Multistage Scenarios for the Evolution of Polymorphisms in Birds

by

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Abstract

One of the most striking characteristics of the animal kingdom is the extent of phenotypic differentiation between the sexes. Selection acts on the phenotype and sex dependent polymorphisms, such as sexual-size dimorphism, result from selection favouring different optima for males and females. Macroevolutionary patterns of morphological variation are inherently fascinating and warrant explanation because they contribute importantly to the phenotypic component of biodiversity. Understanding the evolution of these patterns is complicated because the majority of the genome is shared between males and females, and this could constrain the evolution of sex-specific phenotypes. In this thesis, I develop tools and use existing evolutionary theory to derive testable predictions regarding the evolution of a small but diverse set of morphological polymorphisms in birds. First, I develop tools, in the form of explicit phylogenetic hypotheses, to analyze the evolution of sexual-size dimorphism (SSD) within the order Galliformes (landfowl). I examine the indirect effect that sexual selection acting on males can have on female life history traits (egg size and clutch size), and the roles that sexual selection and divergence in the reproductive roles of males and females contribute to SSD. Second, I evaluate whether intersexual competition on the wintering grounds is implicated in the evolution of disproportionate bill-length dimorphism, which is common in migratory shorebirds (Charadriiformes). To do this, I test predictions derived from the niche differentiation hypothesis, using the western sandpiper (*Calidris mauri*) as a model shorebird differential migrants. Finally, I consider an enigma in avian reproductive biology, the evolution of an extreme form of intraclutch egg-size dimorphism that is unique to the six species of *Eudyptes* penguins (Sphenisciformes). Through these examples I provide evidence that apparently simple polymorphisms, such as SSD, often require multistage explanations.

Keywords: sexual dimorphism, niche differentiation, intraclutch egg-size dimorphism, *Eudyptes*, Galliformes, differential migration

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Chapter 1.

Introduction

Systematic morphological variation within species constitutes a class of phenotypic variation known as polymorphism. Sexual dimorphism refers to the specific case where morphological variation is sex dependent. Sexual-size dimorphism (SSD) is nearly ubiquitous in the animal kingdom; however, SSD does not evolve directly (Price 1984). SSD arises when selection favours different body-size optima for males and females (Price 1984). Once SSD has arisen, additional selection pressures can accentuate sex-dependent variation for a specific morphological character, resulting in disproportionate dimorphism for individual characters (Price 1984). As a consequence, the extent of intraspecific morphological differentiation between males and females can be pronounced. Macroevolutionary patterns of morphological variation are inherently fascinating and warrant explanation because they contribute importantly to the phenotypic component of biodiversity (Fairbairn et al. 2007). The genetic mechanisms underpinning sex-dependent phenotypic differentiation are complex because the majority of the genome is shared between males and females, and this could generate conflicts and constraints on the evolution of sex-specific phenotypes (Arnqvist and Rowe 2005; Fairbairn et al. 2007).

In this thesis, I test process-based explanations for some of the most prevalent morphological polymorphisms known in birds. Selection acts on the phenotype and the polymorphisms considered in this thesis are evaluated at this level. I do not measure selection directly, rather I identify proxies for the strength of selection and use these proxies as explanatory variables or I make comparisons to empirical null models intended to reflect the absence of selection. The first polymorphism that I consider is SSD, which takes two forms. The most common form of SSD is where the female is the larger sex, and this is referred to as female-biased SSD (Fairbairn et al. 2007). The alternate case, where the male is the larger sex, is referred to as male-biased SSD. Four

main hypotheses have been proposed to explain SSD, and they are not mutually exclusive. The first hypothesis, fecundity selection acting on females, has been proposed to explain female-biased SSD (Fairbairn et al. 2007). The second hypothesis, intrasexual competition among males for access to females, has been proposed to explain male-biased SSD (Darwin 1871). These first two hypotheses are incomplete: each only considers the effect of fecundity selection acting on one sex. Implicit in both of these hypotheses is the assumption that large body size is costly and that natural selection opposes the evolution of large size unless the costs are offset by fecundity gains. Sexual conflict theory addresses this deficit explicitly, proposing that SSD results from a difference in the effects of natural selection acting on survival and sexual selection acting on fecundity in males and females (Arnqvist and Rowe 2005). For simplicity I refer to this as the sexual conflict hypothesis but it really involves multiple hypotheses because the effects of natural selection and sexual selection are considered explicitly. The fourth hypothesis, intersexual competition for access to food resources, has typically been proposed to explain female-biased SSD, but its application is not exclusive to this case (Hedrick and Temeles 1989). Only the sexual conflict and intersexual competition hypotheses consider explicitly processes operating on males and females. SSD results from selection for different sex-specific body-size optima, and this suggests that processes affecting males and females must be considered together.

The second polymorphism that I examine is an example of disproportionate dimorphism of a functional morphological character used in foraging, bill length. Typically the direction of dimorphism for an exaggerated functional morphological character follows the direction of SSD, but this is not always the case (Hedrick and Temeles 1989). The intersexual competition hypothesis proposed above to explain SSD has also been proposed to explain disproportionate dimorphism of functional morphological characters used in foraging, whereby disruptive natural selection acts on bill length and results in disproportionate bill-length dimorphism. Sex-specific differences in foraging behaviour are often associated with disproportionate dimorphism of functional foraging characters, and together they constitute niche differentiation, which is why this hypothesis is often referred to as the niche differentiation hypothesis (Fairbairn et al. 2007). The final polymorphism that I examine is an example of intraclutch egg-size variation. Two hypotheses have been proposed to explain systematic intraclutch egg-size variation and

both are applicable to reproductive strategies where incubation commences before the last egg is laid, such that hatching is asynchronous. The brood-size reduction hypothesis suggests that if the food supply during chick rearing cannot be reliably predicted when the eggs are being laid that laying a small final egg can provide an adaptive strategy to facilitate brood-size reduction when food is scarce (Lack 1954). The insurance-egg hypothesis suggests that if fertility is low or if egg loss is common that it can be adaptive to lay more eggs than is optimal for the parents to provision (Dorward 1962). If it is common for all of the eggs to hatch, such that there are more chicks than the parents can successfully provision, then this scenario can favour intense sibling competition, potentially leading to the evolution of obligate siblicide, as a mechanism for brood reduction (Anderson 1989; Anderson 1990).

Evolution and diversification of avian polymorphisms

In this thesis I consider the evolution and diversification of polymorphism in three distinct taxonomic groups and at several taxonomic levels across the avian phylogeny. Formal statistical comparative analyses among species require an explicit phylogenetic hypothesis, which is used to account for shared ancestry and the inherent lack of statistical independence between species (Felsenstein 1985). Where necessary, I generated the phylogenetic hypotheses required to test predictions in a comparative framework and this became an appreciable portion of the thesis. The majority of the thesis involves constructing phylogenetic hypotheses for the order Galliformes (landfowl), and then using these phylogenetic hypotheses as analytical tools for examining the causes and consequences of SSD within the order. The Galliformes is composed of five families, 84 genera and > 280 species that are distributed across all of the continents with the exception of Antarctica (del Hoyo et al. 1994). Species-specific mean body mass within the Galliformes varies between 40 g and 8,000 g and, remarkably, the extremes of body size are represented within a single family, the Phasianidae. SSD tends to be male biased and, due to the prevalence of extravagant ornamentation within the Phasianidae, Darwin (1871) recognized the Galliformes as a model system for sexual selection over a century ago.

Next, I consider a distinct but related sex-dependent polymorphism, disproportionate bill-length dimorphism, which is common in shorebirds (Scolopacidae; sandpipers and allies; Jehl and Murray 1986). The Scolopacidae is one of 18 families in the Charadriiformes (auks, gull, shorebirds and allies), which includes approximately 350 species (del Hoyo et al. 1996). SSD is common and diverse within the Charadriiformes, with some taxa exhibiting female-biased SSD and other taxa exhibiting male-biased SSD (Székely et al. 2004). In addition, SSD in the Charadriiformes spans the entire range of SSD evident in birds (Székely et al. 2004). In a phylogenetic comparative analysis of SSD in the Charadriiformes, proxies of sexual selection that explained body mass and wing cord SSD could not account for bill length SSD (Székely et al. 2004). These results suggest that while sexual selection has been an important driver of SSD in the Charadriiformes, additional factors may be required to explain bill-length SSD (Székely et al. 2004). Many of the smaller sandpipers (< 200g) exhibit female-biased SSD, disproportionate bill length dimorphism and differential migration, such that males and females overwinter at different latitudes, on average. However, there can be extensive overlap in the winter distribution of the sexes, and this suggests that intersexual competition for access to food resources on the wintering grounds may have been an important driver in the evolution and diversification of disproportionate bill-length dimorphism in shorebirds.

The final polymorphism that I examine is intraclutch egg-size dimorphism in *Eudyptes* penguins. Like most penguin species, all six species of *Eudyptes* penguins have a determinate two-egg clutch (Williams 1995). In *Eudyptes*, one egg can be twice as large as the other (Williams 1995), making this the most extreme form of intraclutch egg-size dimorphism documented in birds (Slagsvold et al. 1984). Pronounced intraclutch egg-size dimorphism is exceptionally rare in birds with two-egg clutches (Slagsvold et al. 1984); however, in the cases where it does occur hatching is always asynchronous (Mock 1984). Pronounced intraclutch egg-size dimorphism occurs in two species of eagle (genus *Aquila*) with two-egg clutches (Slagsvold et al. 1984), and in both instances the first-laid egg is larger (Slagsvold et al. 1984). A larger first-laid egg in combination with asynchronous hatching is consistent with an adaptive strategy. Obligate siblicide has evolved in both species, and this is consistent with insurance against loss of the larger first-laid egg (Mock 1984). In the six species of *Eudyptes*

penguins the second-laid egg is always larger, and systematic loss of the smaller first-laid egg prior to hatching is common (Williams 1995). In the cases where the smaller first-laid egg is retained to hatching, it hatches approximately one day later than the larger second-laid egg via an exceptionally rare and poorly understood phenomenon termed reversed hatching asynchrony (St. Clair 1996). The many hypotheses proposed to explain the evolution of extreme intraclutch egg-size dimorphism in *Eudyptes* penguins have focused on adaptive explanations for the smaller first-laid A-egg; however, the primary candidate hypotheses, brood reduction (Lack 1954) and insurance against egg loss (Dorward 1962), have not received empirical support. In a review of the adaptive significance of intraclutch egg-size dimorphism in birds, Slagsvold et al. (1984) excluded penguins from their analyses specifically because the larger second-laid egg in *Eudyptes* spp. seemed to contradict theoretical predictions. Subsequently, St. Clair et al. (1995) reported that systematic loss of the smaller first-laid egg in *Eudyptes schlegeli* was the result of maternal egg ejection and argued that this constituted an exceptionally rare case of maternal infanticide. Given that egg size and clutch size are two of the best-studied life history traits, I used classical life history theory to derive testable predictions regarding the unique and extreme form of intraclutch egg-size dimorphism characteristic of *Eudyptes*.

Thesis Objectives

The title of the thesis begins with the words “multistage scenarios,” and by this I am suggesting that diverse and or complex polymorphisms are expected to have complex explanations. For example, the combination of small body size, female-biased SSD and disproportionate bill length dimorphism in many species of sandpipers (Jehl and Murray 1986) should not, and apparently does not have a single explanation (Székely et al. 2004). A complex phenotype, such as this, is expected to evolve sequentially, in a series of tractable steps. While this theme is not central to every chapter in this thesis, it is a unifying thread. Together, chapters 2-4 explore the Galliformes as a model system for sexual selection in a comparative phylogenetic context. Chapter 2 examines the impact of sexual selection, indexed by SSD and social mating system, on female life history traits, specifically body size, egg size and clutch size. Chapter 2 was a collaborative effort, and my primary contribution was the supertree

phylogeny used as the basis of the comparative analyses. Chapter 3 is a foray into statistical, molecular phylogenetics that substantially advances current knowledge of the phylochronology of the Galliformes as well as the diversification dynamics of this ancient bird order. Chapter 4 uses the molecular phylogeny inferred in Chapter 3 and exploits a novel form of evolved weaponry, tarsal spurs, in combination with data on mode of parental care to provide a trait-based explanation for the evolution of SSD in the Galliformes. Chapters 5 is an intraspecific study that tests predictions derived from the intersexual competition hypothesis to assess whether niche differentiation on the wintering grounds can explain differential bill-length dimorphism in a small-bodied differential migrant, the western sandpiper (*Calidris mauri*), with female-biased SSD. Chapter 6 presents a small-scale comparative analysis testing whether the unique and extreme intraclutch egg-size dimorphism characteristic of *Eudyptes* penguins can be explained by an interaction between a life history slowdown, associated with a transition to a pelagic life style, and canalization of a two-egg clutch.

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Chapter 2.

Can sexual selection drive female life histories? A comparative study on Galliform birds

Kolm, N., R.W. Stein, A.Ø. Mooers, J.J. Verspoor and E.J.A. Cunningham. 2007. Can sexual selection drive female life histories?: A comparative study on Galliform birds. Journal of Evolutionary Biology 20(2): 627-638.

N. Kolm designed the study, assembled the trait data, conducted the comparative analyses and drafted the majority of the manuscript. R.W. Stein assembled the source phylogenies for the genera-level supertree analyses, conducted the supertree analyses and drafted the associated portion of the manuscript.

Abstract

Sexual selection has been identified as a major evolutionary force shaping male life history traits but its impact on female life history evolution is less clear. Here we examine the impact of sexual selection on three key female traits (body size, egg size and clutch size) in Galliform birds. Using comparative independent contrast analyses and directional DISCRETE analyses, based on published data and a new genera-level supertree phylogeny of Galliform birds, we investigated how sexual selection [quantified as sexual size dimorphism (SSD) and social mating system (MS)] affects these three important female traits. We found that female body mass was strongly and positively correlated with egg size but not with clutch size, and that clutch size decreased as egg size increased. We established that SSD was related to MS, and then used SSD as a proxy of the strength of sexual selection. We found both a positive relationship between SSD and female body mass and egg size and that increases in female body mass and egg size tend to occur following increases in SSD in this bird order. This pattern of

female body mass increases lagging behind changes in SSD, established using our directional DISCRETE analysis, suggests that female body mass increases as a response to increases in the level of sexual selection and not simply through a strong genetic relationship with male body mass. This suggests that sexual selection is linked to changes in female life history traits in Galliformes and we discuss how this link may shape patterns of life-history variation among species.

Introduction

Sexual selection is an important driver of many of the most spectacular morphological traits found in the animal kingdom (for example see Andersson 1994). As such, sexual selection is most often emphasized as a driver of the evolution of male-specific traits because sexual selection often acts more strongly on males (Andersson 1994). However, recent studies have shown that sexual selection on male characters also can influence female life history traits, both directly (e.g. Cunningham and Russell 2000; Sheldon 2000; Kolm 2001) and indirectly, potentially through genetic correlations for traits among the sexes (e.g. Weatherhead and Teather 1994; Young 2005). Here we focus on three female traits that are closely linked to fitness in a range of taxa: body mass, egg size and clutch size (e.g. Roff 1992; Stearns 1992; Heath and Blouw 1998; Roff 2002). Recent studies suggest that these traits are particularly strong candidates for being influenced by sexual selection (review by Sheldon 2000; Kolm 2001; review by Kolm and Ahnesjö 2005).

Birds have been studied extensively in relation to both life history evolution and sexual selection. As birds show high variation in these traits, they are well suited for analyses looking at broad scale evolutionary patterns. This has been done both across major lineages of birds (e.g. Bennett and Owens 2002) as well as on a finer, family, genus or species level scale (e.g. Blackburn 1991a; Weatherhead and Teather 1994; Lindenfors et al. 2003; Figuerola and Green 2006; Martin et al. 2006); however, the evolutionary relationships between body size, egg size and clutch size, and the relationships of these with sexual selection, remain unclear. Although most studies agree that female size is positively related to egg size (Bennett and Owens 2002 and references therein, Martin et al. 2006), the relationship to clutch size varies: Bennett and

Owens (2002) found no relationship between body size and clutch size across major lineages of birds whereas Martin et al. (2006) recently found a negative relationship between body size and clutch size among passerines. Although some studies have found a negative relationship between egg size and clutch size (Blackburn 1991a,b; Figuerola and Green 2006; Martin et al. 2006) indicating a trade-off between the two traits, other studies have failed to detect such a relationship (e.g. Saether 1987; Rohwer 1988; Poiani and Jermiin 1994; Bennett and Owens 2002).

The two most common measures of sexual selection are sexual size dimorphism (SSD) and social mating system (MS) (Bennett and Owens, 2002). The most common type of SSD in birds, where the male is larger than the female, is believed to be generated under sexual selection because of selection for increased male size, or more rarely because of a decrease in female size (Andersson 1994). As polygynous species tend to involve more male–male competition [which selects for increased male size (Andersson 1994)] when compared with monogamous species, it is not surprising that level of polygyny and SSD are related (e.g. Owens and Hartley 1998; Dunn et al. 2001, Bennett and Owens 2002). Regarding the link between measures of sexual selection and life histories, Weatherhead and Teather (1994) found that egg size increased with increased levels of SSD across six groups of birds and Figuerola and Green (2006) recently found the same pattern in Anseriformes. However, Bennett and Owens' (2002) larger scale analyses across major lineages of birds did not detect any relationship between sexual selection and female life history traits.

Here we examine the impact of sexual selection on female size, egg size and clutch size among Galliform birds, the group from which all our commercially important poultry and game birds are derived. Importantly here, Galliform birds produce precocial young and hence any trade-off between egg size and egg number is less likely to be confounded by the later evolution of extensive parental care strategies which can shift investment from egg allocation to parental allocation stages. Moreover, Galliform birds show a substantial variation in life history traits as well as levels of sexual selection and are therefore well-suited for comparative analyses on the link between sexual selection and life histories.

