution that the UV component of plumage reflectance is especially likely to influence human assessments, since humans cannot detect UV wavelengths (Eaton,

2005; Armenta *et al.*, 2008b; Seddon *et al.*, 2010). However, most galliform species do not have a strong ultraviolet component in their colouration (in the 300 to 400 nm range, pers. obs.), so our findings are unlikely to represent failure of the assessor to perceive the actual plumage colouration. Instead, we suggest that human trichromatic vision may not be as sensitive to differences in colouration as birds with four colour sensitive cones. Our findings suggest that using an objectively measured and modelled measure of plumage dichromatism may be a more accurate way to quantify differences in colouration between the sexes, and, at least in some cases, may be critical to testing associations between colouration and ecological and life history traits.

We confirmed previous findings in Galliformes and other taxa that sexual size dimorphism was related to mating system (Owens & Hartley, 1998; Figuerola & Green, 2000; Dunn *et al.*, 2001; Lislevand *et al.*, 2009). In our study, all four of our measures of size dimorphism were related to mating system, implying that males in more polygynous mating systems are under selection for overall larger body sizes. Larger morphometric features would give males a greater advantage in more polygynous mating systems, where intrasexual competition tends to be more intense (Darwin, 1871; Andersson, 1994). Lislevand *et al.* (2009) found that mating system predicted size dimorphism in mass, but not wing length, in the Phasianidae, emphasizing the importance of using multiple measures of size dimorphism. Some measures of size dimorphism, such as tail length, can be exaggerated as a result of ornamentation selection in more polygynous mating systems rather than selection for increased body size (Björklund, 1990). As a result, certain morphological measurements have the potential to be less reliable indicators of size dimorphism than others (Björklund, 1990), and this may have been the case for our measure of body length dimorphism, which could have been influenced by variation in tail length in a group where elaborate tails are quite common. We believe our results

differed from Lislevand *et al.* (2009) because of the mating system classification. Lisleland *et al.* (2009) used a binary coded mating system classification: nonlekking, or lekking. Although this broad classification may have been biologically relevant to the objective in their study, it may not be sensitive enough to reveal relationships between less extreme measures of dimorphism and mating systems (Lislevand *et al.*, 2009). Many genera, such as *Lophophorus*, *Gallus*, and *Polyplectron*, are highly dimorphic in multiple morphometric features, but have a mixture of monogamous and polygynous males (Ali & Ripley, 1980; Cramp & Simmons, 1980; Madge & McGowan, 2002).

We determined that increasing mass and body length dimorphism were related to decreasing levels of paternal care in the Galliformes, but we found no relationship between our other two measures of size dimorphism: wing and tarsus length and paternal care. It may not be energetically profitable for males to maintain a larger body size than females if they are focused on raising young biparentally instead of seeking out additional mating opportunities (Emlen & Oring, 1977). Our findings strengthen Lislevand *et al.*'s (2009) argument that examining multiple morphological measurements is necessary to fully understand what traits are under selection. Wing and tarsus length could be under other selection pressures. Many Galliformes are weak or infrequent flyers, so wing size may not be under strong selective pressure with respect to paternal care, although many grouse and pheasants beat their wings to make a drumming noise during mating displays (Beebe, 1926). The Megapodes (family Megapodiidae), use their feet to construct large piles of litter to incubate eggs (Elliot, 1994), which results in proportionally large tarsi and feet. Tarsus length may also be restricted by the presence of tarsal spurs in many species in this order (Sullivan & Hillgarth, 1993). Without controlling for phylogeny, Sigurjónsdóttir (1981) found that increased size dimorphism using wing length and mass was correlated to decreased levels of paternal care in the

Galliformes. This implies that some morphological measurements, such as wing length, may be more constrained by phylogeny than others, such as mass and body length.

We were interested in investigating the relationship between mating system, parental care, and iridescent plumage dichromatism in Galliformes, given that many males incorporate iridescent plumage in mating displays, and iridescent plumage in this group produces some iconic examples of sexual selection, such as the peacock's tail (Cramp & Simmons, 1980; Madge & McGowan, 2002). We did not find a relationship between iridescent dichromatism and mating system or parental care. These findings are difficult to interpret, however, since our measure of iridescence dichromatism relied on visual assessments, which may have been subject to the limitations described above. In addition, iridescent species made up nearly half of the species in our dataset. Variation in dichromatism among iridescent species therefore contributed to the overall relationship between spectral dichromatism and mate choice and paternal care. Several intraspecific studies suggest that females do choose mates based on iridescent plumage colouration (e.g., Omland, 1996; Bennett *et al.*, 1997; Bitton *et al.*, 2007), including iridescent occelli in peacock (*Pavo cristatus*) tail coverts (Loyau *et al.*, 2007), but others have failed to find an association, including among junglefowl (*Gallus gallus*; Ligon *et al.*, 1998). In Ring-necked Pheasants (*Phasianus colchicus*), iridescent plumage does not appear to function in female choice (Mateos & Carranza, 1995) but may be important in intrasexual competition (Mateos & Carranza, 1997). Some studies also suggest that iridescent plumage may be an honest indicator of male quality through trade-offs with hydrophobicity (Eliason & Shawkey, 2011), or association with parasite load or condition (e.g., McGraw *et al.*, 2002; Doucet, 2002; Doucet & Montgomerie, 2003), including Wild Turkeys (*Melagris gallopavo*; Hill *et al.*, 2005). As with any comparative study, it is difficult to interpret which traits are under current

selection, and which traits may have been favoured in the past. For example, the more derived peacock-pheasants (genus *Polyplectron*) have fewer iridescent occelli than more ancestral peacock-pheasant species (Kimball *et al.*, 2001), suggesting the recent reduction of a sexually dimorphic iridescent plumage trait.

There are few, if any, instances of carotenoid-based plumage in the Galliformes. Thus, aside from iridescent plumage and other structural colours, the relationships between spectral dichromatism and mating system and paternal care must be caused in part by melanin-based plumage. Species-specific studies within the order have examined, and found that melanin-based plumage can be a signal of individual condition and be indicative of stress level (Bortolotti *et al.*, 2006; Svobodová *et al.*, 2013), and could therefore be favoured through honest advertisement models of sexual selection (Hill, 2006).