Comparative analyses that control for shared ancestry between species are often used in order to investigate covariation among different life history traits in evolutionary biology (see e.g. Harvey and Pagel 1991). Together with the analysis of independent contrast (Felsenstein 1985), the *Discrete* method (e.g. Pagel 1994; Pagel 1997; Pagel 1999), based on a simple Markov model for trait evolution, is particularly appealing for these types of studies because it allows for analyses of directionality of evolution among correlated traits. This means that it is possible not only to investigate if traits evolve in relation to other traits but also to investigate if changes in one trait tend to precede changes in another trait and so to order the events involved in correlated evolution. Different orderings may support different causal explanations. *Discrete* has been successfully used to disentangle the association among various traits in different taxa (e.g. birds: coloniality, territoriality and habitat, Rolland et al. 1998; mate fidelity and site fidelity, Cézilly et al. 2000; breeding strategy, breeding range, diet and egg size, Krüger and Davies 2002; fish: body size, egg size and clutch size, Kolm et al. 2006). Here, we use this method to disentangle the direction of events in scenarios where sexual selection may drive the evolution of life histories.

To investigate this in Galliformes, we (R.W.S., J.J.V. and A.Ø.M.) assembled a new genus-level supertree for Galliformes and we established a database of life history traits and various measures of sexual selection for the 79 genera from this group. The complex associations between different life history traits in birds make it important to investigate the link between life history traits prior to investigating any link between sexual selection and life histories. Hence, we first investigated the relationships between female size, egg size and clutch size using both analyses of raw data and phylogenetically independent contrasts (PICs). We then examined if MS was related to the degree of SSD and how SSD was linked to these female traits using both correlation analyses (based on raw data and contrasts) as well as directional DISCRETE analyses.

Methods

Data compilation

We constructed a database for 82–214 species (32–63 genera) for which we could find information pertaining to any of the characters of interest: these included male body weight (grams: 180 species from 60 genera), female body weight (grams: 164 species from 53 genera), clutch size (number of eggs in clutch: 214 species from 63 genera), egg size (cm^3: 74 species from 40 genera) and social MS (82 species from 32 genera). These data originated from Cramp and Simmons (1980); Dunning (1993); del Hoyo et al. (1994) and Geffen and Yom-Tov (2001). The data for the variables under investigation were calculated using the average of the species for which we had data and all analyses were performed at the genus level. Body size was quantified as body mass. Egg size was quantified as total egg volume but sometimes, when only measures of egg length and width could be obtained from the literature, we used the Hoyt (1979) egg volume equation with the constant set to 0.5025 [average constant over Galliform genera given in Hoyt (1979)] to calculate egg volume. Clutch size was estimated as number of eggs laid per reproductive event. SSD was calculated using the common $\log_{10}$ (male weight/female weight) calculation (e.g. Fairbairn 1997; Young 2005). For a genus where males are larger than females this yields a positive value for this measure of SSD. For MS, we used a majority rule and scored a genus according to the majority of the species for which we had access to data. For simplicity, we only scored genera as monogamous or polygamous. This means that genera normally considered promiscuous will fall into the polygamous category in our analyses. As we only use MS as a measure of sexual selection, this procedure should not introduce any biases. The only genus recognized as polyandrous (genus *Alectura*) was removed from all analyses of MS to ensure a binary state for this variable. For the *Discrete* analyses, which require binary coded characters, we transformed the continuous variable SSD into a binary variable by scoring all genera above the mean (across all genera) as 1 and all genera below the mean (across all genera) as 0. For female body mass, egg size and clutch size we scored all genera above the mean as 1 and all genera below the mean as 0.

Supertree phylogeny

We collected 40 previously published or 'in press' studies from a variety of sources that reported 72 phylogenetic hypotheses, hereafter referred to as source trees (STs), for genera-level matrix representation with parsimony (MRP) supertree analyses. These studies were screened for data duplication and quality prior to a more rigorous evaluation of their contained STs (see below). Three of these studies were excluded because of complete data duplication with subsequent, more inclusive studies by the same authors. In all instances, we preferred more recent molecular studies using cladistic methodologies (deemed higher quality) to older morphometric studies using either clustering algorithms or no formal analysis; this resulted in the exclusion of three additional studies. With one exception (see below), we limited our selection to studies published after 1966.

We used the 'garbage in, garbage out' protocol of Bininda-Emonds et al. (2004) as our criteria for ST selection. We selected the most comprehensive ST presented in each study; the only exceptions to this occurred when multiple STs were reported and there was data duplication involving the most comprehensive ST. Independence among STs was assessed conservatively, STs were excluded on the basis of relatively minor data duplication among studies. We identified 17 independent STs (Holman 1961; Crowe 1978; Gutiérrez et al. 1983; Helm-Bychowski and Wilson 1986; Sibley and Ahlquist 1990; Randi et al. 1991; Zink and Blackwell 1998; Armstrong et al. 2001; Birks and Edwards 2002; Dimcheff et al. 2002; Drovetski 2002; Pereira et al. 2002; Sorenson et al. 2003; Chubb 2004; Nishibori et al. 2004; Pereira and Baker 2004; Crowe et al. 2006). In addition, we also identified two nucleotide sequences, mitochondrial control region and cytochrome *b*, that were recycled extensively across a further 16 studies; this resulted in two sets of nonindependent STs, one for each of these mitochondrial sequences. For these two sets, we followed the recommendation of Bininda-Emonds et al. (2004), and conducted an interim 'mini supertree' analysis on all of the available STs (control region: Fumihito et al. 1995; Kimball et al. 1997; Kimball et al. 1999; Lucchini et al. 2001; Drovetski 2002; and cytochrome *b*: Kornegay et al. 1993; Ellsworth et al. 1996; Kimball et al. 1997, Kimball et al. 1999, Kimball et al. 2001; Bloomer and Crowe 1998; Munechika et al. 1999; Gutiérrez et al., 2000; Armstrong et al. 2001; Bush and Strobeck 2003; Zhan et al. 2003; Shibusawa et al. 2004; Wen et al. 2005) and included the

resulting ‘mini supertrees’ as STs in the main supertree analyses. Because of insufficient overlap among taxa, there were four, rather than two, resulting ‘mini supertrees’. In an attempt to balance the quality of the included STs with taxonomic coverage, we included one osteological taxonomy of the Odontophoridae (Holman 1961); it was highly congruent with two less complete STs from molecular studies that address relationships among genera (Zink and Blackwell, 1998) and also with other families (Gutiérrez et al. 1983). Prior to coding the STs for MRP, nodes with published bootstrap support values < 50% were collapsed.

Wilkinson et al. (2005) recently compared the properties of 14 supertree methods and demonstrated systematic biases in the way conflicts are resolved among STs: binary coding tends to resolve conflicts in favour of unbalanced STs, whereas additive binary coding tends to resolve conflicts in favour of balanced STs (Wilkinson et al. 2005). We therefore used both coding methods [in RADCON (Thorely and Page 2000)] to generate matrix representation of STs: binary coding (Baum 1992; Ragan 1992) and Purvis’ modification of this method (additive binary coding; Purvis 1995), which eliminates redundancy inherent to binary coding (Purvis, 1995). Our MRP ‘mini supertree’ and main supertree analyses were conducted using the parsimony ratchet (Nixon 1999), which increases the efficiency of heuristic searches for candidate trees, as implemented by PAUPRAT (Sikes and Lewis 2001) in PAUP* (Swofford 2002). STs were weighted uniformly, that is to say that the initial weight of all characters was set to 1, which is the default setting of PAUPRAT (Sikes and Lewis 2001). For each of the MRP matrices we ran 30 independent searches consisting of 200 iterations, with 15% of the characters perturbed between iterations. After the 30 independent searches we extracted the set of optimal candidate trees (shortest length) and removed duplicates trees. The ratchet searches returned 893 and 338 unique optimal candidate trees for the binary coded and the additive binary coded STs respectively. We generated the 50% majority rule consensus supertrees from these two sets of unique optimal candidate trees in PAUP*.

The 50% majority rule consensus supertrees from the two coding methods were highly congruent and both contained a large polytomy associated with the most recent radiation, the Phasianidae. Although Numididae was consistently placed as sister to Odontophoridae and Phasianidae in both 50% majority rule consensus supertrees, the

support for this node was relatively weak (binary coding: 58% and additive binary coding: 54%). To account for this family-level uncertainty, we resolved both candidate supertrees (binary and additive binary) in each of two ways: with Odontophoridae as sister to Numididae and Phasianidae, and with Numididae as sister to Odontophoridae and Phasianidae. Although highly congruent, minor discrepancies did exist between the supertree topologies. So, we consulted the underlying STs, and, in all instances, the supertree constructed from additive binary coded STs matched the STs better. Because of the lack of redundancy in coding, additive binary coding is arguably a better representation of the STs than binary coding (Purvis 1995). We performed the comparative analyses on both candidate supertrees and the results did not differ, so we only present results from analyses based on the supertree constructed from additive binary coded STs.

We attempted to provide resolution to the polytomy encompassing the Phasianidae, as follows. First, we assumed monophyly for each unambiguous branch of the polytomy, and resolved discrepancies between the supertree topologies conservatively. As a result, *Alectoris*, *Pternistis*, *Rollulus*, (*Xenoperdix* with *Arborophila*), and (*Coturnix* with *Margaroperdix*) were each considered as additional branches. Because of inconsistent or ambiguous affinities among the STs, *Meleagris*, *Perdix*, *Tragopan*, (*Afropavo* with *Pavo*), and (*Rheinardia* with *Argusianus*) were also considered as separate monophyletic branches. This increased the size of the polytomy to 18 branches. We then obtained sequence data from GenBank for six genes or introns: three mitochondrial (cytochrome *b*, ND2 and 12S rDNA) and three nuclear (ovomucoid intron G, WPG pseudogene and zona pellucida C). For each sequence, we generated a consensus sequence for each of the 18 branches; this facilitated a more thorough search of tree space at the level of the polytomy. We made a global alignment for all of the sequences for each gene in kPrank (Higgins et al. 2005; Loytynoja and Goldman 2005), using a guide tree imported from ClustalX (Thompson et al. 1997). From this global alignment, we then generated a consensus sequences for each branch of the polytomy. Preliminary work revealed that an 80% threshold for representation in the consensus sequence resulted in > 99% identity to ancestral sequences inferred using Bayesian methods (results not shown). Ambiguous sites in the consensus sequences, i.e. when no single nucleotide met the 80% threshold, were represented by the

corresponding IUB DNA symbol (Cornishbowden 1986). We used MRMODELTEST 2.2 (Nylander 2004) to determine the best model of nucleotide evolution for each partition. Based on a concatenated partitioned alignment of all six sequences and with *Numida meleagris* as outgroup, we inferred a phylogeny for these 18 clades using MRBAYES 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). We ran four chains simultaneously in a Metropolis coupled MCMC search of tree space in two independent iterations of 10 million generations, using default settings. After a burn in of 2.5 million generations, we sampled trees every 1000 generations; this resulted in 7500 candidate trees from which we constructed a 50%majority rule consensus.

We grafted the resulting genera-level phylogeny of the Phasianidae onto the supertree constructed from the additive binary coded STs (Figure 2.1C), producing a composite supertree phylogeny (Figure 2.1B). From the composite supertree phylogeny, we also generated a fully resolved supertree topology based on our 'best informed guess' (BIG) of the remaining unresolved relationships (Figure 2.1A). Finally, we allowed for the same family-level uncertainty in the relationships among Numididae, Odontophoridae and Phasianidae, resolving both the composite and BIG phylogenies in each of the two possible ways.

Analyses

Bivariate contrast analyses on the relationships between all variables were performed both using raw data and Model 1 regression through the origin using PICs (Felsenstein 1985). For the analysis of the relationship between egg size and clutch size we also controlled for female body mass by including all three variables in a multiple regression including the 33 genera for which we had data for all three variables. All branch lengths were set equal to one for the PIC analyses and polytomies were resolved to zero-length branch lengths for the analyses based on the consensus trees (the BIG trees were fully resolved). We then tested for correlations between contrasts and their SD to check whether branch length transformations were needed to avoid type I error (Diaz-Uriarte and Garland 1998). As we did not detect any relationships between absolute values for the contrasts and their SD for any of the analyses, no transformations were needed. As our supertree analyses generated eight different trees [two fully resolved BIG trees based on the additive binary coded supertree (Figure 2.1A),

two composite super trees with the additive binary supertree (Figure 2.1B), two supertrees based on additive binary coding (Figure 2.1C) and two supertrees based on binary coding (not presented here)], we performed all analyses on all eight trees to investigate if our analyses were sensitive to which tree we used. As the results were similar (i.e. no results changed from significance to nonsignificance or vice versa) with only two exceptions (see Results) regardless of which tree was used, we only report the results from the phylogenetic analyses based on the BIG tree. For the analysis of the relationship between SSD and MS, the low sample size did not allow for a phylogenetically independent matched pairs analysis (see Harvey and Pagel 1991) as it only yielded very few matched pairs. Instead, we used a normal ANCOVA with SSD as the dependent continuous variable and MS (monogamy or polygamy) as a categorical independent variable and mean body mass (sexes pooled) as a covariate for the raw data at the genus level. This allowed us to estimate the relationship between these variables while controlling for body mass. All data were $\log_{10}$ transformed prior to raw data analyses and before calculations of independent contrasts to ensure normality. Independent contrasts were calculated using the PDAP:PDTREE module within Mesquite (Midford et al. 2002; Maddison and Maddison 2004).

For the analyses of directional evolution of SSD in relation to female life history traits, we used DISCRETE (4.0) (Pagel 1994; Pagel 1997). This program is based on a Markov model for trait evolution and allows for estimation of ancestral states, investigation of correlated evolution between two traits, investigation of the directionality of changes in traits, and how changes in one trait precedes changes in another trait. A likelihood ratio test is used to compare the maximum likelihood fits of a model that only allows for independent evolution of two traits to a model that allows for dependent evolution between two traits. The likelihood ratio test statistic is χ^2 distributed with d.f. = 4 for the comparison between the independent and the dependent model (Pagel 1997).

One can investigate the pattern of co-evolution for a pair of traits through investigation of the relative magnitudes of their joint transition rates (see Figure 2.2). So, for example, one can ask which path (upper vs. lower) from ‘low-small’ to ‘high-large’ is most likely on the data. One approach (see e.g. Cézilly et al. 2000; Kolm et al. 2006) is to ask which of the eight joint rates (represented by the arrows in Figure 2.2) are indistinguishable from zero, using a likelihood ratio test of the nested models (focal rate

= 0) vs. (focal rate = ML estimate). Rates that are indistinguishable from zero suggest that these paths are unlikely. If one can identify the ancestral states (i.e. which box in Figure 2.2 is ancestral), a full description of the likely evolutionary paths through time is possible.

Following transformation of female body mass, egg size and clutch size into discrete characters, body mass and egg size showed a perfect relationship (i.e. for the 34 genera for which we had data on these traits, all genera with larger than average females had larger than average eggs and vice versa). As DISCRETE assumes no simultaneous changes in two traits, we could thus not disentangle the directional evolution of these two traits in relation to each other. Moreover, this perfect correlation also meant that we only performed a discrete analysis on SSD in relation to female body mass. As the bivariate contrast analyses did not show any relationship between female body mass and clutch size, we did not perform any DISCRETE analysis for this combination of traits. Further, although it would be very interesting to perform a DISCRETE analysis on clutch size in relation to egg size to disentangle the evolution of these two traits for Galliformes (as done for cichlid fishes by Kolm et al. 2006), DISCRETE requires larger sample sizes (N. Kolm, personal observation) than we had for robust tests. To investigate whether the relative frequency of trait values might affect our results (Nosil and Mooers 2005) for our data, we randomized the distribution of female body mass and SSD (from Figure 2.5) across one of our BIG trees 100 times and then performed directional DISCRETE analyses to investigate how often chance alone would yield the same result as from our DISCRETE analyses based on the actual transitions in the tree. Only one of our 100 randomized datasets produced the same significant set of transitions, suggesting this was not a problem.

Results

Analyses based on raw data

Genera with higher female body mass had larger eggs (Figure 2.3A). There was a nonsignificant trend suggesting a negative relationship between female body mass and clutch size (Figure 2.3B). Egg size was negatively correlated to clutch size (Figure

2.3C), and this result was robust also when we controlled for female body mass using a multiple regression analysis (Multiple r^2 = 0.91, $F_{2,30}$ = 148.1, p < 0.0001; partial r = -0.63, t_{30} = 4.5, p = 0.0001). Both female body mass and egg size were positively related to SSD (Figure 2.3D,E). Clutch size was independent of SSD (Figure 2.3F). Polygynous genera had higher levels of SSD than monogamous genera [polygynous genera (mean SSD ± SE): 0.15 ± 0.02; monogamous genera: 0.06 ± 0.02, $F_{1,21}$ = 10.3, p < 0.01]. This result remained statistically significant also when analysed using an ANCOVA with the mean body mass of males and females as a covariate ($F_{1,20}$ = 6.0, p< 0.05).

Phylogenetic analyses

The PIC analyses supported those on the raw data. Genera with higher female body mass had larger eggs and egg size was significantly negatively related to clutch size for all but one of the BIG trees (Figure 2.4A,C). This negative relationship held consistently across analyses for all trees when controlling for female size in a multiple regression analysis (Multiple r^2 = 0.87, $F_{2,30}$ = 104.9, p < 0.0001; partial r = -0.45, t_{30} = 2.8, p < 0.01). However, there was no significant relationship between female body mass and clutch size (Figure 2.4B). Female body mass and egg size were positively related to SSD (Figure 2.4D,E) but again, there was no relationship between clutch size and SSD (Figure 2.4F).

Few contemporary Galliform genera consisted of large-bodied females with low SSD or small-bodied females with high SSD after these variables were transformed into binary characters (Figure 2.5). The DISCRETE analysis of female body mass and SSD therefore confirmed the contrast analyses on the relationship between these variables. The dependent model provided a better fit than the independent model, which is consistent with correlated evolution of these two traits (the log-likelihood for the independent model was -64.1 compared with -58.1 for the dependent model, LR = 11.9, p < 0.05). The 'local' ancestral state (Pagel 1999) for female body mass could not be determined with certainty (high female body mass: 58% posterior probability; low female body mass: 42% posterior probability; LR = 0.63, d.f. = 1, p = 0.43). The ancestral state for SSD could also not be determined with certainty although the trend suggested that the ancestral state was more likely to be low SSD (high SSD: 21% probability; low SSD: 79%; LR = 2.1, d.f. = 1, p = 0.13).

The evolutionary pathways between female body mass and SSD in relation to each other is presented in a flow diagram (Figure 2.6). The results from this analysis show that the most likely path from small-bodied females with low SSD towards a large body with high SSD is via the bottom path, such that SSD increases first. The only P-value that changed (i.e. from $p < 0.05$ to $p > 0.05$ or vice versa) depending on which tree was used across all analyses was for the transition between a large bodied female with high SSD towards a small bodied female with high SSD [i.e. transition q_{34} (Figure 2.6) changed from $p < 0.05$ for all other trees to $p = 0.10$ for one of the BIG trees]. However, the transition rate parameter for this transition was higher than the lowest significant transition rate parameter for any other transition also for this tree (see Kolm et al. 2006 for discussion on this). We therefore suggest that this transition is most likely to be common. In contrast, the most likely route for large bodied females with high SSD towards a small body with low SSD seems via an initial decrease in body mass. Hence, increases in female body size have followed after increases in the levels of sexual selection resulting in increased SSD whereas decreases in SSD have only followed after decreases in female body size. As all genera with large bodied females also lay large eggs and vice versa (see comment on this in the Methods), it means that the DISCRETE analyses would yield similar results if female body mass was exchanged with egg size.

Discussion

Our results showed that egg size is more strongly related to female body mass in Galliformes than is clutch size. Sexual selection was strongly linked to female body mass and egg size in this group and our DISCRETE analysis showed that increases in female body mass and egg size most likely occurred following initial increases in SSD. This suggests that sexual selection has been an important determinant in driving the evolution of these female traits and that these traits have not just been driven by a genetic correlation between male and female body size. Egg size and clutch size were negatively related suggesting a trade-off between these traits in this group.

Our analyses of the interactions among these female traits show, in agreement with many other studies on birds, a strong positive relationship between egg size and female size in Galliformes. Despite the generality of this pattern across taxa (e.g. Roff

1992), the ultimate causes to this relationship are still poorly known. However, physiological limitations may hinder small species from evolving large eggs, as has been suggested in cichlid fishes (Kolm et al. 2006). Also, the egg to body size ratio is generally high in Galliformes, as might be expected in a group that produces precocial young, making them more likely to reach their physiological limit than many other avian groups.

Our analysis of SSD in relation to MS indicate that SSD indeed is a good measure of the level of sexual selection in this group, particularly as this result held also after controlling for female size (Björklund 1990; Bennett and Owens 2002). Both female body mass and egg size were positively related to SSD in agreement with other studies (Weatherhead and Teather 1994; Figuerola and Green 2006). However, our DISCRETE analysis suggests that increases in sexual selection have preceded increases in female size. Although we could not establish the ancestral states of female body mass and SSD with certainty, we found a trend suggesting that the ancestral Galliform genus had a low level of SSD. Hence, as can be seen in Figure 2.6, the most likely route from a small female body mass to a large female body mass (and small egg size to large egg size) is via an increase in SSD. However, the opposite route, from the combination of a large female with high SSD to that of a small female body with low SSD is also possible (Figure 2.6) and indeed may often occur by changes in size preceding changes in SSD. These two routes are consistent with the idea that evolutionary transitions happen relatively freely between these combinations of states. Moreover, because our directional analysis suggests that because increases in female size have occurred after and not only at the same time as increases in SSD, female body mass likely does more than just co-vary with male size through a strong genetic correlation (e.g. Weatherhead and Teather 1994). Together with our results on the life history interactions, this points towards the Galliform genera having evolved along a continuum between two strategies: (1) genera with low levels of sexual selection, small females, large clutches and small eggs and (2) genera with high levels of sexual selection, large females, small clutches and large eggs. Our dataset is somewhat limited as it does not include data on the number of clutches that are produced per year. This additional variable would be beneficial to include in future studies. However, we believe that our description of the evolution along this continuum is at least indicative for Galliformes. Although it is well

known that birds have evolved along a slow–fast continuum and that the positioning along this continuum is related to body size and egg size (fast: small body size, small eggs, fast reproduction; slow: large body size, large eggs, slow reproduction) (e.g. Roff 1992; Roff 2002 and references therein), we are not aware of anyone implicating sexual selection as an evolutionary driver affecting movement along this particular continuum.

Why might female body mass increase after increases in SSD if not only through a strong genetic correlation between female body mass and male body mass? Given the interaction between female size, egg size and clutch size in Galliformes, we cannot be certain whether it is female body size per se or positive selection for egg size (and/or negative selection for clutch size) that covaries with increases in SSD. For instance, increased levels of sexual selection in males often co-vary with decreases in male care in birds (e.g. Andersson 1994 and references therein). Precocial chicks will need a larger egg investment and if smaller clutches of larger eggs require less care than larger clutches of small eggs, increases in SSD could well co-vary with changes in MS from monogamy to polygamy (as suggested by our analysis of SSD in relation to MS). Hence, for genera evolving from monogamy to polygamy, increasing body mass and egg size at the cost of clutch size could be a way for females to maximize offspring success when minimal paternal care is available. Future analyses on systems where data on paternal care is readily available could address how changes in these female traits may have occurred along changes in paternal care to test this hypothesis. An alternative explanation that we find interesting is that increases in SSD may lead to increases in female–female competition and hence selection for increased female size. This hypothesis was originally put forth by Langston et al. (1990) who suggested that a major cost of polygyny for females is competition for resources. Hence, increases in SSD (coupled to increases in the level of polygyny) may lead to increased competition for resources among females, which in term selects for increased female size if larger females are better competitors (Langston et al. 1990). Supporting this, such a pattern has been found in red-winged blackbirds (*Agelaius phoeniceus*; Langston et al. 1990) and also in other groups of birds (e.g. dunnocks, *Prunella modularis*; Langmore et al. 2002).

Few studies have detected a negative relationship between egg size and clutch size in birds, which raises the question of why it is evident in Galliformes. Galliform birds

are precocial with relatively little post-hatching parental care and the majority of a female's investment goes into eggs, which tend to be disproportionately large compared with those laid by altricial birds. Building on the arguments by Lack (1967), we suggest that this causes a constraint in resources which cannot be mediated at the parental care stage (as can occur in altricial species) leading to a clear trade-off between egg size and egg number in this group of birds. Supporting this, negative relationships between egg size and egg number in birds have in fact most often been found in precocial groups of birds (Lack 1968; Blackburn 1991a; Rohwer 1991; Figuerola and Green 2006; this study; but see Blackburn 1991b). Given the differences between different studies of different groups of birds, future broad scale analyses, using similar data sets to that of Bennett and Owens (2002) or meta-analyses, would prove fruitful to identify the ultimate causes to why some, but not all, groups of birds show a trade-off between egg size and egg number (see also Blackburn 1991b and Martin et al. 2006, for discussion on this). Predation should also be considered more carefully.

To conclude, based on a new genus level supertree of Galliformes and DISCRETE analyses, our results suggest that sexual selection leading to higher levels of SSD has been an important driver of female body mass and egg size in this group. Moreover, our results show that these increases in female size are not simply by-products of selection for increased male size through a strong genetic correlation. We suggest that future studies should incorporate sexual selection to a much higher degree in order to fully understand the reasons for the extreme variation in female life histories among contemporary taxa.

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Figures

Figure 2.1. Genera-level supertrees for the Galliformes based on additive binary coding of source trees: fully resolved 'best informed guess' topology (A), composite supertree phylogeny (B), and 50% majority rule consensus supertree (C). Each of the three topologies was also resolved so that the Odontophoridae was sister to the Numididae and the Phasianidae, by switching the placements of the Odontophoridae and the Numididae at the node marked with an asterisk (*).

Figure 2.1A

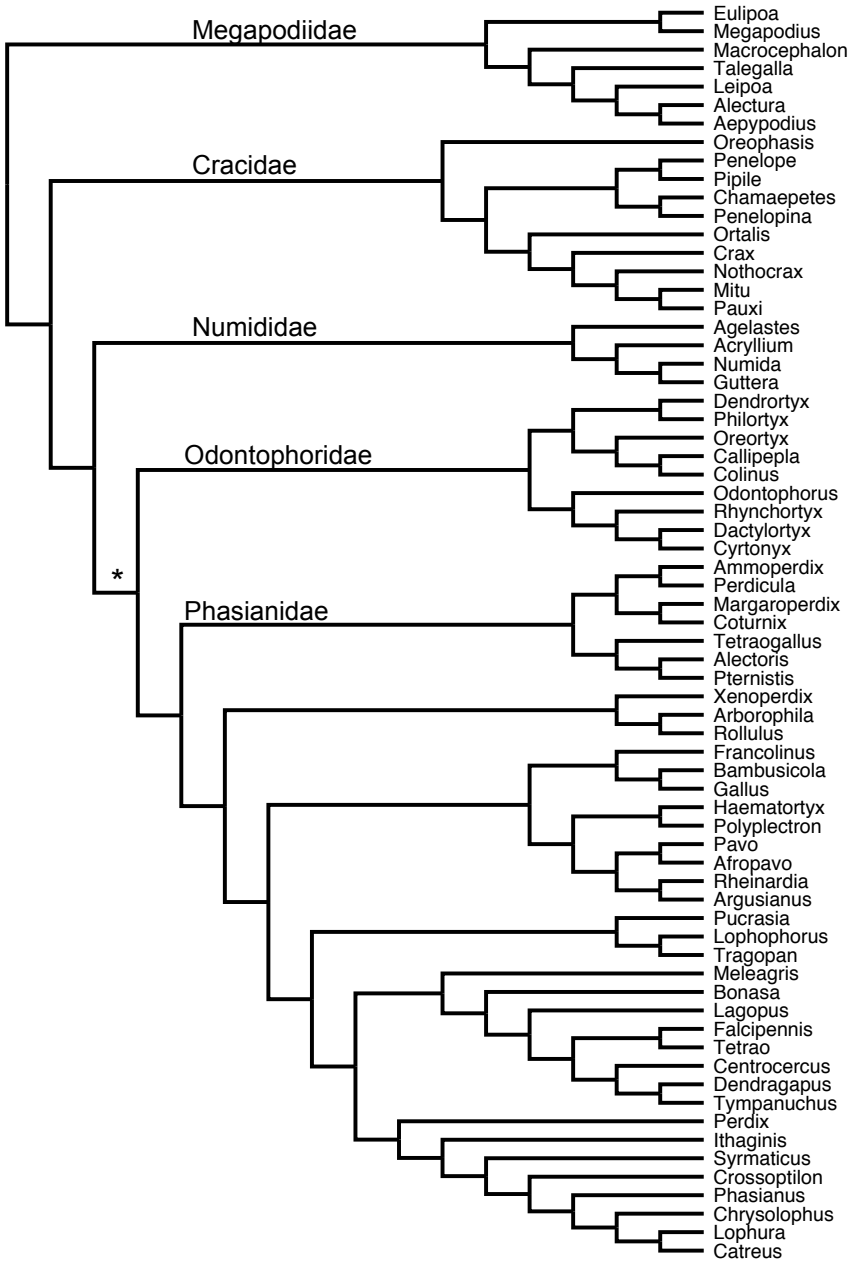


Figure 2.1B

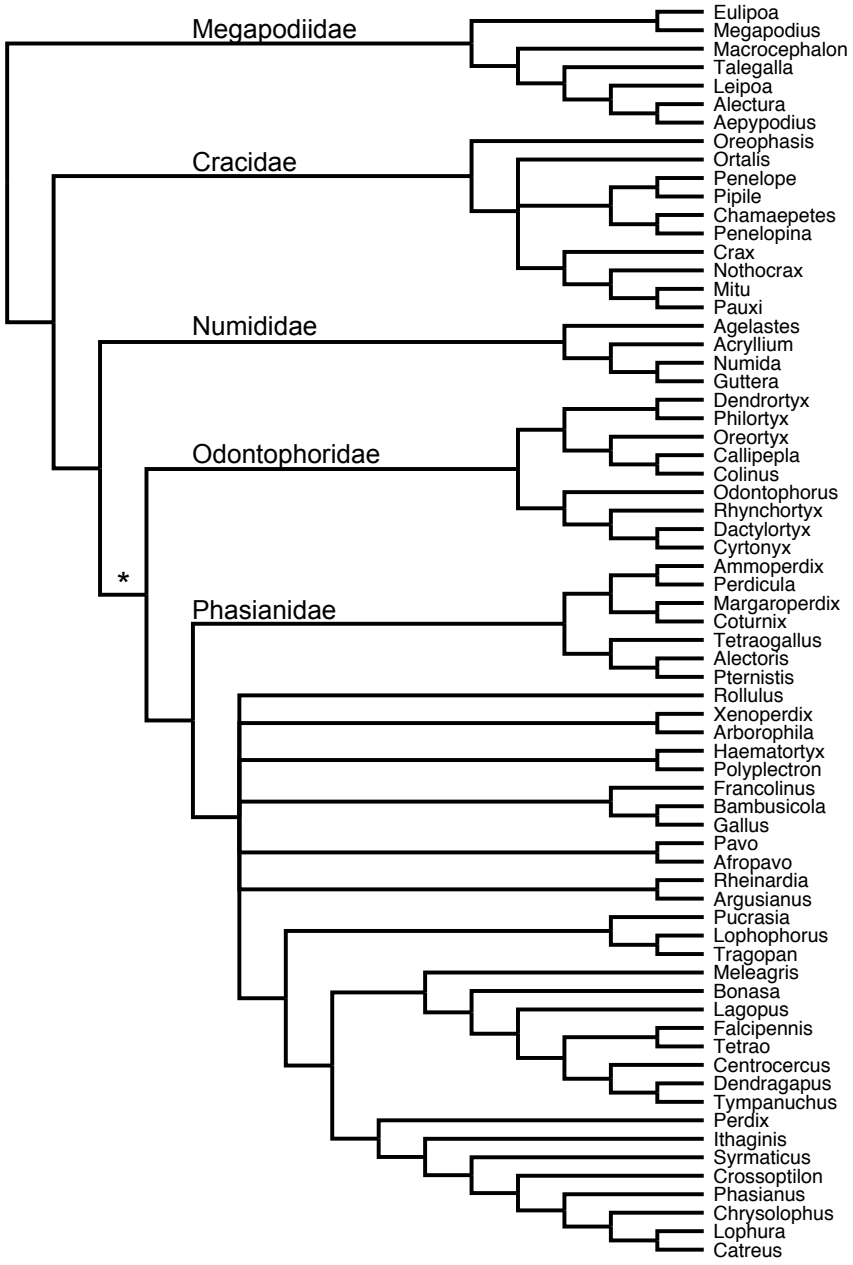

Megapodiidae
Cracidae
Numididae
Odontophoridae
Phasianidae
*
Eulipoa
Megapodius
Macrocephalon
Talegalla
Leipoa
Alectura
Aepypodius
Oreophasis
Ortalis
Penelope
Pipile
Chamaepetes
Penelopina
Crax
Nothocrax
Mitu
Pauxi
Agelastes
Acryllium
Numida
Guttera
Dendrortyx
Philortyx
Oreortyx
Callipepla
Colinus
Odontophorus
Rhynchortyx
Dactylortyx
Cyrtonyx
Ammoperdix
Perdicula
Margaroperdix
Coturnix
Tetraogallus
Alectoris
Pternistis
Rollulus
Xenoperdix
Arborophila
Haematortyx
Polyplectron
Francolinus
Bambusicola
Gallus
Pavo
Afropavo
Rheinardia
Argusianus
Pucrasia
Lophophorus
Tragopan
Meleagris
Bonasa
Lagopus
Falcipennis
Tetrao
Centrocercus
Dendragapus
Tympanuchus
Perdix
Ithaginis
Syrmaticus
Crossoptilon
Phasianus
Chrysolophus
Lophura
Catreus