In our study, sexual dichromatism in facial fleshy ornaments was not related to either mating system or paternal care. These findings are surprising since experiments in the Galliformes have shown that females use fleshy ornaments in mate choice in multiple genera (Brodsky, 1988; Buchholz, 1995; Ligon *et al.*, 1998; Mateos, 1998; Rintamäki *et al.*, 2000). Facial fleshy ornaments, which are present in many extant species, are thought to have originally evolved for thermoregulation in a basal ancestor to the Galliformes and have been co-opted as sexual traits (Kimball & Braun, 2008). There is strong evidence to suggest that facial flesh is an honest signal of male health and quality (Brodsky, 1988; Buchholz, 1997; Mateos, 1998; Rintamäki *et al.*, 2000; Pérez-Rodríguez & Viñuela, 2008). Moreover, fleshy ornaments can change based on an individual's health over a matter of days, a much faster rate compared to plumage, which reflects a male's health at molt days, weeks, or months previously (Pérez-Rodríguez & Viñuela, 2008). If fleshy ornaments are particularly honest, females may use this trait to assess males in all types of mating

systems, which may explain why we did not find a relationship between dichromatism in fleshy ornaments and life history traits.

Although tarsal spurs have the potential to be a secondary sexual character in the Galliform order (Badyaev *et al.*, 1998), we found no relationship between tarsal spur dimorphism and mating system or paternal care. Our findings are in agreement with the most recent comparative work on tarsal spur dimorphism within this order by Sullivan and Hillgarth (1993), but not with Davidson's (1985) study. Davidson (1985) concluded that there was a high correlation between spur length and body size, and that polygamy was associated with the presence of single or multiple spurs . However, as Sullivan and Hillgarth (1993) point out, Davidson's (1985) dataset was biased towards monogamy and did not control for phylogeny. It would be interesting to examine spur dimorphism specifically in relation to male-male competition: even in polygynous mating systems, males vary in the frequency of physical aggressive interations (e.g., Davidson, 1981; Davidson, 1983). Spurs might also be maintained in part by natural selection if they function in defense against predators, although no one has tested this idea (Caro, 2005). Our dataset does not allow us to determine whether tarsal spurs are under currently under natural or sexual selection in the Galliformes, are artefacts from a distant ancestor, or are under different selection pressures that are specific to certain species or genera.

In this study, we found that spectral dichromatism, which we modelled in tetrachromatic space based on receiver visual sensitivity, was related to both mating system and paternal care in the Galliformes, whereas a visually-assessed measure of dichromatism was not related to either life history trait. Our findings caution against relying on visual assessments of plumage colouration and highlight the need for objective assessments of animal colouration. In corroboration with previous work, we also found that sexual size dimorphism was related to both mating system

and paternal care. Taken together, our findings suggest that sexual selection on males, perhaps in combination with opposing natural selection on females, has played an important role in the evolution of sexually dichromatic plumage and sexually dimorphic body size in Galliformes. Surprisingly, we found no evidence that other well-known sexual ornaments in this group were related to mating system or parental care, including fleshy ornaments, and tarsal spurs. The Galliformes contain an unusually large number of species that exhibit multiple sexual ornaments, but many of these ornaments may not be under current sexual selection across the entire order. Species-specific studies that isolate and manipulate individual ornaments may provide further resolution to generalized phylogenetic patterns.

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Tables and Figures

Table 3.1. Criteria used for ranking the mating system used by each galliform species. Ranks are based on criteria outlined in Owens and Hartley (1998), Dunn *et al.* (2001), and MacFarlane *et al.* (2007).

rank	description
0 – polygamy not reported/rare	Less than 1% of bonds are polygamous. In most cases the species is monogamous, one male pair-bonded with one female, with rare cases of polygamy (polyandry [one female pairs with more than one male] and/or polygyny [one male pairs with more than one female]).
1 – occasional facultative polygamy	1% -15% of bonds are polygamous. In most cases the species is monogamous, one male pair-bonded with one female, with some cases of polygamy, which can be in the form of polyandry, polygyny, promiscuity (no pair bond is formed; males and females meet only for courtship and copulation), or cooperative breeding (more than two birds of the same species provide parental care at a single nest).
2 – frequent facultative polygamy	More than 15% of bonds are polygamous. Most bonds are considered polygamous, but with some cases of social monogamy.
3 – obligate polygamy	Only polygamous systems are used: polygyny, polyandry, and/or promiscuity.

Table 3.2. Criteria used for ranking male and female role in parental care in galliform species, based on the efforts made by both sexes after copulation (Owens & Bennett, 1994).

rank	nest building	incubation	brood-provisioning	passive brood defense	active brood defense
0	female chooses nest site and builds it alone	female incubates alone	female feeds hatchlings alone	only female defends offspring by brooding	only female employs specific actions designed for predator deterrence
1	female and male choose nest site and build nest	female and male incubate the clutch	female and male feed hatchlings	female and male defend offspring by brooding	both female and male will employ specific actions designed to deter predators
2	male chooses nest site and builds it alone	male incubates alone	male feeds hatchlings alone	only male defends offspring by brooding	only male employs specific actions designed for predator deterrence

Table 3.3. Criteria used for categorizing intraspecific sexual dimorphism in plumage, facial fleshy ornaments, and tarsal spurs in Galliformes. Plumage dichromatism was categorized by visual inspection. Facial flesh and tarsal spur dimorphism were assessed only in species with facial fleshy ornaments and/or tarsal spurs in at least one sex. Rankings are based on criteria in Owens and Hartley (1998) for plumage dichromatism; facial flesh and tarsal spur rankings are based on the soft part variable ranking scale established by Sigurjónsdóttir (1981).

rank	plumage region	facial flesh	tarsal spurs
0	no difference in colour, colour intensity, or pattern between males and females; monochromatic	no difference between male and female flesh in size, shape, or colour; monomorphic	no difference between male and female spur size or shape; monomorphic
1	males and females differ in the intensity of plumage colour; dichromatic	flesh is different in size, shape and/or colour between males and females; dimorphic	spurs are present in both males and females, but differ in size and/or shape; dimorphic
2	plumage colour and/or pattern is different between males and females; completely dichromatic	flesh is present in males and absent in females; completely dimorphic	Spurs are present in males and absent in females; completely dimorphic

Table 3.4. Results of MCMC glmm analyses assessing the relationship between mating system, paternal care, and six measures of sexual dimorphism in Galliformes. The parameter estimate is the mean of the posterior distribution. The 95% confidence interval reports the lower and upper boundary values of the posterior distribution. A 95% confidence interval that encompasses only negative values only is interpreted as a negative correlation between the variable and the fixed effect; a posterior distribution above zero is interpreted as a positive correlation between the variable and the fixed effect. Bolded measures and p-values highlight statistically significant relationships.