Figure 2.1C

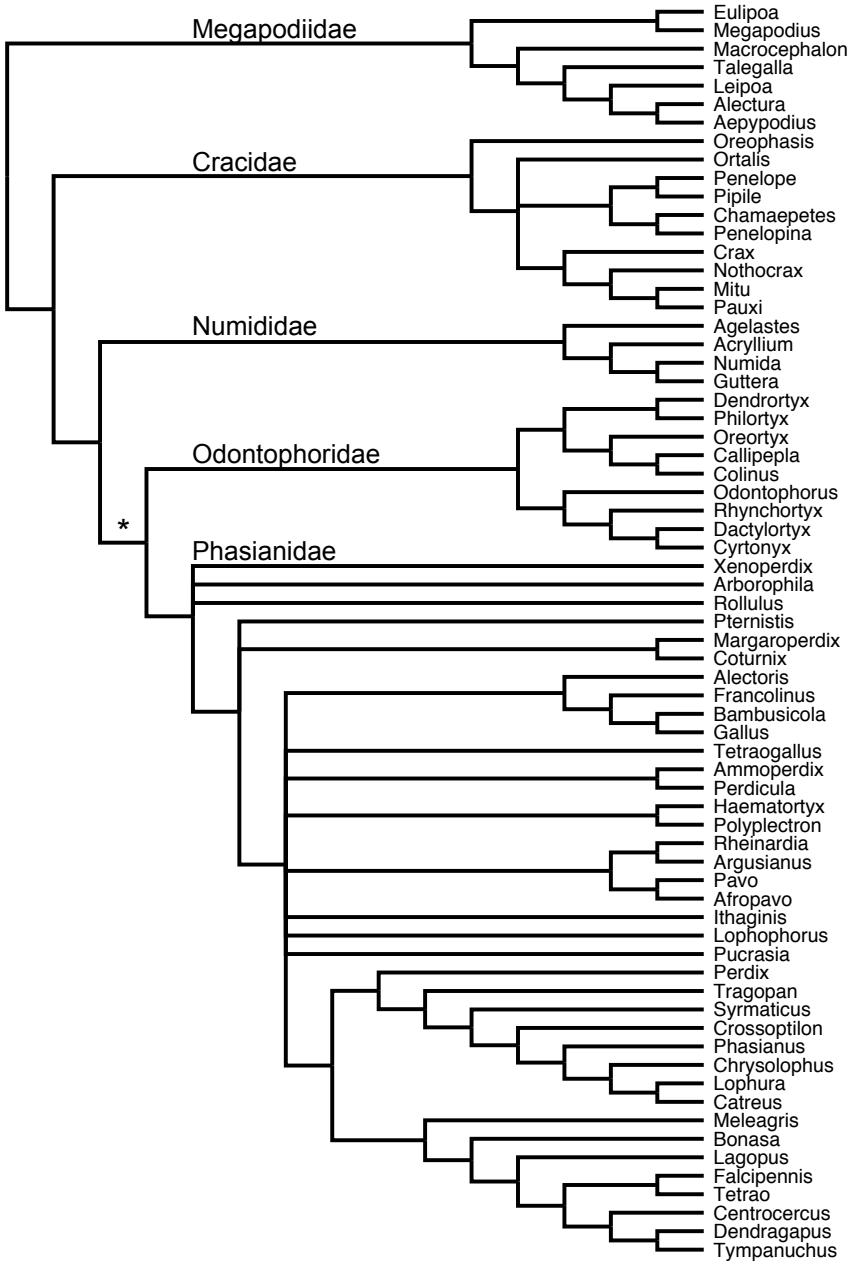

Megapodiidae
Cracidae
Numididae
Odontophoridae
*
Phasianidae
Eulipoa
Megapodius
Macrocephalon
Talegalla
Leipoa
Alectura
Aepypodius
Oreophasis
Ortalis
Penelope
Pipile
Chamaepetes
Penelopina
Crax
Nothocrax
Mitu
Pauxi
Agelastes
Acryllium
Numida
Guttera
Dendrortyx
Philortyx
Oreortyx
Callipepla
Colinus
Odontophorus
Rhynchortyx
Dactylortyx
Cyrtonyx
Xenoperdix
Arborophila
Rollulus
Pternistis
Margaroperdix
Coturnix
Alectoris
Francolinus
Bambusicola
Gallus
Tetraogallus
Ammoperdix
Perdicula
Haematortyx
Polyplectron
Rheinardia
Argusianus
Pavo
Afropavo
Ithaginis
Lophophorus
Pucrasia
Perdix
Tragopan
Syrmaticus
Crossoptilon
Phasianus
Chrysolophus
Lophura
Catreus
Meleagris
Bonasa
Lagopus
Falcipennis
Tetrao
Centrocercus
Dendragapus
Tympanuchus

Figure 2.2. Flow diagram of the possible transitions of a hypothetical model of dependent correlated evolution of two traits (a and b) that can take two stages each (low or high; small or large). Each potential transition is given by q_{ab}.

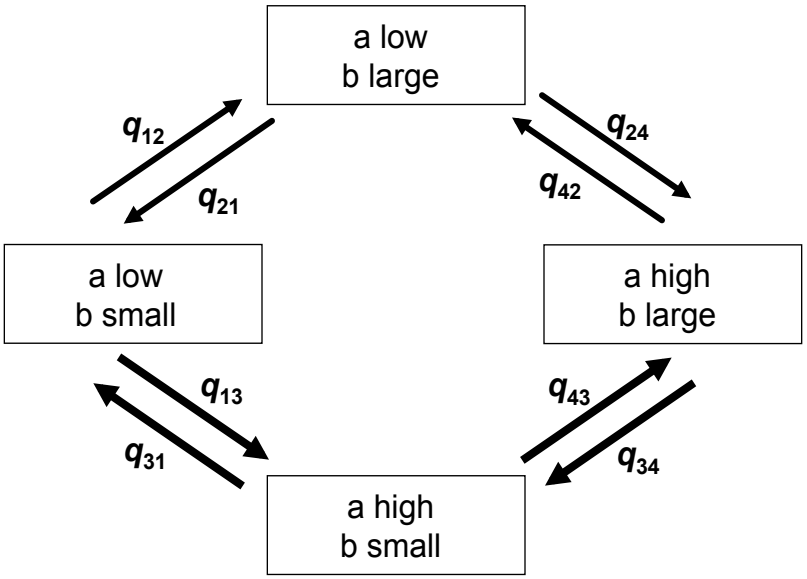


Figure 2.3. Bivariate correlations for raw data. (A) Egg size vs. female body mass (Pearson correlation: n = 34, r = 0.92, p = 0.0001). (B) Clutch size vs. female body mass (Pearson correlation: n = 48, r = -0.26, p = 0.077). (C) Egg size vs. clutch size (Pearson correlation: n = 38, r = -0.44, p = 0.006). (D) Female body mass vs. sexual size dimorphism (SSD) (Pearson correlation: n = 53, r = 0.38, p = 0.005). (E) Egg size vs. SSD (Pearson correlation: n = 34, r = 0.49, p = 0.004). (F) Clutch size vs. SSD (Pearson correlation: n = 48, r = 0.10, p = 0.49).

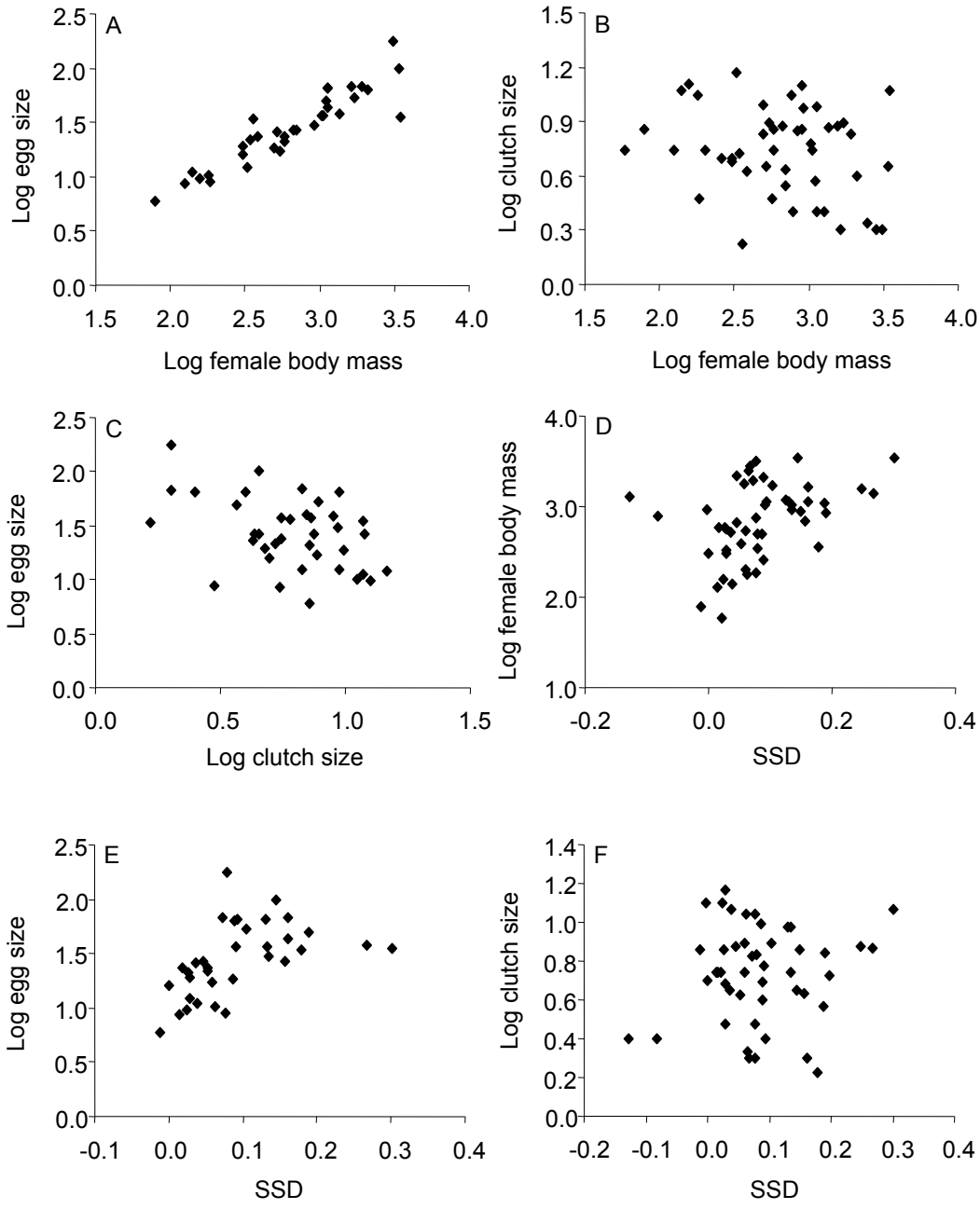


Figure 2.4. Bivariate regressions based on independent contrasts and performed through the origin, with the independent variable positivized. The solid gray line represents y = 0. The solid black line is the fitted regression model. (A) Egg size vs. female body mass (t_{32} = 12.5, r = 0.91, p < 0.0001). (B) Clutch size vs. female body mass (t_{45} = 0.17, r = -0.03, p = 0.87). (C) Egg size vs. clutch size (t_{36} = 1.9, r = -0.31, p = 0.06 (note that this negative relationship was statistically significant at p < 0.05 for all other trees, see text for details)). (D) Female body mass vs. sexual size dimorphism (SSD) (t_{49} = 3.9, r = 0.49, p = 0.0003). (E) Egg size vs. SSD (t_{32} = 2.7, r = 0.43, p = 0.01). (F) Clutch size vs. SSD (t_{45} = 0.02, r = -0.003, p = 0.98).

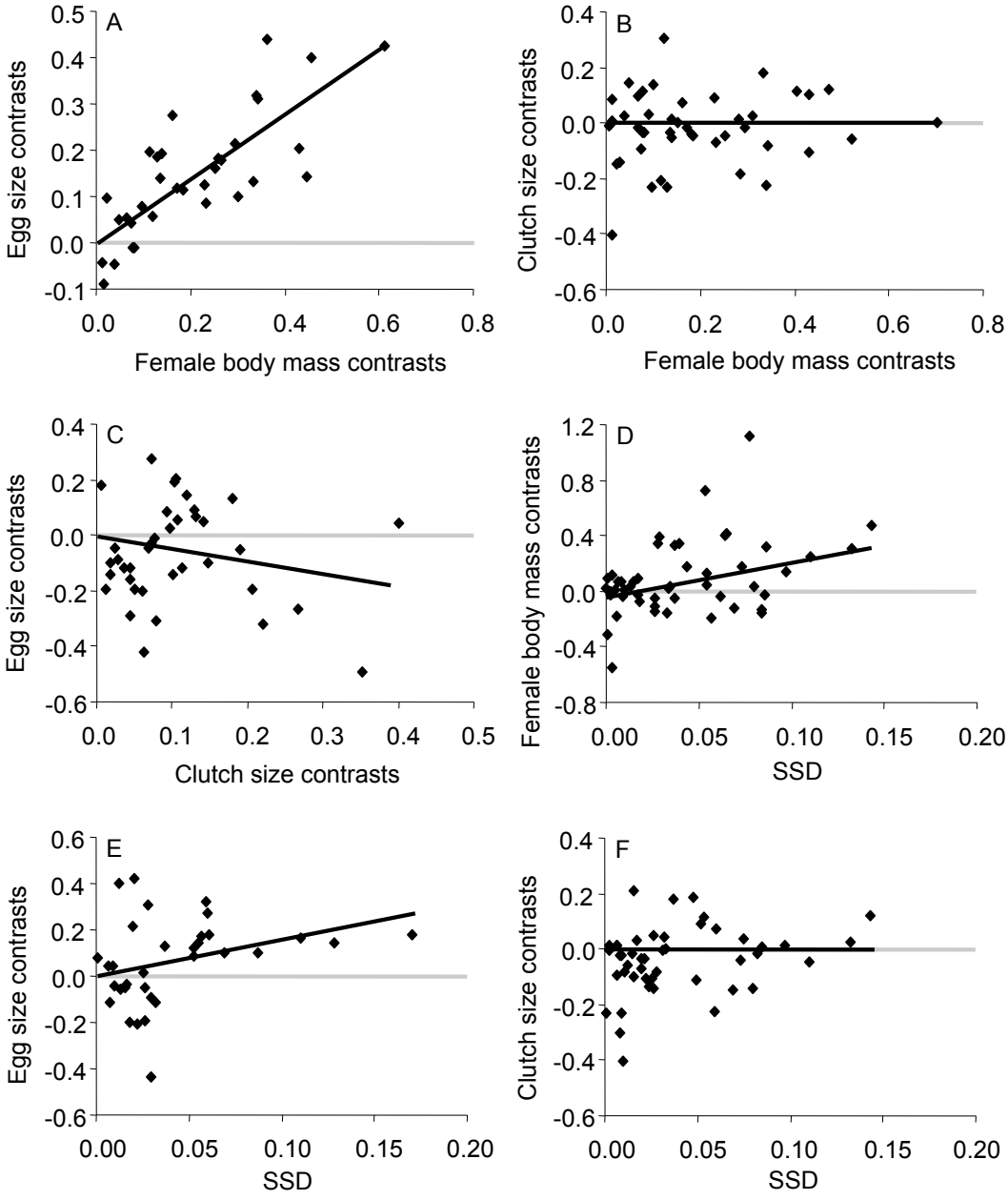


Figure 2.5. Current combinations of states of female body mass (= egg size) and sexual size dimorphism for Galliform genera.

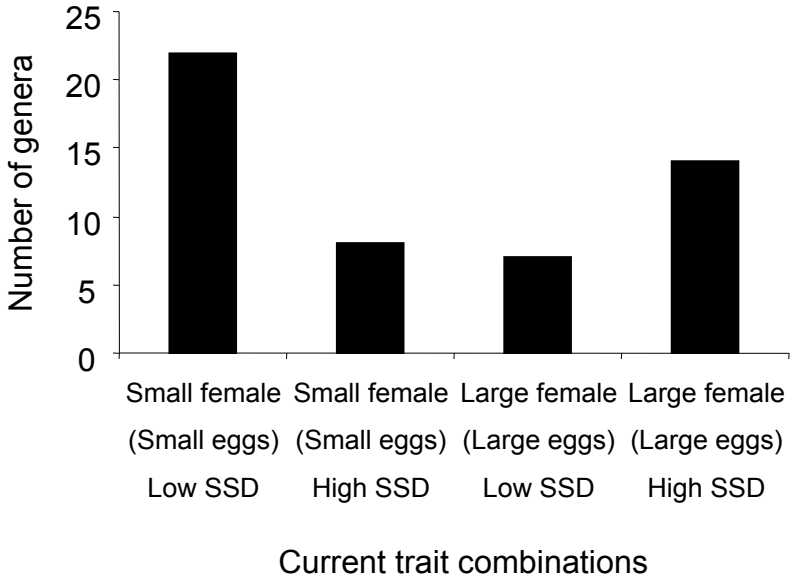
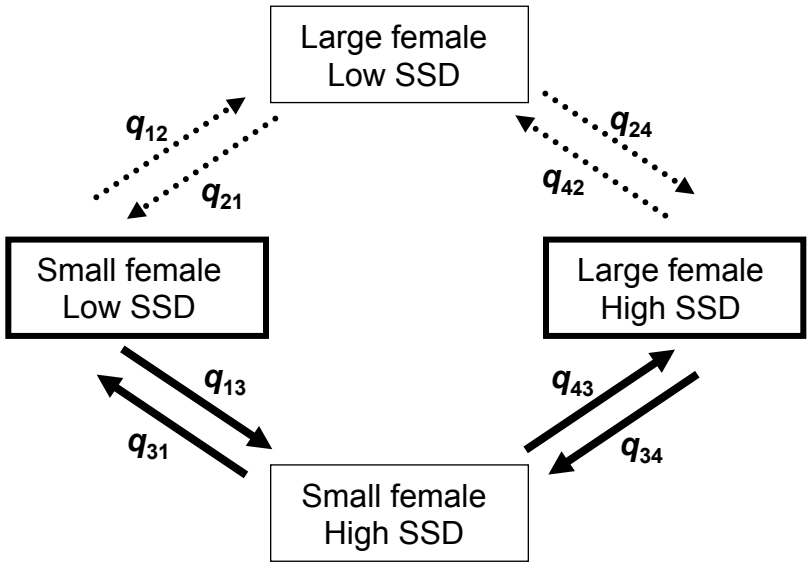


Figure 2.6. Flow diagram over the evolutionary transitions between female body mass and sexual size dimorphism (SSD). The common current states are high lighted by boldly lined boxes. Significant transitions are marked by solid arrows, whereas nonsignificant and thus unlikely transitions, are marked by dotted arrows. Statistics for individual transitions are as follows (n = 51): q_{12} (transition rate parameter) = 0.05, LR = 2.8, p = 0.09; q_{21} = 0.24, LR = 1.5, p = 0.22; q_{24} = 0.10, LR = 2.2, p = 0.14; q_{42} = 0.00007, LR = 0.06, p = 1.0; q_{34} = 0.46, LR = 2.6, p = 0.10 (note that this transition rate parameter was high and also statistically significant across all other trees), q_{43} = 0.70, LR = 4.8, p = 0.03; q_{31} = 0.96, LR = 8.6, p = 0.003; q_{13} = 0.41, LR = 5.8, p = 0.02. Note that a similar relationship would be obtained between egg size and SSD as all genera with high female body mass also laid large eggs and *vice versa*.



Chapter 3.

A molecular time scale for the Galliformes: Cretaceous origins and diversification in the Paleogene and Neogene

Unpublished. Intended for submission to Molecular Phylogenetics and Evolution.

R.W. Stein, J.W. Brown and A.Ø. Mooers.

R.W. Stein designed the study, assembled the molecular, fossil and taxonomic data, conducted the phylogenetic analyses, conducted the diversification analyses in LASER, and drafted the manuscript. J.W. Brown provided critical input on the phylogenetic analyses and the fossil calibration priors, and conducted the diversification analyses in MEDUSA.

Abstract

The phylogeny of the Galliformes (landfowl) has been studied extensively; however, the previously inferred chronologies have been questioned recently due to misplaced or misidentified fossil calibrations, and the diversification dynamics of this order have never been characterized. Using an alignment consisting of 14539 bp of mitochondrial and nuclear DNA sequence data, Bayesian uncorrelated lognormally distributed relaxed clock models and fossil calibration, I reconstruct the phylochronology of 223 of 291 extant Galliform species. I demonstrate that three stem-group lineages arose in the Cretaceous and crossed the Cretaceous-Paleogene (K-Pg) mass extinction (65.5 Ma). In a second series of analyses I used taxonomic constraints to include 68 data-deficient species and generated a large distribution of candidate phylogenies for all 291 extant Galliform species. I conducted diversification analyses on 10000 phylogenies

sampled from this distribution and show that the diversification of Galliform birds is best explained by a model that includes 1 – 3 clade-specific increases in diversification rate. The most frequently inferred increase is associated with an episode of explosive diversification (49.2 – 64.3 Ma) that coincides with a period of climate warming in the Paleogene (65.5 – 33.9 Ma) and gave rise to the crown group including Numididae, Odonotphoridae and Phasianidae. The second most frequently inferred increase in diversification rate is associated with a second episode of explosive diversification (18.8 – 24.1 Ma) in the Neogene (33.9 – 2.5 Ma) that gave rise to the Cracidae crown group. The tempo and mode of diversification in the Galliformes birds conforms to a two-pulse model, with stem lineages arising in the Cretaceous and post-K-Pg diversification occurring in the Paleogene and the Neogene.

Introduction

Fossil evidence of neornithine (modern) birds from the Cretaceous is extremely limited, and this has hindered our understanding of the temporal origin of modern birds and the impact of the Cretaceous-Paleogene (K-Pg) mass extinction event (65.5 Ma) on their evolutionary history (Dyke and van Tuinen 2004). The initial phylogenetic analyses designed to reconstruct the evolutionary history of modern birds provided disparate chronologies, fueling a “rocks vs. clocks” debate over which type of data and methodology (strict fossil record vs. molecular sequence data calibrated with strict molecular clock) provided the most appropriate means of inferring the diversification chronology of modern birds (Ericson et al. 2006, 2007; Brown et al. 2007, 2008). Donoghue and Benton (2007) argued for a united approach where “rocks” provide minimum and “clocks” maximum age estimates for calibrated nodes (Brown and van Tuinen 2011). Two simultaneous developments facilitated this united approach. The first was the development of Bayesian relaxed clock methodologies (Drummond et al. 2006 and Drummond and Rambaut 2007) including sophisticated and flexible means of temporal calibration (Ho and Phillips 2009). The second was the discovery of two key fossils that have certain affinities with extant neornithine orders and are dated either immediately before or immediately after the K-Pg boundary: *Vegavis iaai* is dated to the late Maastrichtian in the Cretaceous (66 Ma) and has clear affinities within the Anseriformes crown group (Clarke et al. 2005) and *Waimanu manneringi* is dated to the

early Paleocene in the Paleogene (60.5 Ma) and has clear affinities with the Sphenisciformes stem group (Slack et al. 2006). These fossils, and associated phylogenetic chronologies, provide strong evidence indicating a Cretaceous origin for modern birds.

A Cretaceous split within the Galloanserae, between the waterfowl and landfowl, is unambiguous because *Vegavis* has been subject to rigorous phylogenetic analysis and has clear affinities with the Anatidae stem group, within the Anseriformes crown group (Clarke et al. 2005). Nevertheless, divergence time estimates for the basal split in the Galloanserae from recent studies using Bayesian dating methods and *Vegavis* as a calibration still provide disparate chronologies (83 Ma, Haddrath and Baker 2012; 108 Ma, Crowe et al. 2006). This is surprising because Galloanserae is one of the most consistently and highly supported clades in the phylogeny of modern birds (Cracraft and Clarke 2001; Fain and Houde 2004; Ericson et al. 2006; Slack et al. 2007; Pacheco et al. 2011; Haddrath and Baker 2012; Jetz et al. 2012). Moreover, the divergence time estimates for Galliformes reported by van Tuinen and Dyke (2004), Pereira and Baker (2006) and Crowe et al. (2006) have been criticized because of incorrect and (or) misplaced fossil calibrations, which could produce overestimated divergence times (Mayr 2006; Ksepka 2009), and similar criticisms could be levied against more recent work (e.g. Kan et al. 2010). This suggests that the persistence of disparate chronologies within Galloanserae may be due to a combination of among-study variation in taxon sampling, amount and type of sequence data (mitochondrial and (or) nuclear) analyzed, fossil calibrations and the phylogenetic inference and dating methods used. With that in mind, detailed analyses within Galloanserae still offer promise for resolving whether the diversification of crown-group Galliformes commenced before or after the K-Pg mass extinction, and this is one of the goals of this chapter.

While a Cretaceous origin for the Galliform stem lineage is certain, it remains unclear whether any Galliform crown-group lineages arose in the Cretaceous and crossed the K-Pg boundary (Ksepka 2009). Here, I use a large alignment of mitochondrial and nuclear DNA sequence data, fossil calibration and Bayesian dating methods to reconstruct the phylochronology of 223 of 291 (77%) species from the Galliformes. This extensive taxa set includes species from each of the 5 families and from 75 of the 84 (89%) currently recognized genera (see below). If Galliform crown-

group lineages arose in the Cretaceous, as has been suggested (Ksepka 2009), then the K-Pg mass extinction could have influenced their subsequent diversification dynamics. Van Tuinen et al. (2006) proposed a two-pulse model to account for the tempo and mode of diversification for modern non-passerine birds, with ordinal diversification in the Cretaceous and post-K-Pg diversification via either a “short fuse” in the Paleogene (65.5 – 33.9 Ma) or a “long fuse” in the Neogene (33.9 – 2.5 Ma). A complete phylogenetic hypothesis is required to accurately characterize diversification dynamics, so I used taxonomic affinities to incorporate 68 data-deficient species and subsequently reanalyzed the DNA data matrix for this expanded taxa set (for a related approach see Jetz et al. 2012). These analyses generated a large distribution of candidate phylogenies that account for uncertainty in local topology and divergence times for data-deficient species. I use a sample of 10000 candidate phylogenies from this distribution to characterize diversification dynamics of the Galliformes and to assess whether their diversification chronology corresponds to the two-pulse model proposed by van Tuinen et al. (2006).

Methods

BEAST (Drummond et al. 2006) is the only Bayesian phylogenetic inference platform that allows simultaneous estimation of topology and chronology (Brown and van Tuinen 2012), and this is the platform that I used to reconstruct Galliformes phylogeny, to estimate divergence times and to construct the trees required for diversification analyses. The tree-building analyses presented here were designed to facilitate two goals: 1. To assess whether any of the extant crown-group lineages of Galliformes arose in the Cretaceous and survived the K-Pg mass extinction; and 2. To characterize diversification dynamics of Galliform birds throughout their evolutionary history. The requisite phylogenetic analyses proceeded in three stages. First, I selected a series of mitochondrial and nuclear loci, downloaded sequence data from GenBank and the Barcode of Life Data System, aligned the sequences, inferred models of molecular evolution and conducted extensive preliminary analyses to assess topological support. Second, I tested rate constancy (molecular clock), incorporated fossil calibrations and performed phylochronological reconstruction for 223 of 291 ingroup taxa. Third, I developed taxonomically informed constraint structures to facilitate inclusion of 68 data-

deficient species, and then I repeated phylochronological reconstruction for all 291 spp. of extant Galliformes. This final approach exploits the parameters estimated from the sequence data to assign branch lengths to data-deficient species. Using this approach I was able to generate a large distribution of candidate phylogenies for the entire Order that account for uncertainty in the affinities and divergence times of data-deficient taxa. I conduct diversification analyses on 10000 trees sampled from the posterior distribution of this final analysis.

Taxa set

As considered here, the order Galliformes is composed of five families, 84 genera and 291 species (Appendix 1). I used the most recent species accounts available for Galliformes to determine the ingroup taxa set (Carroll 1994; de Juana 1994; del Hoyo 1994; McGowan 1994; Martínez 1994; Porter 1994; Jones, Dekker and Roselaar 1995; Delacour and Amadon 2004). I excluded one previously recognized taxon, *Lophura imperialis*, which has recently been identified as a hybrid taxon (Hennache et al. 2003). I included seven additional taxa that were subsequently identified as valid species in molecular phylogenetic analyses: *Coturnix australis* (Seabrook-Davison et al. 2009), *Crossoptilon harmani* (Chang et al. 2008), *Dendragapus fuluginosus* (Barrowclough et al. 2005), *Falcipennis franklinii* (Gutierrez et al. 2000), *Lagopus scoticus* (Gutierrez et al. 2000), *Polyplectron katsumatae* (Chang et al. 2008) and *Xenoperdix obscurata* (Bowie and Fjelda 2005). I used the most recent version of the International Ornithologists Union's master list of bird names (IOC Master List v3.3; Gill and Donsker 2013) to assign names to taxa. I include four outgroup taxa from the Anseriformes (Carbonera 1992). Together, these comprise the taxa set and taxonomic data.

Molecular data matrix

I assembled a 14539 bp alignment from pre-existing GenBank and Barcode of Life Data records (Appendix 2) for 229 of 291 (79%) ingroup taxa (Galliformes). This sample includes representatives from each of the five families (Megapodiidae, Cracidae, Niumididae, Odontophoridae and Phasianidae) and from 75 of the 84 genera (89%). The sample of outgroup taxa includes representatives from each of the three Anseriform families (Anatidae, Anhimidae and Anseranatidae). The alignment is composed of a

novel set of 6-protein coding (CO1, Cyt *b*, ND1, ND2, ND4 and ND5; 7827 bp; Table 3.1) and 3-nonprotein coding (control region, 12S and 16S rDNA; 2642 bp) mitochondrial loci in combination with 1-protein coding (587 bp) and 4-nonprotein coding (3483 bp) nuclear loci. I aligned the sequence data from each loci with SATé-II (Liu et al. 2012) and I subsequently removed start and stop codons from protein-coding sequences. Due to the presence of a single 579 bp insertion and associated microinversions (Kimball and Braun 2008), I excluded b-fibrinogen intron 7 sequences from representatives of three genera, *Oreortyx*, *Callipepla* and *Colinus*, within the Odontophoridae. Non-protein coding sequences are subject to high frequencies of insertions and deletions, making some regions impossible to align reliably. This issue is particularly acute for the mitochondrial control region, so I excluded the hyper-variable domains previously identified for Galliformes (Lucchini and Randi 1999; Pereira et al. 2004; Crowe et al. 2006). Consistent with this approach, I also excluded regions from nonprotein coding sequences that would not align; in these instances I aligned, edited and realigned the sequences until the alignment stabilized.

After the alignments were complete, I inferred the best-fit model of molecular evolution for each locus using MrModelTest2.0 (Nylander 2004), PAUP*4.0 (Swofford 2003) and AIC model selection criteria. I inferred gene trees using RAxML (Stamatakis 2006), inspected the resulting topologies for consistency, and then conducted concatenated analyses to assess topological support across the entire alignment. Temporal calibration under Bayesian phylogenetic inference requires topological constraints on taxa sets, so I verified that candidate calibration nodes had high bootstrap support in the concatenated RAxML analysis ($\geq$ 99%; Table 3.2). I tested rate constancy (molecular clock) at each locus using PAUP*4.0 (Swofford 2003) before assigning molecular clock models. Likelihood ratio tests rejected rate constancy at all loci except the nuclear exon, c-mos (df = 35, p = 0.30; all other loci: df = 43–209, $p \leq 0.0004$). Nucleotides at third-codon positions evolve much faster than those at first and second positions. Codon-position models unlink the mutation rates of slower-evolving first and second positions from faster-evolving third positions (Shapiro et al. 2006). So, I specified codon-position models for all protein-coding loci to account for this among site rate heterogeneity. Again, I used MrModelTest2.0 (Nylander), PAUP* (Swofford 2003) and AIC model to infer best-fit models for first- and second- vs. third-codon positions.

Ideally, calibration fossils should be subjected to phylogenetic analysis to ensure correct node calibration; however, this has been done for relatively few fossils from Galloanserae (Ksepka 2006). So, I selected outgroup taxa so I could include *Vegavis* as a basal calibration. *Vegavis* has clear affinities with stem Anatidae (Clarke et al. 2005) and provides calibration for the Anseriformes crown group. Due to the importance of calibrating the root node, I also used *Vegavis* to calibrate the split between the Anseriformes and the Galliformes (see below). I selected three fossils assigned to crown Galliformes as calibrations (Table 3.2). Fossils from the genus *Palaeortyx* have a well-developed intermetacarpal process on the carpometacarpus (wing bone), and this process on the carpometacarpus is diagnostic of Odontophoridae and Phasianidae (Mayr 2009). So, I used *P. gallica* (Mayr, Poschmann and Wuttke 2006), which is dated to the Oligocene ($\geq$ 24.7 Ma) and represented by a nearly complete, articulated skeleton, to calibrate the node uniting Numididae, Odontophoridae and Phasianidae. The two other fossils, *Boreortalis* spp. (Brodkorb 1964) and *Rhegminornis calobates* (Olson and Farrand 1974; Steadmann 1980), are dated to the lower Miocene (16 Ma) and represented by partial tibiotarsus or tarsometatarsus (leg and foot bones) or carpometacarpus. Following Ho and Phillips (2009), I used the minimum age of each fossil to set the hard minimum bound of a lognormal calibration prior (Table 3.2). For internal calibrations, I selected a mean and standard deviation such that 95% of the probability density was bounded by the 25-Ma interval that preceded the fossil's minimum age. I chose a 25-Ma interval because the basal node of Galliformes is inferred to be ~100 Ma (Brown et al. 2008) and I used four fossils that are distributed across the phylogeny. This approach is intended to place equal weight on each of the internal calibrations. For the split between the Anseriformes and the Galliformes, I selected a mean and standard deviation such that 95% of the probability density was bounded by a 44-Ma interval; here, I chose 44 Ma because the split between the Anseriformes and the Galliformes has been dated at ~110 Ma (Brown et al. 2008) and *Vegavis* is dated at 66 Ma (Clarke et al. 2005).

I used BEAUti v1.6.1 to generate command files for BEAST v1.6.1 (Drummond and Rambaut 2007). Here, I specified topological constraints, molecular models, clock models, a node heights model, calibration priors, starting trees and MCMC parameters (Tables 3.1 and 3.2; see below). Here, I specified five topological constraints: one to

enforce monophyly on the Galliformes ingroup and four associated with the fossil calibrations. I specified the best-fit models of molecular evolution for non-protein coding loci and the best-fit codon position models for all protein-coding loci, a strict-clock model for c-mos and uncorrelated lognormal relaxed clock models for all other loci. I grouped the nine-mitochondrial loci under a single relaxed-clock model because the mitochondrion is inherited as a unit. I specified temporal calibration by setting a birth-death prior on node heights and calibration priors based on four dated fossils. I performed analyses on three different starting trees to ensure that initial conditions did not bias results. For each starting tree I performed four preliminary 40-million step MCMC searches with different initiation seeds, sampled the MCMC chain every 10000 steps and examined the sampled parameter values in Tracer v1.5.0 (Rambaut and Drummond 2012). I discarded the first 15 million steps as burnin and combined the post-burnin sample of trees and associated parameter values for each starting tree; this resulted in a sample of 10000 trees and associated parameter values. I inspected the post-burnin sample of parameter values in Tracer v1.5.0 to assess convergence, mixing and effective sample size (ESS) for each parameter, and then I generated maximum clade credibility (MCC) trees from the composite tree file in TreeAnnotator v1.6.1 (Drummond and Rambaut 2007).

Preliminary analyses revealed two problems. First, topological support was unexpectedly low across the Phasianidae and within two genera of the Cracidae. Inspection of the DNA data matrix revealed eight taxa (Phasianidae: *Caloperdix oculeus*, *Galloperdix lunulata*, *Meleagris ocellata*, *Rhizothera longirostris* and *Tragopan melanocephalus*; Cracidae: *Ortalis cinereiceps*, *Penelope albipennis* and *Penelope marail*) with limited sequence data (184-822 bp) that might be eroding node support. Taxa with limited little sequence data contribute negligibly to the tree likelihood and, as a consequence, they can “float” in the analysis and erode node support. I used RAxML to assess the impact of these floating taxa, both individually and in combination with one another. These RAxML analyses revealed that *Galloperdix lunulata*, *Rhizothera longirostris*, *Tragopan melanocephalus*, *Ortalis cinereiceps*, *Penelope albipennis* and *Penelope marail* did not have stable affinities; these taxa were considered to be floaters and were subsequently treated as data-deficient. Second, despite substantial sampling from the MCMC chain the ESSs for two key parameters, posterior and

treeModel.rootHeight, were well below the minimum acceptable effective sample size (100) indicative of sufficient sampling from the posterior. This problem is associated with attempting to estimate parameters from a sparse data matrix; in effect, there are many clock rate combinations that provide equally good fits to the sequence data and this precludes convergence. This problem is exacerbated by the MCMC algorithm's single-parameter per-step updating routine.