fixed effect	n	dimorphism or dichromatism measure	parameter estimate	95% confidence interval	P-value
mating system	61	**spectral dichromatism**	0.1372	0.0133, 0.2585	**0.031**
	61	visually assessed dichromatism	0.0323	-0.0525, 0.1108	0.435
	61	iridescent dichromatism	0.4939	-0.2156, 1.2509	0.199
	61	**size dimorphism (mass)**	0.1307	0.0367, 0.2181	**0.007**
	61	**size dimorphism (body length)**	0.1006	0.0252, 0.1734	**0.009**
	61	**size dimorphism (wing length)**	0.0286	0.0110, 0.0457	**0.001**
	61	**size dimorphism (tarsus length)**	0.0341	0.0046, 0.0623	**0.028**
	61	facial flesh dimorphism	-0.0380	-0.1623, 0.0810	0.524
	61	tarsal spur dimorphism	-0.0122	-0.1805, 0.1483	0.887
paternal care	52	**spectral dichromatism**	-0.1855	-0.2904, -0.0706	**0.002**
	52	visually assessed dichromatism	-0.0671	-0.1510, 0.0064	0.098
	52	iridescent dichromatism	-0.4312	-1.0692, 0.2221	0.197
	52	**size dimorphism (mass)**	-0.1000	-0.1856, -0.0058	**0.028**
	52	**size dimorphism (body length)**	-0.1277	-0.2033, -0.0548	**0.001**
	52	size dimorphism (wing length)	-0.0165	-0.0350, 0.0019	0.074
	52	size dimorphism (tarsus length)	-0.0217	-0.0480, 0.0060	0.117
	52	facial flesh dimorphism	0.0540	-0.0782, 0.1906	0.409
	52	tarsal spur dimorphism	-0.0608	-0.2165, 0.0780	0.404

CHAPTER 4

General discussion

Iridescence produces some of the most spectacular visual displays in nature, and is not limited to bird plumage: it exists in beetle elytra, butterfly wings, and flower petals (Vukusic & Sambles, 2003; Seago *et al.*, 2009). However, iridescent colours in different taxa are produced using different nanostructural strategies, and we have only recently begun to understand the mechanistic basis of this highly specialized colouration (Vukusic & Sambles, 2003; Prum, 2006; Seago *et al.*, 2009). Moreover, relatively little is known about how iridescent colouration evolves and the selective factors favoring its evolution and maintenance in different groups (Doucet & Meadows, 2009). The purpose of my thesis was to investigate the evolution of iridescent plumage in birds by characterizing proximate mechanisms and ultimate functions. My research focused on the Galliformes, an order of birds with multiple sexually dimorphic traits including extensive variation in iridescence. To understand the evolution of iridescence, I first produced a phylogeny to define the relationships between 70 galliform species. I used this phylogeny to examine evolutionary gains and losses of iridescent plumage and barbule nanostructure. I used visual modelling to examine the range of colours produced by different nanostructures. Finally, while controlling for phylogenetic relationships, I examine the relationship between six measures of sexual dimorphism and mating system and paternal care. My research contributes to our understanding of the evolution of the complex nanostructures required to produce iridescent plumage, and provides insight into the diverse selection pressures that influence the gain and loss of these highly specialized colours.

My research shows that iridescence is a highly labile trait, which is common for sexually selected ornaments (Hill *et al.*, 1998), but is also likely to be under considerable phylogenetic influence, as has been found for carotenoid-based plumage (Hofmann *et al.*, 2006). Using ancestral state reconstruction, I estimated the ancestral lineages of extant iridescent species. Instead of a small number of common iridescent ancestors, I found that iridescent plumage has evolved independently multiple times, usually from non-iridescent ancestors. Different nanostructural types appear to have evolved directly from non-iridescent ancestors, independent of melanosome morphology and organizational complexity. Very few other studies have estimated the evolutionary gains and losses of iridescent plumage among such a large number of species. Iridescent plumage was also shown to exhibit multiple gains and losses in the blackbird family (Icteridae; Shawkey *et al.*, 2006) and African Starlings (Sturnidae; Maia *et al.*, 2013). However, these studies also suggested a transitional state between non-iridescent and iridescent nanostructures (Shawkey *et al.*, 2006; Maia *et al.*, 2011; Maia *et al.*, 2013), which I did not find in Galliformes. This could suggest that selective pressures are different in my study group; in order for a transitional state to be detected, it must be fixed in an ancestral species, and remain present in extant taxa (Coyne & Orr, 2004). In the Galliformes, transitional states with a distinct phenotype may not have been fixed in ancestral species and subsequently maintained in extant taxa, because they were energetically unfavorable (Eliason & Shawkey, 2012), or selected against through natural or sexual selection (Wiens, 2001).

Future studies could benefit from expanding this nanostructural dataset to include more male Galliformes with glossy black and matte black plumage, in addition to female plumage. Some brown female plumage has a very faint iridescent shine, which I particularly noted in female Green Junglefowl *Gallus varius* dorsal tail feathers, as had another galliform expert (pers. comm. R.T. Kimball). Expanding this

dataset would increase the resolution of proximate evolutionary patterns. Furthermore, in the blackbirds mentioned above, there is only one iridescent common ancestor, which implies that all of the iridescent nanostructures are highly related (Shawkey *et al.*, 2006). In contrast, iridescent Galliformes have multiple ancestors, and it is therefore likely that multiple ancestors underwent different nanostructural innovations leading to iridescence. This pattern lowers the likelihood of iridescence following the same evolutionary pathways in this order, compared to groups with a single ancestral iridescent innovation. That hollow melanosomes have evolved three times independently is even more striking, and suggests that the step from solid to hollow melanosomes could require a relatively small innovation to melanosome development.

Melanins that are directly deposited as pigments into growing feathers produce a very restricted range of colours (Stoddard & Prum, 2011). In contrast, carotenoids produce an intermediate range of colours, and structural colours produce the broadest range of colours (Stoddard & Prum, 2011). Galliformes only use melanin-based and structural colouration mechanisms, and thus are more restricted in plumage colouration compared to clades such as blackbirds, which use all three types of mechanisms (McGraw, 2006; Shawkey *et al.*, 2006). Iridescent nanostructures could be less important to exploiting novel colouration, resulting in lower selection pressure, in clades with multiple colouration mechanisms. Future studies could test this hypothesis by comparing speciation and extinction rates between clades that vary in colour mechanism strategies, and the extent to which they exploit tetrahedral colour space volume. Comparing the speciation, extinction and diversification rates for multiple clades may provide more insight into the selection pressures influencing iridescent plumage.