In addition to the specifications described above for the preliminary analyses, I modified the final analysis of the molecular matrix to resolve these problems and extended the MCMC search. First, I excluded the six data-deficient species identified as floaters in the preliminary analyses. Second, I introduced a compound parameter that specifies a set of clock rates; this facilitate simultaneous updating of clock rates in the MCMC search. Third, for each of the three starting trees I performed one 120-million step MCMC search with a different initiation seed and sampled the MCMC chain every 10000 steps. After discarding the first 15-million steps as burnin, I inspected the sampled parameters in Tracer v1.5.0 to ensure adequate mixing and ESSs; each analysis appeared to have sampled the posterior sufficiently (ESS > 200; posterior (536-619) and treeModel.root.Height (221-315)). The three analyses converged on similar topologies and parameter estimates, so I combined 10000 post-burnin samples from each starting tree and this resulted in a sample of 30000 trees and associated parameter values from the posterior. Finally, I conducted prior-only analyses as follows: I fixed the topologies of the three inferred MCC trees, used them as starting trees, and estimating branch lengths for these topologies while sampling only from the "tree prior." The tree prior is the product of the calibration priors and the birth-death prior on node heights and it provides an important reference for assessing the extent to which node age estimates are determined by the sequence data vs. the tree prior (Drummond and Rambaut 2007).

Complete taxa set

I incorporated taxonomic constraints to facilitate the inclusion of 68 data-deficient species (six floaters with very limited sequence data and 62 species with no sequence data). Four of the six floaters are from three genera (*Tragopan*, *Ortalis* and *Penelope*) represented in the molecular matrix and I constrained each species to its named genus. The two remaining floaters are from genera not represented in the molecular matrix

(*Galloperdix* and *Rhizothera*). RAxML analyses demonstrated that *Galloperdix* has affinities with *Haematortyx* and *Polyplectron* (97% bootstrap support) and that *Rhizothera* has affinities with *Pucrasia* (50% bootstrap support). So, I specified topological constraints to enforce these affinities. Fifty-three of the 62 data-deficient species are from 18 genera represented in the molecular matrix, and I constrained each species to its named genus (Appendix 1). The nine remaining data-deficient species are from seven genera not represented in the molecular matrix. Six of these nine data-deficient species are from four genera (*Dactylortyx*, *Dendrortyx*, *Philortyx* and *Rhynchortyx*) in the Odontophoridae. I used Holman's (1961) genus-level taxonomy of Odontophoridae, which is based on osteology and agrees with the relationships that we inferred for five genera and 12 species within Odontophoridae, to constrain these four genera.

The three remaining data-deficient species are from monotypic genera, *Anurophasis*, *Lerwa* and *Melanoperdix*, within the Phasianidae. The taxonomic affinities of these monotypic genera are less clear, but there is a good candidate clade for each species. *Anurophasis monorthonyx* is distributed in the Snow Mountains of Papua New Guinea and colonization from south east Asia would involve a > 35 km flight over the deep ocean channel that marks Wallace's line. Very few species of extant Phasianidae are capable of such a long flight; the exception is a group of four genera within the sub-family Coturnicinae: *Coturnix*, *Excalfactoria*, *Margaroperdix* and *Synoicus*. Species from within *Coturnix*, *Excalfactoria* and *Synoicus* are distributed across Australia and or Papua New Guinea, suggesting that their ancestors colonized Australasia from southeast Asia (McGowan 1994; Seabrook-Davison et al. 2009). Similarly, *Margaroperdix madagarensis* is endemic to Madagascar, suggesting that its ancestor colonized Madagascar from Africa (McGowan 1994). Taken together, the distribution of this clade suggests a tendency toward long-distance dispersal, and *Anurophasis monorthonyx* has historical taxonomic affinities with this clade (McGowan 1994). So, I constrained *Anurophasis* to the clade containing *Coturnix*, *Excalfactoria*, *Margaroperdix* and *Synoicus* and simultaneously enforced genus-level contraints to maintain monophyly within each of these genera. *Lerwa lerwa* has a large, but isolated distribution in the alpine and sub-alpine zones of the Himalayas (Potapov 2000). However, *Lerwa* has no clear systematic affinities within Asia (Potapov 2000). Mey

(2006) reported that *Lerwa* shares two unique species of chewing lice (*Ischnocera*) with *Meleagris* and with some taxa in the Odontophoridae and Cracidae. Like *Meleagris*, *Lerwa* has a single pair of tarsal spurs, which are only present in males in both genera (Davison 1985); however, tarsal spurs are completely lacking within the Odontophoridae or Cracidae (Davison 1985). The combination of the unique, shared species of chewing lice and tarsal spurs in males suggest affinities between *Lerwa* and *Meleagris*. So, I constrained *Lerwa* and *Meleagris* to be sister genera and simultaneously enforced monophyly of *Meleagris*. Finally, *Melanoperdix niger* is distributed across south east Asia (Borneo, Malaysia and Sumatra). *Rollulus* and *Caloperdix* share this distribution and are sister genera in the data-only MCC tree. *Rollulus* is missing the hallux nail, the hallux nail is greatly reduced in *Melanoperdix* (Jerdon 1864) and *Melanoperdix* has historical affinities with *Rollulus* (McGowan 1994). So, I constrained *Caloperdix*, *Melanoperdix* and *Rollulus*, which are all monotypic genera, to a single clade.

After incorporating the 68 data-deficient species into the alignment, I specified the additional taxon-set constraints required to include the data-deficient species and assigned the same molecular models, clock models, node heights model and calibration priors used with the molecular matrix. Constraining *Lerwa* to be the sister genus to *Meleagris* required one additional calibration prior; the reason for this is that *Lerwa* is endemic to Asia and *Meleagris* is endemic to North America. *Rhegminornis calobates* places a hard minimum bound of 16 Ma on the prior (Table 3.2) and the time to most recent ancestor inferred for the clade including *Meleagris* and Grouse is 26.7 Ma (95% CI: 23.1 – 30.5 Ma). Under the hypothetical scenario of *Lerwa* and *Meleagris* as sister genera, their ancestral lineage would have diverged 16.0 – 30.8 Ma ago and the ancestor of *Meleagris* would have arrived in North America > 16 Ma ago. This time frame of arrival in North America coincides with the faunal interchange that occurred between Asia and North America 18 – 21 Ma ago via the Bering land bridge (Qiu 2003). In an attempt to accommodate these chronologies and to minimize interactions between calibration priors on adjacent nodes (both associated with *Rhegminornis*), I specified a soft maximum bound of 26 Ma and specified a mean and standard deviation for the lognormal distribution accordingly (Table 3.2).

To ensure a diverse sample of trees for the complete taxon set I conducted 20 MCMC searches for 65 million steps each and sampled the chain every 100000 steps,

instead of every 10000 steps, and combined the results of these analyses. BEAST v1.6.1 accepts incompletely resolved starting trees, which it randomly resolves prior to initiating the MCMC search. I included an incompletely resolved starting tree and conducted MCMC searches on 20 different starting trees (verified at the end of the search), and I initiated each search with a different starting seed. I discarded the first 15 million steps as burnin and combined the post-burnin samples for each starting tree; this resulted in a posterior distribution of 10000 candidate trees and associated parameter estimates. I inspected the composite file of sampled parameter values in Tracer v1.5.0 to ensure adequate mixing and sufficient sampling (ESS $\geq$ 200) to indicate convergence on the posterior.

Diversification analyses

I conducted diversification analyses on a sample of 10000 trees drawn from the posterior distribution of the analyses that included all 291 species of extant Galliformes. I compared the single-rate diversification models implemented in the R package LASER (Rabosky 2006) with the rate-shift model implemented in MEDUSA (Alfaro et al. 2006; Brown et al. 2012). LASER implements 7 single-rate diversification models: pure birth, birth-death, density dependent logistic, density dependent exponential, speciation variable (exponential speciation with constant extinction), extinction variable (constant speciation with exponential extinction), and speciation and extinction variable (exponential speciation and extinction). The rate-shift model implemented in MEDUSA allows pure birth and birth death to be modeled simultaneously on different parts of a single phylogeny. In addition to the parameters estimated for the pure birth and birth death models, MEDUSA's rate-shift model is penalized for the parameter that identifies rate-shift locations. The likelihood (and AIC calculations) in LASER and MEDUSA differ by a constant (birth-death model evaluated on the 291 species MCC tree: LASER lnLikelihood = 387.203, MEDUSA lnLikelihood = -970.816, lnLikelihood difference = 1358.019; ΔAIC is exactly twice this), so it is straightforward to correct for this difference before comparing results from the two methodologies (see Supplementary Material in Jetz et al. 2012).

Results

The phylochronological reconstructions presented here require 147 parameters to be estimated, and this is accomplished through extensive MCMC sampling from the posterior distribution of the sequence data under the molecular and clock models. The ESS for several key parameters, posterior distribution (536-619), prior distribution (243-309), tree likelihood (1308-1647) and age of the root node (221-315), indicate adequate sampling for parameter estimation (i.e. ESS > 200 in each of the 3 replicates). Approximately 5% of the parameters (7–8) had ESS < 200, but most of these were > 100. All 3 replicates converged on similar parameter estimates, topologies and chronologies.

Before evaluating whether any Galliform crown-group lineages arose in the Cretaceous, I first confirmed that the chronology inferred from the molecular matrix does not simply reflect expectation from the tree prior. To do this I combined 10000 post-burnin samples from each of the three replicate analyses of the molecular matrix and thinned this set from 30000 to 10000 samples, and then I compared node age estimates and their associated 95% credibility intervals (CI) for the 5 calibrated nodes with the prior-only estimates. ESSs derived from the molecular matrix indicated that the posterior distribution (1169), prior distribution (579), tree likelihood (3445) and age of the root node (596) were sampled sufficiently, and each of the 147 parameters had an ESS > 200. Node age estimates derived from the molecular matrix for the Galloanserae and Numididae splits were 12.8 and 9.8 Ma (15 – 20%) older and the node age estimate for crown Cracidae was 12.8 Ma (38%) younger than expected under the tree prior (Table 3.3). In addition, the 95% CIs for node age estimates derived from the molecular matrix for the Numididae split, crown Cracidae and turkeys (Meleagridinae) together with grouse (Tetraoninae) were 10.5, 19.4 and 5.1 Ma (33 – 79%) younger than expected under the tree prior (Table 3.3). Although the molecular matrix is sparse, these comparisons indicate that the inferred rates of molecular evolution were influential in establishing the timeline of the evolutionary history of Galliformes presented here.

The topology inferred from the combined sample of trees derived from the three replicate analyses of the molecular matrix is, in general, very well supported (Figure 3.1, Table 3.4). The major divergence events that form the backbone of the tree and lead to

the 5 extant families and the nodes that unite each of the 5 families have posterior probability support of 1.0 (Table 3.4; excluding constrained nodes). As a consequence, topological uncertainty has no influence on the node age estimates and the 95% CI associated with assessing which, if any, lineages of extant Galliformes originated in the Cretaceous and crossed the K-Pg boundary (65.5 Ma). Four nodes are critical for making this assessment: the Cracidae split, the Numididae split, crown Megapodiidae and crown Cracidae (Figure 3.1 “D”, “E”, “G” and “H”, respectively; Table 3.4). The Cracidae split is uncalibrated and has a posterior support probability of 1.0. I inferred that this split occurred 75.7 – 98.5 Ma ago, and this pre-dates the K-Pg boundary by 10.2 Ma. In the Bayesian analyses the Numididae split is calibrated with a hard minimum bound of 24.7 Ma; however, RAxML analyses indicate 100% bootstrap support (Table 3.2). I inferred that the Numididae split occurred 49.4 – 64.5 Ma ago, and this post-dates the K-Pg boundary by at least 1.0 Ma. Crown Megapodiidae is uncalibrated and has a posterior support probability of 1.0. I inferred that diversification of crown Megapodiidae commenced 24.3 – 35.5 Ma ago and this post-dates the K-Pg boundary by 30.0 Ma. Crown Cracidae is calibrated with a hard minimum bound of 16 Ma and RAxML analyses indicate 100% bootstrap support (Table 3.2). I inferred that the diversification of crown Cracidae commenced 18.8 – 24.1 Ma, and this post-dates the K-Pg boundary by 41.4 Ma. These results indicate that 3 lineages of extant Galliformes originated in the Cretaceous and survived the K-Pg mass extinction, and that all of the family-level crown groups diversified in the Paleogene and the Neogene.

After incorporating the 68 data-deficient species I ran an extended series of MCMC searches to ensure a diverse sample of trees to account for topological uncertainty in the complete taxa set. Again, ESS indicated that the posterior distribution (2371), prior distribution (1796), tree likelihood (6166) and root node age (1611) were sampled sufficiently, and all 147 parameters had ESS values > 200. Node age estimates from the taxon-complete tree set were, on average, 0.55 ± 0.11 Ma older than those derived from the molecular matrix (Table 3.5). This suggests that adding the 68 data-deficient taxa had little effect on the inferred chronology of Galliform diversification. Diversification analyses on a sample of 10000 taxon-complete trees show that lineage accumulation stalled for ~40 Ma, coincident with the K-Pg mass extinction (Figure 3.2). Lineage accumulation resumed after the mass extinction, proceeded at an

approximately constant rate for ~40 Ma and appears to have increased slightly between 15 – 5 Ma ago. In an attempt to explain these whole-tree patterns of diversification across the evolutionary history of Galliformes, I used AIC model selection criteria to compete a set of 8 diversification models. All 7 of the single-rate diversification models implemented in LASER ran on all 10000 trees except for the speciation-variable model, which failed to run on 924 trees. Among the 7 single-rate diversification models implemented in LASER, the speciation variable model had the lowest AIC score on 9044 of the 9076 trees (99.6%) that it ran on. The speciation-variable model and the speciation- and extinction-variable model are nested. Likelihood ratio tests performed on the results of the 9076 trees where the speciation-variable model ran show that it was strongly preferred to the speciation- and extinction-variable model (mean difference in logLikelihood = 0.003 ± 0.001; p = 0.99 ± 0.00). The density dependent exponential and the speciation-variable models provided equally good fits to the data (mean ± stderr; ΔAIC = 1.28 ± 0.50). However, and importantly, MEDUSA's clade-specific rate-shift model out-performed LASER's speciation-variable model on 9999 of the 10000 trees (Table 3.6; ΔAIC = 12.7 ± 0.2). MEDUSA analyses revealed substantial variation in mean diversification rate across the 10000 trees and favoured a combination of pure-birth and birth-death models for all trees (Figure 3.3). Of the 10000 trees analyzed, 63% exhibited 1 rate-shift, 35% exhibited 2 rate-shifts and 2% exhibited 3 rate-shifts.

Discussion

I provide evidence, derived from fossil-calibrated Bayesian uncorrelated lognormally distributed relaxed clock analyses of a > 14500 bp alignment of mitochondrial and nuclear DNA sequence data from 223 ingroup taxa, that intra-ordinal diversification of the Galliformes proceeded well before the K-Pg mass extinction (Figure 3.1; Table 3.4, 95% credibility intervals) such that three stem lineages crossed the K-Pg boundary. The Galliformes diverged from Anseriformes 86.6 – 110.2 Ma ago and that the first split within Galliformes occurred 83.6 – 108.3 Ma ago, giving rise to the ancestor of the Megapodiidae and to the common ancestor of all other Galliformes. A second split occurred 75.7 – 98.5 Ma ago, giving rise to the ancestor of the Cracidae and to the common ancestor of Numididae, Odonotophoridae and Phasianidae. This second, more recent split pre-dates the K-Pg mass extinction by 10.2 Ma. Crown Megapodiidae arose

24.3 – 35.5 Ma ago and crown Cracidae arose 19.8 – 24.1 Ma ago; these 95% credibility intervals post-date the K-Pg mass extinction by > 30 Ma. The split between Numididae vs. Odonotphoridae and Phasianidae occurred 49.4 – 64.5 Ma ago and post-dates the K-Pg mass extinction event by at least 1 Ma. Taken together, these results suggest that, while three Galliform crown lineages originated in the Cretaceous, all of the extant families diversified after the K-Pg mass extinction, either in the Paleogene (65.5 – 33.9 Ma) or in the Neogene (33.9 – 2.3 Ma).

Using 10000 taxon-complete trees, I characterized the temporal and global diversification dynamics of Galliform birds throughout their ~100 Ma evolutionary history. Lineage accumulation was strongly attenuated through the K-Pg mass extinction (65.5 Ma ago), but it resumed 49.4 – 64.5 Ma ago and proceeded at a near constant rate for ~40 Ma. Lineage accumulation appears to have increased slightly between 5 – 15 Ma ago (Figure 3.2). This global, whole-tree pattern of lineage accumulation in Galliformes is best explained by striking among-clade variation in mean diversification rate (Figure 3.3). Crown Megapodiidae and the pre-K-Pg stem lineages exhibited the lowest diversification rates, while crown Cracidae and crown Phasianidae exhibited the highest. The temporal pattern of diversification represented in the lineage-through-time plot captures tree-wide rather than clade-specific changes in diversification rate. Although crown Cracidae and crown Odonotophoridae have broadly similar geographic distributions, they differ substantially in their mean diversification rates (Figure 3.3). Taken together, these observations suggest that a combination of temporal and spatial factors may be required to explain the complex pattern of among-clade variation in diversification rates observed here, within the Galliformes (see below).

Phylochronology of the Galliformes

Inferences regarding the tempo and mode of diversification rely squarely on the topological support and inferred chronology of the analyzed phylogenies. I used BEAST to infer the timeline of diversification for the Galliformes because this is the only Bayesian phylogenetic inference platform that allows simultaneous inference of topology and chronology (Drummond et al. 2006; Brown and van Tuinen 2011). This is important because all of the other available methods use a two-step procedure where a topology is first inferred and then a time-line is inferred on the constrained topology. When I used

BEAST to infer chronology on a constrained topology the 95% credibility intervals for our node ages were appreciably smaller, suggesting that this may be a source of bias in two-step dating methods. The phylogenies presented here account for topological uncertainty and molecular rate variation among loci and, for protein-coding loci, among codon positions. Calibration is a critical part of every phylochronological reconstruction and fossil selection and placement has been a contentious issue in previous reconstructions of Galliformes diversification chronology (Mayr 2008; Ksepka 2009). Following the recommendation of Ksepka (2009), I used *Vegavis iaai* to calibrate the root node of Galloanserae; as a consequence, this key Anseriform fossil sets the inferred timeline for Galliform diversification. I also used *Palaeortyx gallica* as an internal calibration. Although *P. gallica* has not been subjected to formal phylogenetic analyses, it exhibits a diagnostic skeletal character that places it within either the stem or crown group that includes Odonotphoridae and Phasianidae (Mayr 2009); so, I used *P. gallica* to calibrate the node that unites Numididae, Odontophoridae and Phasianidae (Mayr 2009). In both cases the age estimates inferred by the molecular data for the calibrated nodes were appreciably older than those derived from the prior alone. The affinities of the two other fossil calibrations, *Boreortalis* spp. (Brodkorb 1964) and *Rhegminornis calobates* (Olson and Farrand 1974; Steadmann 1980), are less certain; however, the node ages derived from the sequence data for both of the calibrated nodes are within their calibration priors.