Sexual selection has led to the elaboration of multiple different types of ornaments in some species, although whether some or all of those ornaments are

under current selective pressure is poorly understood (Møller & Pomiankowski, 1993; Møller & Petrie, 2002; Papeschi & Dessì-Fulgheri, 2003; Husak & Swallow, 2011; Kimball *et al.*, 2011). To help clarify the selection pressures on multiple ornaments in the Galliformes, I divided multiple traits into six categories of dichromatism and dimorphism, and examined their relationships to mating systems and paternal care. My results indicated that spectral dichromatism was related to both mating system and paternal care in the Galliformes. Species that used more polygynous mating systems were more likely to have a higher measure of spectral dichromatism. Male reproductive skew is more pronounced in polygynous mating systems as a result of choosier females, which allows for the elaboration and exaggeration of the traits females find attractive (Darwin, 1871; Andersson, 1994). Spectral dichromatism was also related to the level of paternal care. Unfortunately, we cannot determine from our data set if this is an artefact of the significant relationship between increasing polygynous mating systems and decreasing level of paternal care, or from additional selection pressures. The level of parental care has the ability to constrain plumage towards cryptic colouration, especially in females, through natural selection (Wallace, 1889; Martin & Badyaev, 1996) as a result of predation (Ghalambor & Martin, 2001). If female plumage becomes increasingly drab, and male plumage remains the same, dichromatism would also increase. However, in a male-biased population of peacocks, cryptically plumaged peahens experienced a higher predation rate (Takahashi, 2008), but whether this was confounded by females having peachicks was not specified. Although there are many studies that examine the relationship between plumage dichromatism and mating system, very little research has examined this relationship with paternal care. To better understand what measures of sexual dichromatism are under sexual selection pressures in Galliformes, I suggest future studies manipulate plumage patterning and colouration to isolate multiple ornaments in an attempt to establish

the precedence of plumage types used in female choice, and establish the effect of colouration on predator detection.

Importantly, neither of my visually assessed dichromatism measures was related to either mating system or paternal care, despite the fact that both iridescent dichromatism and visually assessed dichromatism were highly correlated with spectral dichromatism. A study comparing similar methods for quantifying dichromatism in antbirds also found that spectral and visually assessed measures were highly correlated (Seddon *et al.*, 2010), although they did not expand their analyses to investigate if the same correlations were found with an additional variable across dichromatism measures. Comparing human visual rankings and spectral dichromatism, authors have come to different conclusions: human visual assessment is not substantially different from objective measurements (Armenta *et al.*, 2008; Seddon *et al.*, 2010), and human assessment is an inadequate method of quantifying dichromatism (Eaton, 2005). Unlike these studies that were limited to a direct comparison of different dichromatism measures, I applied these two methods of quantifying dichromatism to a large-scale comparative analysis, and found that they do not produce the same results. I feel this provides strong evidence that for studies that rely on quantifying dichromatism, human visual assessments should complement an objectively measured and visually modelled method.

Given that selection pressures act differentially on individual traits and morphological measurements (Björklund, 1990; Lislevand *et al.*, 2009), I used four different morphological measurements as proxies for size dimorphism. All four measures were related to mating system, indicating there is intense selection pressure for larger males in more polygynous species. Polygynous males generally experience higher male-male competition (Andersson, 1994). Heavier, larger males would be more visually threatening to opponents, and have an advantage in physical combat (e.g. Hagelin, 2002). When traits are all highly correlated, such as my four

different measures of size dimorphism, differential selection pressures are much more cryptic. In contrast to relationships with mating system, I found that only body length dimorphism and mass dimorphism were related to paternal care. Wing length and tarsus length may be under different selective pressures. Alternatively, wing and tarsus length may be under stronger phylogenetic influence than the other two measures, and thus more constrained in their ability to be selected upon (Badyaev & Hill, 2003). In parallel with Lislevand *et al.* (2009), my findings demonstrate the importance of using multiple measurements when examining relationships concerning size dimorphism, and future studies should continue this practice.

Recent work warned against assuming that an uncomplicated trade-off exists between mating system and level of paternal care (Stiver & Alonzo, 2009), where an increase in polygyny necessarily leads to a decrease in paternal care. Many studies make this assumption, which could explain why so little work examines sexual dimorphism in relation to parental care separately from mating system. Galliform hatchlings are well-developed and quite independent, which could lessen the pressure for paternal involvement, and ultimately dilute the relationship between sexual dimorphism and paternal care. However, the riskiest form of parental care is feeding and protection (Owens & Bennett, 1994). Therefore, females can benefit from paternal care by sharing this risk. Parental protection may be more important in this group compared to other bird families, since most Galliformes are preyed on by multiple species (Beebe, 1926). In the future, an effort should be made to observe more wild Galliformes: many species are endangered, yet we still lack information on mating system, reproductive timing, duration of the pair-bond, how many eggs are laid, or the level of paternal care for a number of species.

Surprisingly, my study did not show any relationship between fleshy ornaments and mating system or paternal care. This may be indicative of different

selection pressures between genera or clades that are not projected at the order level. For example, fleshy ornaments became specialized, in that they could change shape quickly and reversibly, in a single clade (Kimball & Braun, 2008). Sexual selection could be more intense for these erectile ornaments, and mating system could be significantly correlated to fleshy dimorphism within this clade, whereas fleshy ornaments outside of this clade may be maintained for the ancestral function of heat loss (Buchholz, 1996). Many of the species-specific studies demonstrating female choice for fleshy ornaments are found within this erectile clade (Brodsky, 1988; Buchholz, 1995; Mateos, 1998; Rintamäki *et al.*, 2000), with the exception of eye rings in the Red-legged Partridge (*Alectoris rufa*; Pérez-Rodríguez & Viñuela, 2008). Eye rings is this species are coloured by carotenoids, which could provide valuable mate-choice information to females, since carotenoid-based plumage is not found in the Galliformes. Again, this strengthens the argument that fleshy ornaments could be correlated to mating system within only specific groups of species.

My analyses also failed to show a relationship between tarsal spur dimorphism and mating system or paternal care. Tarsal spurs would be useful as armaments in more polygynous mating systems, where male-male competition is more frequent and intense (Andersson, 1994). Tarsal spurs are also indicative of an individual's health (Badyaev *et al.* 1998). Thus, tarsal spur dimorphism could be a result of sexual selection. While the tarsal spurs of some species exhibited little or no intraspecific variation, other species exhibited dramatic intraspecific variation. For example, in Tragopans, some males had large, sharp tarsal spurs, while other males had a small nub, and some completely lacked spurs (pers. obs.). This could imply that a common ancestor to more derived galliform families had tarsal spurs, which is why they are found so frequently throughout this order (Davidson, 1985). Uniform tarsal spurs in a species could suggest that this trait was once under

selection until it reached phenotypic fixation, either by physiological or ecological contraints (Kirkpatrick, 1982). Sexual selection needs intraspecific trait variation to act (Andersson, 1994). Thus, tarsal spurs that are more uniform in size and shape within a species are unlikely to be under current selection pressures. Tarsal spurs that show intraspecific variation in size are more likely to be under current selection pressures, and show a correlation to mating system, in species with intraspecific variation in shape and size of these armaments.

For my thesis I was interested in the proximate mechanisms and ultimate functions of iridescent plumage. By estimating the pattern of gains and losses of iridescent plumage in relation to barbule nanostructure, I determined that iridescence is a highly labile trait that has evolved independently multiple times, directly from a non-iridescent ancestor in almost all cases. By examining the volume occupied in tetrahedral colour space by different nanostructural types, I determined that organized nanostructures producing iridescence were capable of exploiting a greater diversity of colours than unorganized nanostructures. Similarly, I determined that nanostructures using solid melanosome rods were capable of exploiting a greater diversity of iridescent colours than nanostructures using hollow melanosomes. I also found iridescent species to have higher diversification rates than non-iridescent species. By quantifying six measures of sexual dimorphism, I found that spectral dichromatism, which included iridescence, was related to mating system and paternal care. Likewise, I found that size dimorphism was related to both mating system and paternal care. These results suggest that sexual selection for large colourful males and natural selection for small drab females can explain patterns of dichromatism and dimorphism in this group. By demonstrating that spectrally measured and visually assessed dichromatism yield different results, my findings emphasize the importance of using objective measurements of animal coloration. My study established broad evolutionary patterns for the mechanisms

producing iridescence in the Galliformes, as well as the influence of multiple selective factors on the evolution of sexual dimorphism and dichromatism in this group. These methods can be applied to other taxa in which iridescence has evolved. By combining the evolutionary patterns of iridescence from multiple large scale works, we will achieve greater understanding of how iridescence evolves, and the functions it serves.

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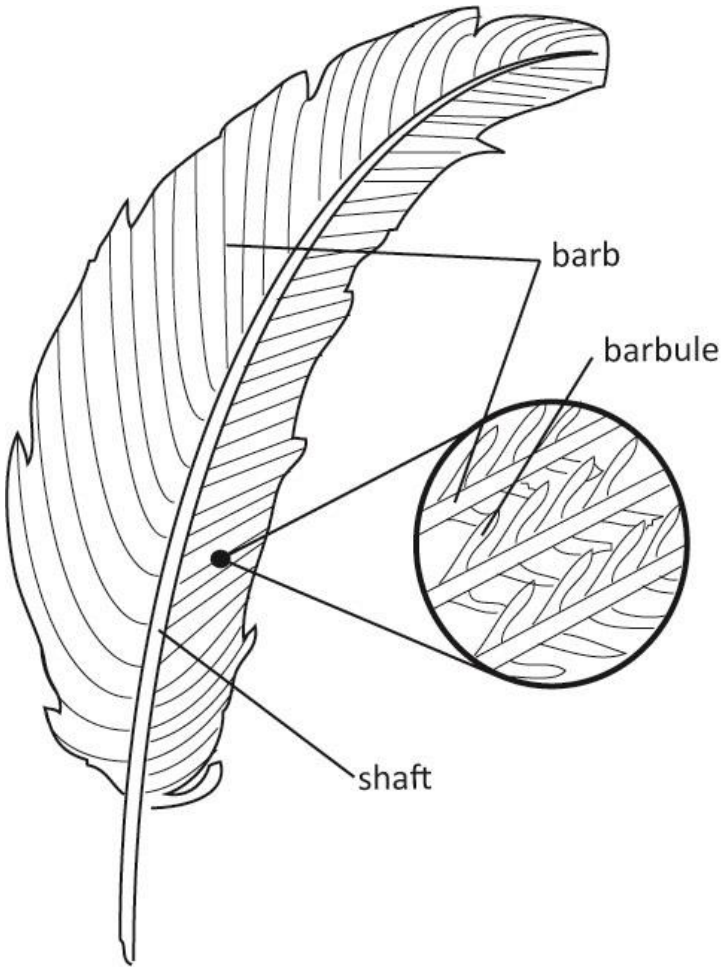
APPENDICES

Appendix A

Anatomy of a feather. A feather is composed of a central shaft, to which barbs are attached. Barbules are attached on either side of each barb. Line drawing by Kevyn Gammie.



Appendix B

Genbank accession numbers for the nuclear and mitochondrial genes used to produce our 70 galliform species phylogeny. For some species, individual mitochondrial genes were trimmed from the complete mitochondrial genome. The first four columns are mitochondrial genes; the last 12 columns are nuclear genes.