The phylogeny of Galliformes has been studied extensively over the last two decades and the family- and subfamily-level affinities are relatively stable (Kimball et al. 1999; Pereira et al. 2002; Pereira and Baker 2006; Crowe et al. 2006; Shen et al. 2010; Cohen et al. 2012). A thorough review of this work is beyond the scope of this study, but Eo et al. (2009) present a synthesis in the form of a supertree phylogeny of Galloanserae; their supertree included 385 source trees from 108 published studies and the majority of these were from the Galliformes. The most recent large-scale phylogenetic hypothesis for the Galliformes is from Crowe et al. (2006), which presents the results of parsimony analyses conducted on 65 genera and 158 species and Bayesian analyses conducted on 66 of these species. Both of these analyses were conducted on a relatively small alignment of sequence data (~4000 bp; mitochondrial: control region, 12s rDNA, cyt *b* and ND2; nuclear: ovomucoid intron 7). The parsimony

analyses also included a matrix of morphological and behavioural characters (Crowe et al. 2006). Although parsimony reconstructions are vulnerable to homoplasy, the family- and subfamily-level affinities inferred by Crowe et al. (2006) are consistent with one another and with those inferred in the present study. The analyses conducted in the present study are strictly sequence-based (with the exception of the data-deficient taxa) and resolve many of the intra-generic relationships (Figure 3.1) not considered by Crowe et al. (2006). I used the same five loci used by Crowe et al. (2006), and I augmented these loci with sequences from 5 additional mitochondrial and 4 additional nuclear loci (> 10000 additional bp). The MCC tree derived from this expanded molecular matrix includes 223 ingroup taxa and 80% of the 222 subtending nodes have posterior support probabilities > 0.95 (Figure 3.1). The majority of the nodes with lower posterior support probabilities are associated with relatively recent diversification events. Due to the rapid accumulation of sequence data and sampled loci, I was able to identify a novel combination of loci that allowed a simultaneously increase taxon sampling and topological resolution (Figure 3.1).

In contrast, the timeline of Galliformes diversification is much less certain (Ksepka 2009). Several studies have attempted to infer the diversification chronology of Galliformes and there has been a general consensus for intra-ordinal diversification in the Cretaceous (Crowe et al. 2006; Pereira and Baker 2006; Cox et al. 2007; Kan et al. 2010); however, these chronologies have been questioned due to controversy over incorrect or misplaced fossil calibrations, which could bias older node age estimates (Ksepka 2009). This critical issue prompted careful comparison between the prior expectation of node ages, based on a birth-death model of node heights and the calibration distributions, and our inferred diversification chronology. The previous, potentially flawed attempts to infer Galliformes diversification chronology provide two other important insights into this complex problem. The precision of node age estimates, as indicated by the width of their 95% credibility intervals, appears to be inversely related to the amount of sequence data (Cox et al. 2007; Kan et al. 2010) and to the size of the taxon set (Crowe et al. 2006; Pereira and Baker 2006). This suggests that the phylochronology presented here provides an important reappraisal of Galliformes diversification chronology. Exploiting BEAST's flexible framework, I used taxonomic constraints to incorporate 68 data-deficient species. Adding these data-deficient species

had a negligible impact on inferred node ages and their associated 95% credibility intervals (Table 3.5).

Diversification rate shifts

The Chicxulub asteroid strike that precipitated the K-Pg mass extinction (Schulte et al. 2010) left an indelible mark on the evolutionary history of birds (Longrich et al. 2011) and this is apparent in the evolutionary history of the Galliformes (Figure 3.2). This is evidenced mostly clearly by the low and homogeneous diversification rates of the three stem lineages that arose in the Cretaceous and crossed the K-Pg boundary (Figure 3.3). The five extant families of Galliformes exhibit > 3-fold variation in diversification rate and I inferred that 1 – 3 increases in diversification rate are required to account for this variation in diversification rate (Figure 3.3). The most frequently inferred rate shift corresponds to a sustained increase in diversification rate post-K-Pg, in the clade including Numididae, Odontophoridae and Phasianidae. Diversification rate appears to be relatively uniform within each of the five families; however, the pattern of among-family variation is complex. Variation in the timing of diversification in combination with the current geographic distribution of the five families suggest that both temporal and spatial factors may be required to account for the inferred rate shifts and the complex pattern of among-family variation in diversification rate. While a formal analysis is limited by the number of lineages represented in the present study, below I highlight the potential importance of environmental stability, ecological opportunity, niche filling and dispersal as explanatory factors of the variation in diversification rate among the five extant families of the Galliformes.

The Megapodiidae crown group (megapodes) originated in the Oligocene (24.3 – 35.5 Ma ago), includes 7 genera and 22 species and exhibits the lowest diversification rate (~0.03 lineages × Ma^{-1}; Figure 3.3) among the five extant Galliform families. The megapodes are confined to Indo-Australia, primarily east of Wallace's line (Jones, Dekker and Roselaar 1995). Much of the Sahul shelf, which connects Australia with New Guinea, is relatively shallow and some of the island complexes inhabited by megapodes are remote (e.g. Niuafo'ou, the Palau and Marina islands, and the Nicobar and Andaman islands; Jones, Dekker and Roselaar 1995). Although megapodes are relatively strong fliers, their occurrence on remote Pacific islands suggests that climate-induced changes

in sea level during the Oligocene may have influenced their current distribution. If this is the case then changes in sea level may have also influenced their diversification. Most species occur on oceanic islands and smaller islands are occupied by only one or two species; even Australia and Papua New Guinea have just three and five species, respectively (Jones, Dekker and Roselaar 1995). Such low species richness suggests that niche filling may have constrained diversification on small oceanic islands (c.f. Rabosky 2009). Consequently, I suggest that environmental instability and niche filling may explain the low diversification rates inferred for the Megapodiidae.

The Cracidae crown group (cracids: chachalacas, guans and curassows) originated in the late Miocene (18.8 – 24.1 Ma ago), includes 11 genera and 50 species and exhibits the highest diversification rate (~0.10 lineages $\times$ Ma^{-1}; Figure 3.3) among the five extant Galliform families. The cracids are distributed across southern North America and South America (Delacour and Amadon 2004); however, the fossil record indicates that the extant radiation of cracids originated in North America (Brodkorb 1964). The current and historical geographic distribution of cracids in combination with the inferred phylogeny (Figure 3.1), suggest a late Miocene diversification in southern North America and subsequent colonization of South America via the Panamanian land bridge. There are two main clades of Cracids (the guans vs. the curassows, chachalacas and horned guan; Figure 3.1) and both are arboreal-habitat specialists (Delacour and Amadon 2004). The Andes is the second largest mountain range in the world, spanning ~55º latitude (9000 km) and rising > 7000 m above sea level. The Andes provides a complex niche landscape that could facilitate diversification of arboreal habitat specialists. Rate shifts inferred by MEDUSA suggest that cracids underwent an explosive diversification when several lineages colonized South America < 2.5 Ma ago.

The crown group including Numididae (guineafowl), Odontophoridae (tooth-beaked quail) and Phasianidae (pheasants, partridges, grouse, turkeys and allies) originated 49.4 – 64.5 Ma in the Paleogene, which coincides with a period of climate warming between 50 – 60 Ma and includes the Paleocene – Eocene thermal maximum (55 Ma). Rate shifts inferred by MEDUSA suggest that this radiation is associated with a sustained increase in diversification post-K-Pg (Figure 3.3). This super-familial clade is a diverse assemblage of 66 genera and 218 species with a geographic distribution that includes all of the continents except Antarctica (Carroll 1994; de Juana 1994; McGowan

1994; Martínez 1994; Porter 1994). Crown-group guineafowl originated in the Miocene (13.6 – 21.7 Ma) and includes 4 genera and 6 species that are restricted to coastal and sub-Saharan Africa (Martínez 1994). Crown-group tooth-beaked quail originated in the Eocene (37.9 – 52.3 Ma) and includes 10 genera and 34 species that are primarily distributed across the Americas (Carroll 1994); however, two species in the genus *Ptilopachus* are African endemics (Cohen et al. 2012). Crown-group Phasianidae originated in the Eocene (40.4 – 52.8 Ma) and includes 52 genera and 178 species, which are distributed across Africa, Eurasia, Australia and North America (de Juana 1994; McGowan 1994; Porter 1994). The diversification rate for the Phasianidae crown group (~0.09 species × Ma^{-1}; Figure 3.3) is approximately twice that for the Numididae crown group (~0.04 species × Ma^{-1}; Figure 3.3), and the diversification rate for the Odonotphoridae crown group is intermediate (~0.07 species × Ma^{-1}; Figure 3.3). This pattern of diversification rates is accounted for, in part, by a corresponding pattern of variation in geographic distribution. The Phasianidae crown group has been exceptionally successful at dispersing into new landscapes and a diverse set of habitats; they have colonized all of the continents inhabited by Galliformes except South America (de Juana 1994, McGowan 1994, Potter 1994).

Van Tuinen et al. (2006) proposed a two-pulse model to account for the tempo and mode of diversification in modern non-passerine birds. Specifically, they proposed ordinal diversification in the Cretaceous, which produced a number of stem-group lineages that crossed the K-Pg boundary, and post-K-Pg diversification via either a "short fuse" in the Paleogene or a "long fuse" in the Neogene (van Tuinen et al. 2006). In addition to the well-known K-Pg mass extinction event (Schulte et al. 2010; Longrich et al. 2011), there was a subsequent, lesser mass extinction event that marks the Paleogene-Neogene boundary (33.9 Ma; van Tuinen et al. 2006), which was marked by a > 8º C decrease in temperature in just 400,000 yrs (Zanazzi et al. 2007). Using a phylogenetic hypothesis composed of 10000 taxon-complete trees for the Galliformes, I provide evidence supporting pre-K-Pg diversification within Galliformes, with three stem lineages crossing the K-Pg boundary. Two of the stem lineages exhibited "long-fuse" post-K-Pg diversification dynamics: both the Megapodiidae and the Cracidae crown groups diversified in the Neogene. The third stem lineage exhibited "short-fuse" post-K-Pg diversification dynamics and an explosive radiation during the Paleogene that

produced the crown group including Numididae, Odontophoridae and Phasianidae. The molecular timeline of diversification presented here for the Galliformes is consistent with van Tuinen et al.'s (2006) two-pulse model and suggests an important role for the Paleogene-Neogene transition in the familial and subfamilial diversification of the Galliformes, and possibly other non-passerine lineages.

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Tables and Figures

Table 3.1. Composition of the molecular data matrix, models of molecular evolution and clock models used in Bayesian phylochronological reconstructions for the Galliformes. The complete alignment spans 14539 bp.

Genome	Locus	Function	Length (bp)	Taxa	Molecular model[2]	Clock model[3]
mitochondrial	Control region[1]	regulatory	669	172	GTR+I+G(4)	UCLD relaxed 1
mitochondrial	12S rRNA	rRNA coding	624	125	GTR+I+G(4)	UCLD relaxed 1
mitochondrial	16S rRNA	rRNA coding	1349	59	GTR+I+G(4)	UCLD relaxed 1
mitochondrial	CO1	protein coding	1545	133	GTR+I+G(4) CP	UCLD relaxed 1
mitochondrial	Cyt *b*	protein coding	1137	214	GTR+I+G(4) CP	UCLD relaxed 1
mitochondrial	ND1	protein coding	966	47	GTR+I+G(4) CP	UCLD relaxed 1
mitochondrial	ND2	protein coding	1035	170	GTR+I+G(4) CP	UCLD relaxed 1
mitochondrial	ND4	protein coding	1374	63	GTR+I+G(4) CP	UCLD relaxed 1
mitochondrial	ND5	protein coding	1770	66	GTR+I+G(4) CP	UCLD relaxed 1
nuclear	B-fibrinogen	intron 7	929	64	GTR+G(4)	UCLD relaxed 2
nuclear	Ovomucoid	intron 7	477	84	GTR+G(4)	UCLD relaxed 3
nuclear	AGRP	introns 1 & 2	1155	35	HKY+G(4)	UCLD relaxed 4
nuclear	Rhodopsin	intron 1	922	57	HKY+G(4)	UCLD relaxed 5
nuclear	C-MOS	protein coding	587	38	GTR+I+G(4) CP[4]	strict 1

Notes.[1]Hypervariable regions were removed in accord with Desjardin and Morias (1990). The included portions of the control region correspond to bases: 13-28, 39-102, 104-116, 118-160, 378-558, 571-596, 602-638, 643-699, 704-846 and 861-945 of *Gallus gallus* from Desjardins and Morais (1990) GenBank accession number NC001323.
[2]CP: abbreviation for codon position.
[3]UCLD: abbreviation for uncorrelated lognormal distribution.
[4]Best fit molecular model: GTR+I for codon positions 1 and 2 and GTR+G(4) for codon position 3.

Table 3.2. Calibration fossils, bootstrap support for calibrated nodes and lognormal calibration parameters used in Bayesian phylochronological reconstructions for the Galliformes.

Fossil	Calibrated node	Figure 3.1 node label	Boot-strap support (%)	Hard minimum bound (Ma)	Soft maximum bound (Ma)	Log-normal Mean	Log-normal STD
Vegavis iaai[2]	Galloanserae	A	NA	66.0	110.0	3.09	0.42
Vegavis iaai[2]	Anseriformes crown	B	100	66.0	91.0	2.52	0.42
Palaeortyx gallica[3,4]	Numididae split	E	100	24.7	49.7	2.52	0.42
Boreortalis sp.[1]	Cracidae crown	H	100	16.0	41.0	2.52	0.42
Rhegminornis calobates[1,5,6]	Meleagridinae & Tetraoninae	*	99	16.0	41.0	2.52	0.42
Rhegminornis calobates[1,5,6]	Meleagridinae & *Lerwa*	NA	NA	16.0	26.0	1.61	0.42

Sources.[1]Brodkorb 1964, [2]Clarke et al. 2005, [3]Mayr, Poschmann and Wuttke 2006, [4]Mayr 2009, [5]Olson and Farrand 1974, [6]Steadman 1980.

Table 3.3. Comparison of time to most recent common ancestor (TMRCA) and its 95% credible interval (CI) from analyses including only calibration priors and analyses including calibration priors and the molecular data matrix.

Parameter	Calibrated node	Figure 3.1 node label	Calibration (Ma)	Lognormal prior (Ma)	Lognormal prior and molecular data (Ma)
TMRCA	Galloanserae	A	84.5	85.0	97.8
	Anseriformes crown	B	76.5	76.4	76.4
	Numididae split	E	35.2	47.5	56.9
	Cracidae crown	H	26.5	34.1	21.3
	Meleagridinae & Tetraoninae crown	*	26.5	25.9	26.7
95% CI	Galloanserae	A	44	23.3	23.6
	Anseriformes crown	B	25	13.4	13.4
	Numididae split	E	25	25.5	15.0
	Cracidae crown	H	25	24.6	5.2
	Meleagridinae & Tetraoninae crown	*	25	12.6	7.5

Table 3.4. Topological support, time to most recent ancestor (TMRCA) and its 95% credibility interval (CI) for major nodes of the Galliformes phylochronology presented in Figure 3.1.

Node	Figure 3.1 node label	Posterior support	TMRCA (Ma)	TMRCA 95% CI (Ma)
Galloanserae*	A	–	97.8	86.6 – 110.2
Anseriformes crown*	B	–	76.4	70.3 – 83.8
Galliformes crown+	C	–	95.9	83.6 – 108.3
Cracidae split	D	1.00	86.4	75.7 – 98.5
Numididae split*	E	–	56.9	49.4 – 64.5
Odontophoridae split	F	1.00	53.6	46.3 – 60.7
Megapodiidae crown	G	1.00	29.5	24.3 – 35.5
Cracidae crown*	H	–	21.3	18.8 – 24.1
Numididae crown	I	1.00	17.6	13.6 – 21.7
Odontophoridae crown	J	1.00	45.2	37.9 – 52.3
Phasianidae crown	K	1.00	46.4	40.4 – 52.8
Arborophilinae crown	L	1.00	39.6	33.4 – 46.3
Coturnicinae crown	M	1.00	34.7	29.7 – 39.9
Pavoninae crown	N	0.99	38.3	33.4 – 43.8
Meleagridinae crown	O	1.00	6.7	1.6 – 12.4
Tetraoninae crown	P	1.00	18.8	15.6 – 22.2
Phasianinae crown	Q	1.00	28.0	24.1 – 31.9

Notes.*Node constrained due to fossil calibration.
+Node constrained to root the tree.

Table 3.5. Comparison of divergence time estimates derived from the molecular data matrix in combination with fossil calibration and the molecular and taxonomic data with fossil calibration.