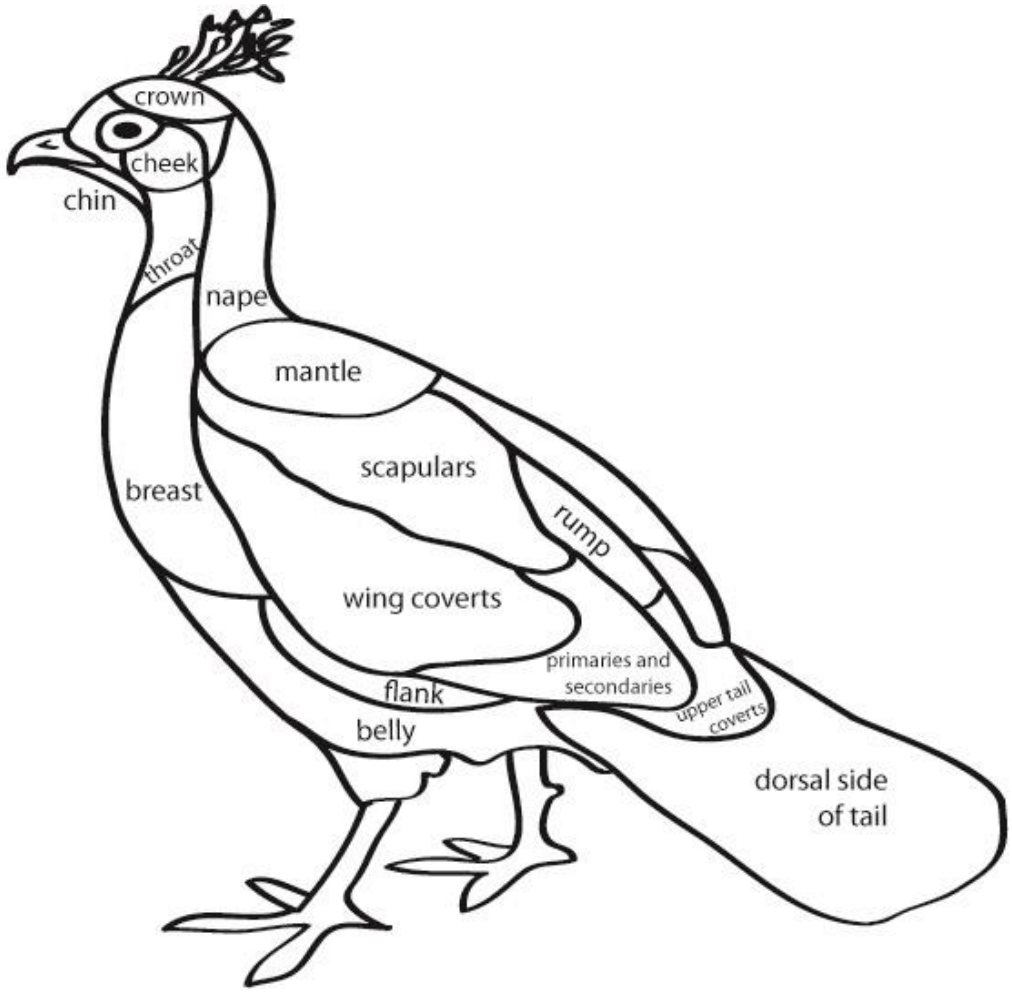
Scientific Name	AGRP	ovomucoid intron G	CYTB	mito D-loop	complete mito genome	TYR	TYRP1	DCT/TYRP2	Rab27a	ND2	12S ribo	rhodopsin intron 1	beta fibrinogen intron 7	CRYAA intron 2	EEF2 exon 6	ALDOB intron 6	ALDOB 3'UTR
Gallus gallus	AB029443.1	AF170979.1	GU261707.1	DQ834510.1	GU261707.1					EU845754.1	DQ885561.1	AY952757.1	AY952658.1	FJ881721.1	FJ881855.1		FJ881776.1
Gallus varius		EF569485.1	NC_007238.1	D64163.1	NC_007238.1					AF222551.1	AF222582.1	EF569444.1	EF569464.1				
Gallus lafayettei		EF569483.1	NC_007239.1	DQ834512.1	NC_007239.1					NC_007239.1		EF569442.1	EF569462.1				
Gallus sonneratii	EF571210.1	EF569484.1	EF571186.1	DQ834511.1	NC_007240.1	EF571135.1	EF571101.1	EF571064.1	FJ449552.1	NC_007240.1		EF569443.1					
Bambusicola thoracica		AF170978.1	EU839452.1	DQ834513.1	EU165706.1					AF222538.1	AF222570.1	EF569437.1	DQ306962.1				
Bambusicola fytchii			AM236891.2	FJ752423.1	FJ752423.1					FJ752423.1							
Francolinus francolinus			AF013762.1	DQ834514.1													
Francolinus pondicerianus	EF571211.1	DQ832081.1	U90648.1	GU213076.1		EF571136.1	EF571113.1	EF571065.1	FJ449560.1	DQ768279.1	DQ832103.1						
Coturnix coturnix	EF571216.1		EU839461.1	DQ834529.1		EF571140.1	EF571106.1	EF571077.1	FJ449553.1	EU845745.1	FN675551.1	EU737202.1			EU738609.1		
Coturnix japonica	AB489989.1	AY952773.1	NC_003408.1	NC_003408.1	NC_003408.1					NC_003408.1	AJ490509.1	AY952756.1	AY952657.1	FJ881718.1			FJ881773.1
Tetraogallus tibetanus			EU839456.1	GQ343551.1						EU845747.1							
Tetraogallus himalayensis			EU839460.1	DQ834520.1						EU845749.1							
Alectoris rufa	EF571223.1		Z48775.1	FN376868.1		EF571144.1	EF571110.1	EF571073.1	FJ449567.1		AM902517.1	EF569436.1	DQ306961.1				
Alectoris graeca			Z48772.1	DQ834524.1													
Alectoris barbara			AM492953.1	FN376870.1							AM944502.1						
Francolinus squamatus		DQ832088.1	AM236904.1	DQ834531.1						DQ768286.1	DQ832109.1						
Francolinus swainsonii		DQ832091.1	AM236907.2	DQ834532.1						DQ768287.1	DQ832110.1						
Pavo muticus	EF571196.1	AF170989.1	AF013763.1	DQ834509.1	EU417811.1	EF571120.1	EF571086.1	EF571050.1	FJ449558.1	EF569478.1		EF569449.1	EF569465.1				
Pavo cristatus	EF571200.1	AF170990.2	DQ010648.1	DQ834508.1		EF571124.1	EF571090.1	EF571054.1	FJ449562.1	AF394612.1	AY952766.1		AY952659.1	FJ881728.1			FJ881783.1

Afropavo congensis	EF571221.1	AF170991.1	AF013760.1	DQ834507.1		EF571146.1	EF571112.1	EF571076.1		DQ768253.1		EF569434.1	DQ306959.1	FJ881714.1	FJ881857.1	FJ881835.1	FJ881769.1
Argusianus argus	EF571222.1	AF331954.1	AF013761.1	DQ834505.1		EF571148.1	EF571102.1	EF571069.1						FJ881715.1		FJ881836.1	FJ881770.1
Rheinardia ocellata			AF330060.1	DQ834506.1													
Chrysolophus amherstiae		DQ832080.1	AB120130.1	AY368067.1	FJ752434.1					DQ768277.1	DQ832102.1						
Chrysolophus pictus		DQ307014.1	EU839476.1	DQ834497.1	FJ752433.1					DQ768255.1		EF569439.1	DQ306964.1	FJ881717.1	FJ881850.1	FJ881838.1	FJ881772.1
Phasianus colchius	EF571199.1	AY952774.1	AF028798.1	DQ834495.1	FJ752430.1	EF571123.1	EF571089.1	EF571053.1		AF222561.1	U83742.1	AY952759.1	AY952661.1	FJ881730.1	FJ881851.1		FJ881785.1
Phasianus versicolor			AY368058.1	AY376866.1	NC_010778.1					NC_010778.1							
Catreus wallichi	EF571213.1	AF170980.1	AF028792.1	DQ834499.1		EF571138.1	EF571104.1	EF571067.1		DQ768254.1		EF569438.1	DQ306963.1	FJ881716.1	FJ881849.1	FJ881837.1	FJ881771.1
Crossoptilon mantchuricum	EF571214.1		AF534553.1	DQ834502.1		EF571139.1	EF571105.1	EF571068.1									
Crossoptilon auritum			AF028792.1	DQ834501.1	JF937589.1					EU845771.1							
Lophura leucomelana			AF314643.1	AJ300153.1													
Lophura nycthemera	EF571205.1	DQ307017.1	EU417810.1	DQ834498.1	EU417810.1	EF571130.1	EF571096.1	EF571059.1		DQ768261.1		EF569447.1	DQ306969.1	FJ881723.1	FJ881853.1	FJ881839.1	FJ881778.1
Lophura edwardsi	EF571208.1		AF314638.1	AJ300148.1		EF571133.1	EF571099.1	EF571062.1	FJ449557.1								
Lophura swinhoii	EF571204.1	DQ307018.1	AF314644.1	AJ300155.1		EF571129.1	EF571095.1	EF571058.1		DQ768262.1		EF569448.1	DQ306970.1				
Syrmaticus humiae		DQ832077.1	AF534560.1	DQ834491.1	NC_010774.1					DQ768293.1	DQ832099.1						
Syrmaticus ellioti		DQ307019.1	AF534559.1	DQ834493.1	NC_010771.1					GU214317.1	DQ832100.1	EF569458.1	DQ306975.1				
Syrmaticus reevesii	EF571192.1		AF028801.1	DQ834492.1	AB164623.1	EF571116.1	EF571082.1	EF571047.1		DQ768271.1		EF569459.1	DQ306976.1	FJ881733.1		FJ881842.1	FJ881788.1
Syrmaticus soemmerringii			AY172840.1	AY368068.1	NC_010767.1												
Perdix perdix	EF571194.1	AF170982.1	AF028791.1	DQ834484.1		EF571118.1	EF571084.1	EF571049.1	FJ449561.1	AF222560.1	AF222590.1	EF569456.1	DQ306971.1	FJ881731.1	FJ881852.1	FJ881841.1	FJ881786.1
Perdix dauuricae			EU839468.1	FJ752431.1	FJ752431.1					AF222559.1	AF222589.1						
Perdix hodgsoniae			EU839472.1							EU845764.1							
Pucrasia macrolopha		AF170983.1	AF028800.1	DQ834490.1	FJ752429.1					DQ768269.1	FR873678.1	EF569457.1	DQ306974.1				
Bonasa umbellus			AF230167.1	AF532416.1						AF222541.1	U83740.1						
Bonasa bonasia			FJ752435.1	AF532418.1	FJ752435.1					AF222539.1	AF222571.1						
Tetrao tetrix	EF571203.1		EF571183.1	AF532458.1		EF571128.1	EF571094.1	EF571057.1	FJ449559.1	AF222564.1	AF222593.1						
Tetrao urogallus	EF571189.1		AB120132.1	AF532466.1		EF571147.1	EF571079.1	EF571044.1		AF222565.1	AF222594.1						
Agriocharis ocellata				AF487121.1													

Meleagris gallopavo	EF571202.1	AF170984.1		AF532414.1	NC_010195.2	EF571126.1	EF571092.1	EF571056.1		AF222556.1	U83741.1	AY144679.1	AY952660.1	FJ881724.1	FJ881856.1		FJ881779.1
Tetraophasis szechenyii			EU839484.1	FJ799743.1	FJ752428.1					EU049326.1							
Tetraophasis obscurus			EU049324.1	JF921876.1	JF921876.1					EU049327.1							
Lophophorus impejanus	EF571207.1	DQ307015.1	AF028796.1	AY376858.1		EF571132.1	EF571098.1	EF571061.1		DQ768259.1	DQ832098.1	EF569445.1	DQ306967.1				
Lophophorus sclateri			FJ752432.1	AY376860.1	FJ752432.1												
Lophophorus lhuysii			EU839487.1	AY376859.1	NC_013979.1					EU845760.1							
Tragopan temminckii	EF571190.1		AF028802.1	DQ834488.1	FJ752427.1	EF571114.1	EF571080.1	EF571045.1		AF222566.1	AF222595.1	AY952760.1	AY952662.1	FJ881734.1	FJ881854.1		FJ881789.1
Tragopan caboti			AF200723.1	NC_013619.1	NC_013619.1					NC_013619.1	AB004240.1						
Tragopan satyra	EF571191.1		AF534555.1	AF532412.1		EF571115.1	EF571081.1	EF571046.1	FJ449568.1								
Tragopan blythii		DQ307021.1	AF200722.1							DQ768272.1		EF569460.1	DQ306977.1				
Ithaginis cruentus		DQ832076.1	AF068193.1	DQ834487.1	JF921875.1					DQ768258.1	JQ796701.1						
Polyplectron bicalcaratum		AF331959.1	AF028799.1	DQ834503.1	EU417812.1					EF569479.1		EF569450.1	EF569466.1				
Polyplectron chalcurum		AF331956.1	AF330061.1	AJ295256.1						EF569480.1		EF569451.1	EF569467.1				
Polyplectron inopinatum	EF571197.1	AF331958.1	AF330064.1	AJ295258.1		EF571121.1	EF571087.1	EF571051.1	FJ449565.1	EF569482.1		EF569454.1	EF569469.1				
Polyplectron germaini		AF331960.1	AF330063.1	AJ295257.1						DQ768266.1		EF569453.1	DQ306972.1				
Polyplectron malacense	EF571195.1	AF331957.1	AF330065.1	AJ295260.1		EF571119.1	EF571085.1	EF571078.1		DQ768268.1		EF569455.1	DQ306973.1				
Polyplectron napoleonis	EF571198.1	AF331955.1	AF330062.1	DQ834504.1		EF571122.1	EF571088.1	EF571052.1		EF569481.1		EF569452.1	EF569468.1	FJ881729.1	FJ881858.1	FJ881840.1	FJ881784.1
Arborophila torqueola			AM236889.1	DQ834475.1													
Arborophila rufogularis			FJ752424.1	FJ752424.1	FJ752424.1												
Acryllium vulturinum	EF571219.1	DQ832070.1	AF536742.1	NC_014180.1	NC_014180.1	EF571143.1	EF571109.1	EF571072.1		AF536745.1	AF536739.1						
Numida meleagris	EF571201.1	AF170975.1	AP005595.1	DQ834466.1	AP005595.1	EF571125.1	EF571091.1	EF571055.1	FJ449563.1	AF222557.1	AF222587.1	EU737246.1	AY952653.1	FJ881725.1	EU738650.1		FJ881780.1
Ortalis vetula		AF170974.1	AY354494.1							AF394614.1	AY952762.1	AY952751.1	AY952651.1	FJ881727.1	FJ881846.1		FJ881782.1
Crax rubra		AY952770.1	AY274029.1							AY952746.1	AY274003.1	AY952750.1	AY952650.1	FJ881719.1	FJ881845.1		FJ881774.1
Alectura lathami			AF082058.2	DQ834465.1	AY346091.1					AF394615.1	AY274004.1	EU737168.1	AY952647.1		EU738574.1		

Appendix C

We collected reflectance spectrometry measurements from 15 plumage regions of male and female museum specimens of 70 species of Galliformes. These same areas were visually categorized as either iridescent or non-iridescent and used for our visual assessment of sexual dichromatism. Line drawing by Kevyn Gammie.



Appendix D

Comparison of maximum likelihood BiSSE models for non-iridescent (state 0) and iridescent (state 1) under no constraints and varying parameter constraints for state changes in the galliform order. Starred constraints are significantly different from the no constraint model. ΔAIC is calculated from the model with the lowest AIC value. Models with ΔAIC less than or equal to 2 are considered equally likely (Burnham & Anderson, 2002).

model	constraints	lambda0	lambda1	mu0	mu1	q01	q10	parameters	lnLik	AIC	ΔAIC
no constraint	none	41.590	24.040	41.433	1.194e-04	6.000e-07	21.152	6	48.128	-84.26	2.00
constraint 1	lambda0 = lambda 1	30.074	30.074	29.660	11.071	0.016	16.331	5	47.137	-84.27	1.99
constraint 2*	mu0 = mu1	27.651	37.612	25.592	25.592	0.128	10.463	5	45.618	-81.23	5.03
Constraint 3*	q01 = q10	32.366	33.435	25.199	35.021	35.021	1.717	5	39.217	-68.43	17.83
constraint 4*	lambda0 = lambda1, mu0 = mu1	31.016	31.016	24.929	24.929	0.266	6.343	4	43.269	-78.58	7.68
constraint 5*	lambda0 = lambda1, q01 =q10	32.868	32.868	25.759	34.530	1.699	1.699	4	39.219	-70.43	15.83
constraint 6*	mu0 = mu1, q01 = q10	37.793	29.756	31.317	31.317	1.501	1.501	4	39.042	-70.08	16.81
constraint 7*	lambda0 = lambda1, mu0 = mu1, q01 = q10	30.911	30.911	24.717	24.717	1.001	1.001	3	37.894	-69.79	16.47
constraint 8	q01 = 0	41.598	24.041	41.442	2.917e-09	0.000	21.154	5	48.128	-86.26	0.00
constraint 9*	q10 = 0	38.928	30.397	31.921	37.887	1.916	0.000	5	37.810	-65.62	20.64

Appendix E

Comparison of maximum likelihood BiSSE models for solid (state 0) and hollow (state 1) melanosomes under no constraints and varying parameter constraints for state changes among iridescent galliform species. ANOVAs revealed none of the models were statistically different from each other. ΔAIC is calculated from the model with the lowest AIC value. Models with ΔAIC less than or equal to 2 are considered equally likely (Burnham & Anderson, 2002).

model	constraints	lambda0	lambda1	mu0	mu1	q01	q10	parameters	lnLik	AIC	ΔAIC
no constraint	none	24.986	9.282	21.050	5.825	1.016	1.945e-08	6	34.856	-57.71	2.71
constraint 1	lambda0 = lambda 1	18.977	18.977	13.457	17.971	2.023	2.170e-09	5	34.070	-58.14	2.29
constraint 2	mu0 = mu1	20.551	14.447	14.788	14.788	1.943	1.368e-09	5	34.451	-58.90	1.53
Constraint 3	q01 = q10	26.128	8.150	22.615	3.022	0.795	0.795	5	34.590	-59.18	1.25
constraint 4	lambda0 = lambda1, mu0 = mu1	18.773	18.772	14.313	14.313	1.538	1.456e-10	4	33.921	-59.84	0.59
constraint 5	lambda0 = lambda1, q01 =q10	18.895	18.895	14.086	15.931	1.522	1.522	4	33.249	-58.50	1.93
constraint 6	mu0 = mu1, q01 = q10	19.934	14.967	14.382	14.382	1.635	1.635	4	33.644	-59.29	1.14
constraint 7	lambda0 = lambda1, mu0 = mu1, q01 = q10	18.756	18.756	14.287	14.287	1.366	1.366	3	33.215	-60.43	0
constraint 8	q01 = 0	22.171	11.599	17.072	5.025	0.000	4.404	5	34.132	-58.26	2.17
constraint 9	q10 = 0	24.987	9.282	21.051	5.824	1.016	0.000	5	34.856	-59.71	0.72

Appendix F

Summary statistics for six parameter MCMC BiSSE models for the non-iridescent and iridescent analysis, and the solid and hollow melanosome analysis using galliform species. λ_0 is the speciation rate for state 0 (either non-iridescent or solid); λ_1 is the speciation rate for state 1 (either iridescent or hollow). λ_1/λ_0 is the speciation rate ratio; the closer this value is to 1 the more similar the speciation rate of the two states are. Q_{10} is the transition rate from state 1 to state 0 (loss of more derived trait – iridescence or hollow melanosomes); q_{01} is the transition rate from state 0 to state 1 (gain of more derived trait). The ratio of the extinction rate of the more derived trait (μ_1) to the speciation rate of the more derived trait (λ_1) indicate a higher rate of speciation than extinction. A diversifiction rate ratio of state 1 (r_1) to the diversification rate of state 0 (r_0) greater than 1 indicates that species with the innovation had higher rates of diversification.

binary states	λ_1/λ_0	prop. of steps with $\lambda_1 > \lambda_0$	q10/q01	prop. of steps with $q_{10} > q_{01}$	q_{10}/λ_1	μ_1/λ_1	λ_1-μ_1 (diversification rate, r_1)	r_1/r_0
non-iridescent vs. iridescent	1.027	0.533	33.456	1.000	0.613	0.307	18.611	6.673
solid vs. hollow melanosomes	0.623	0.181	1.475	0.556	0.299	0.821	2.392	0.394

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