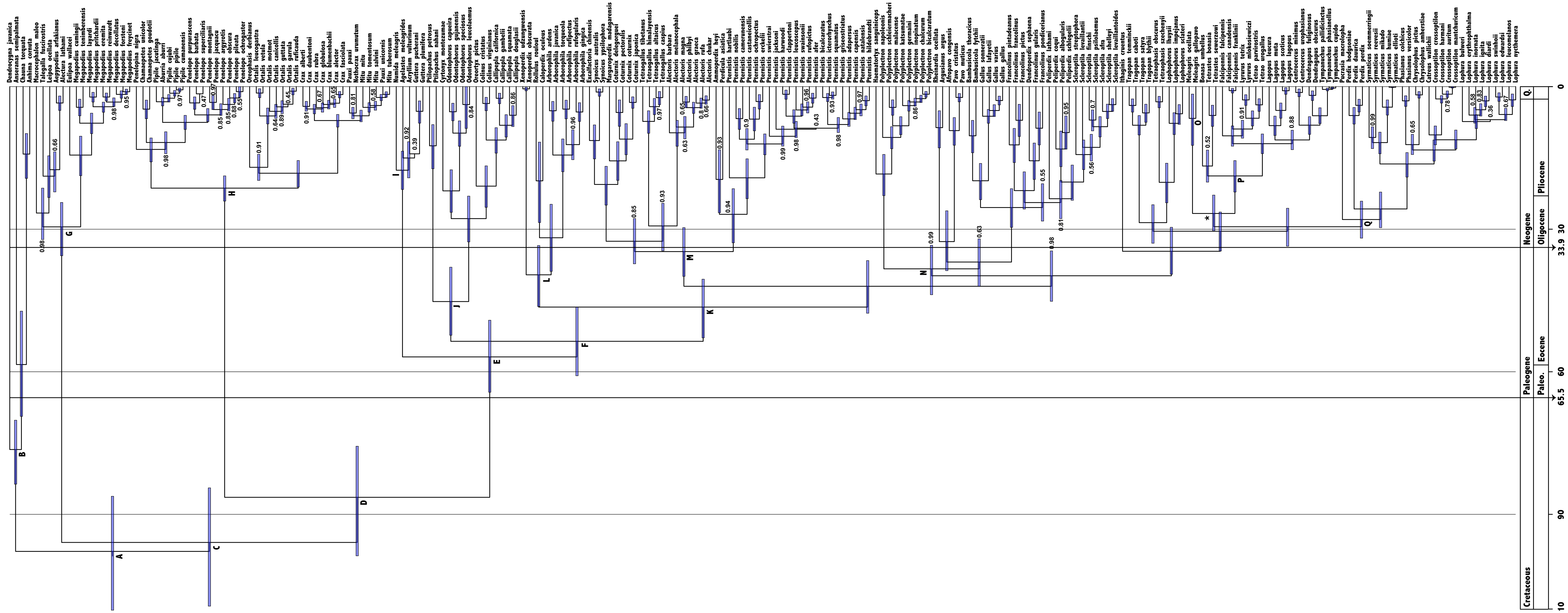
Node	**Figure 3.1 label**	**227 species[1] (mean; Ma)**	**227 species[1] (95% CI; Ma)**	**294 species[2] (mean; Ma)**	**294 species[2] (95% CI; Ma)**
Basis of inference		prior & data	prior & data	prior, data & taxonomy	prior, data & taxonomy
Galloanserae	A	97.8	86.3-109.9	98.8	87.4-111.7
Anseriformes crown	B	76.4	70.3-83.7	76.5	70.2-83.8
Galliformes crown	C	95.9	83.7-108.3	97.0	85.1-110.3
Cracidae split	D	86.4	75.4-98.2	87.4	76.7-100.0
Numididae split	E	56.9	49.4-64.4	57.6	50.1-65.5
Odontophoridae split	F	53.6	46.7-61.1	54.3	47.2-61.8
Megapodidae crown	G	29.5	24.3-35.4	29.5	23.9-35.2
Cracidae crown	H	21.3	18.8-24.0	21.4	18.9-24.1
Numididae crown	I	17.6	13.6-21.6	17.7	13.8-21.8
Odontophoridae crown	J	45.2	38.1-52.3	45.9	39.0-53.3
Phasianidae crown	K	46.4	40.3-52.6	47.1	41.1-53.4

Table 3.6. AIC values for eight diversification models competed on 10000 taxon-complete phylochronologies of the Galliformes. Likelihood values were standardized across analytical methodologies (MEDUSA and LASER) using the likelihood of the homogeneous birth-death model at its MEDUSA-based maximum likelihood parameter estimates.

Model	Model type	lnLK ± sem	AIC ± sem	ΔAIC ± sem
MEDUSA^	clade shift	404.2 ± 0.2	1917.1 ± 0.4	0.0 ± 0.0
Speciation variable*	temporal variation	398.4 ± 0.2	1925.1 ± 0.4	12.7 ± 0.2
Density dependent exponential	temporal variation	394.7 ± 0.2	1930.5 ± 0.4	13.4 ± 0.0
Speciation and extinction variable	temporal variation	396.4 ± 0.2	1931.2 ± 0.4	14.1 ± 0.0
Birth-death	constant-rate	392.5 ± 0.2	1935.1 ± 0.4	18.0 ± 0.0
Extinction variable	temporal variation	392.4 ± 0.2	1937.2 ± 0.4	20.1 ± 0.0
Pure birth	constant-rate	387.3 ± 0.2	1943.4 ± 0.4	26.3 ± 0.1
Density dependent logistic	temporal variation	387.3 ± 0.2	1945.4 ± 0.4	28.3 ± 0.1

Notes. ^with 1 – 3 rate shifts per tree.
*The speciation variable model ran on 9076 of the 10000 trees; ΔAIC is based only on the results from those 9076 trees.
lnLK: abbreviation for loglikelihood.
AIC: abbreviation for Akaike information criterion.
ΔAIC: abbreviation for difference in Akaike information criterion.

Figure 3.1. Phylochronogy of 223 species of ingroup taxa from the Galliformes and 4 outgroup taxa from the Anseriformes derived from concatenated analysis of a 14539 bp alignment of mitochondrial and nuclear DNA sequence data. Posterior probability of node support is only indicated when < 1.00. Node bars indicate 95% credibility interval for mean node age. The Cretaceous – Paleogene (65.5 Ma) and the Paleogene – Neogene (33.9 Ma) boundaries correspond to mass extinction events.



Notes. “Paleo.” is an abbreviation for Paleocene and “Q.” is an abbreviation for Quaternary. Capital letters correspond to taxonomic levels as follows: Super-ordinal (A: Gallonanserae), ordinal (B: Anseriformes, C: Galliformes), familial (G: Megapodiidae, H: Cracidae, I: Numididae, J: Odontophoridae, K: Phasianidae) and subfamilial (L: Arborophilinae, M: Coturnicinae, N: Pavoninae, O: Meleagridinae, P: Tetraoninae, Q: Phasianinae) taxonomic levels. Capital letters along the backbone of the phylogeny correspond to family-level splits as follow: Megapodiidae split (C), Cracidae split (D), Numididae split (E), Odontophoridae and Phasianidae split (F).

Figure 3.2. Lineage through time plot for 10000 taxon-complete candidate phylochronologies encompassing the entire evolutionary history of the Galliformes. Waiting time to speciation for each of the 10000 phylogenies is depicted by the gray lines. Mean waiting time to speciation is represented by the black line.

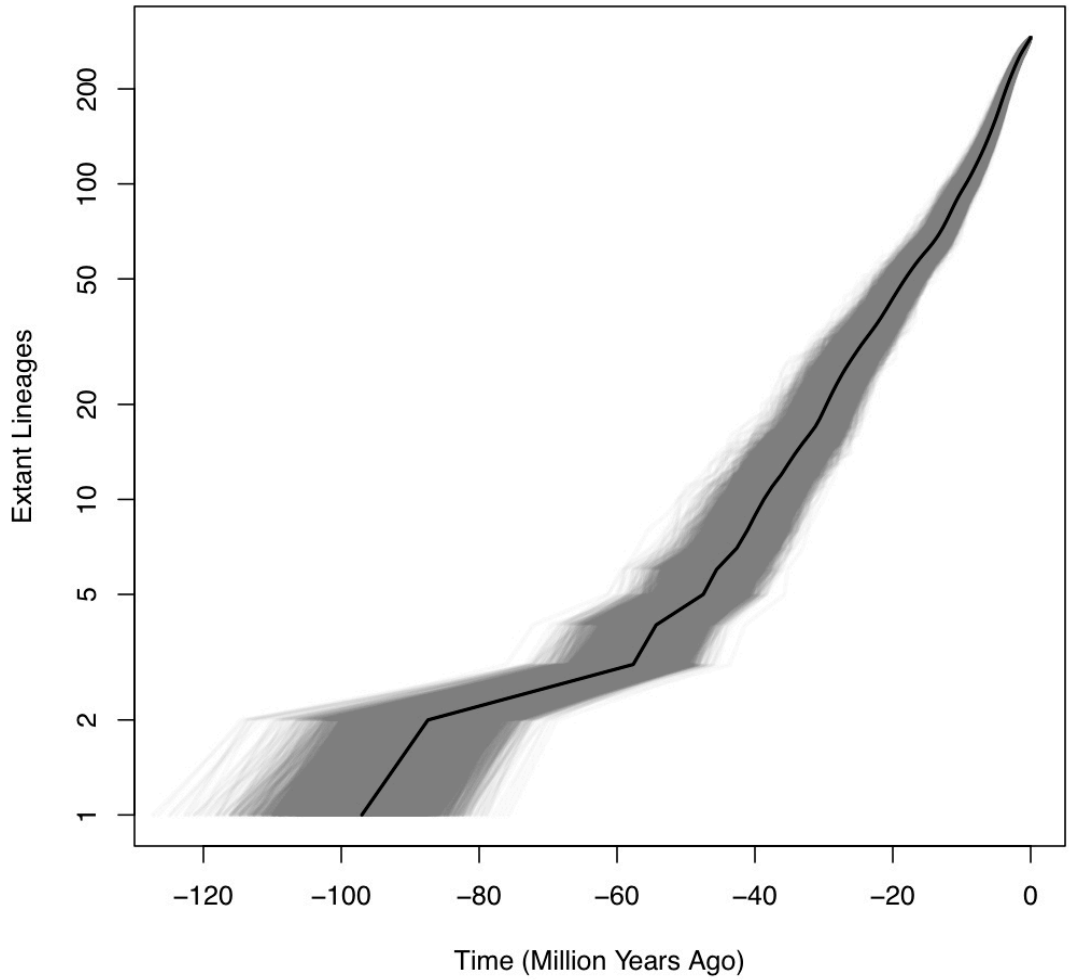
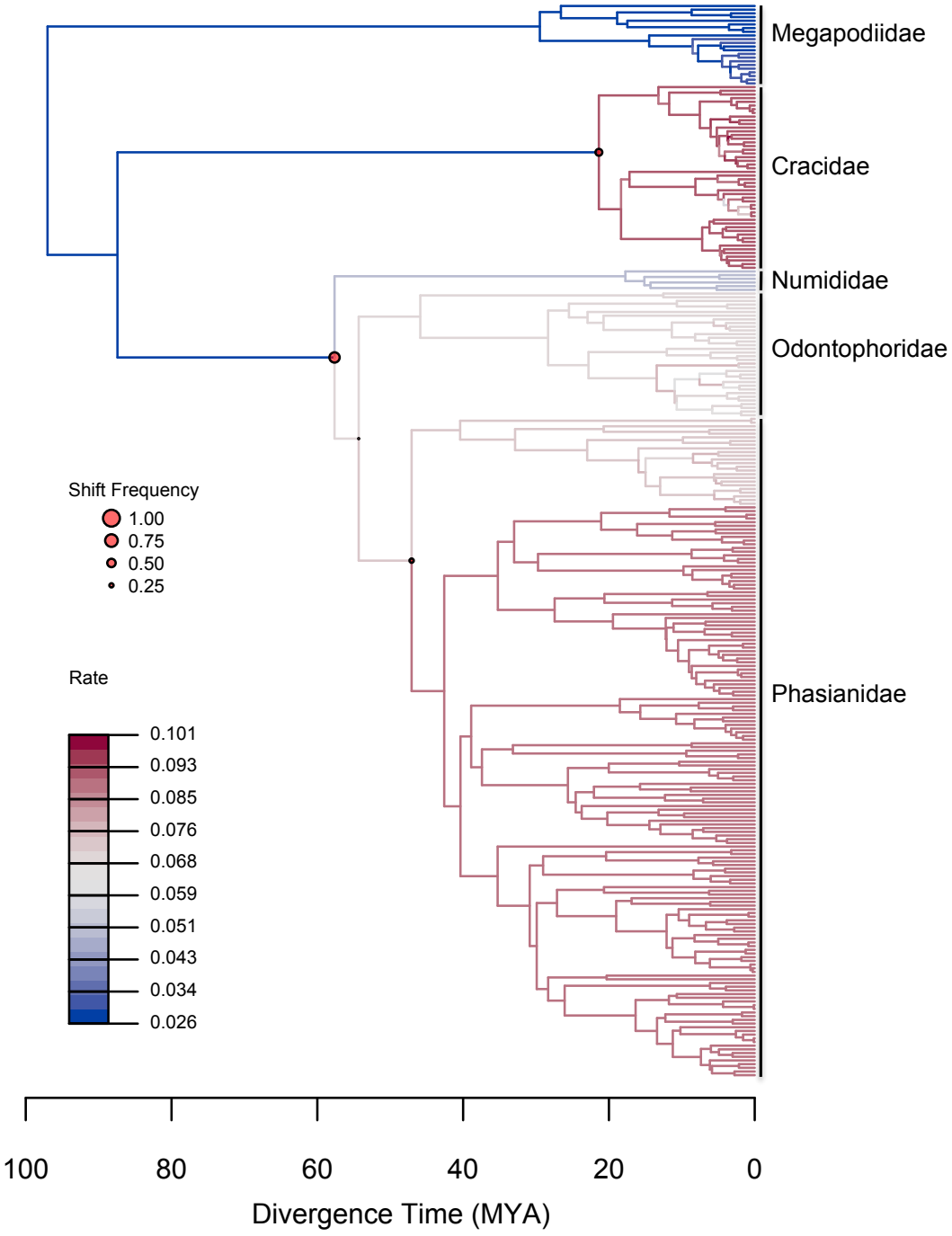


Figure 3.3. Lineage-specific diversification-rate shifts inferred by MEDUSA on 10000 taxon-complete candidate phylochronologies encompassing the entire evolutionary history of the Galliformes.



Appendices

Appendix 3.1. Taxonomy of the Anseriformes and Galliformes taxa included in Bayesian phylochronological reconstructions

Order	Family	Scientific name	Common name	Data deficient	Taxonomic reference
Anseriformes	Anatidae	*Anhima cornuta*	Lesser Whistling Duck	No	3
Anseriformes	Anhimidae	*Chauna torquata*	Horned Screamer	No	3
Anseriformes	Anhimidae	*Anseranas semipalmata*	Southern Screamer	No	3
Anseriformes	Anseranatidae	*Dendrocygna javanica*	Magpie Goose	No	3
Galliformes	Megapodiidae	*Aepypodius arfakianus*	Wattled Brushturkey	No	9
Galliformes	Megapodiidae	*Aepypodius bruijnii*	Waigeo Brushturkey	Yes	9
Galliformes	Megapodiidae	*Alectura lathami*	Australian Brushturkey	No	9
Galliformes	Megapodiidae	*Eulipoa wallacei*	Moluccan Megapode	No	9
Galliformes	Megapodiidae	*Leipoa ocellata*	Malleefowl	No	9
Galliformes	Megapodiidae	*Macrocephalon maleo*	Maleo	No	9
Galliformes	Megapodiidae	*Megapodius bernsteinii*	Sula Megapode	Yes	9
Galliformes	Megapodiidae	*Megapodius cumingii*	Philippine Megapode	No	9
Galliformes	Megapodiidae	*Megapodius decollatus*	New Guinea Scrubfowl	No	9
Galliformes	Megapodiidae	*Megapodius eremita*	Melanesian Megapode	No	9
Galliformes	Megapodiidae	*Megapodius forsteni*	Forsten's Megapode	No	9
Galliformes	Megapodiidae	*Megapodius freycinet*	Dusky Megapode	No	9
Galliformes	Megapodiidae	*Megapodius geelvinkianus*	Biak Scrubfowl	Yes	9
Galliformes	Megapodiidae	*Megapodius laperouse*	Micronesian Megapode	Yes	9
Galliformes	Megapodiidae	*Megapodius layardi*	Vanuatu Megapode	No	9
Galliformes	Megapodiidae	*Megapodius nicobariensis*	Nicobar Megapode	Yes	9
Galliformes	Megapodiidae	*Megapodius pritchardii*	Tongan Megapode	No	9
Galliformes	Megapodiidae	*Megapodius reinwardt*	Orange-footed Scrubfowl	No	9
Galliformes	Megapodiidae	*Megapodius tenimberensis*	Tanimbar Megapode	No	9
Galliformes	Megapodiidae	*Talegalla cuvieri*	Red-billed Brushturkey	Yes	9
Galliformes	Megapodiidae	*Talegalla fuscirostris*	Black-billed Brushturkey	Yes	9
Galliformes	Megapodiidae	*Talegalla jobiensis*	Collared Brushturkey	No	9
Galliformes	Cracidae	*Aburria aburri*	Wattled Guan	No	7
Galliformes	Cracidae	*Chamaepetes goudotii*	Sickle-winged Guan	No	7

Order	Family	Scientific name	Common name	Data deficient	Taxonomic reference
Galliformes	Cracidae	*Chamaepetes unicolor*	Black Guan	No	7
Galliformes	Cracidae	*Crax alberti*	Blue-billed Curassow	No	7
Galliformes	Cracidae	*Crax alector*	Black Curassow	No	7
Galliformes	Cracidae	*Crax blumenbachii*	Red-billed Curassow	No	7
Galliformes	Cracidae	*Crax daubentoni*	Yellow-knobbed Curassow	No	7
Galliformes	Cracidae	*Crax fasciolata*	Bare-faced Curassow	No	7
Galliformes	Cracidae	*Crax globulosa*	Wattled Curassow	No	7
Galliformes	Cracidae	*Crax rubra*	Great Curassow	No	7
Galliformes	Cracidae	*Mitu mitu*	Alagoas Curassow	No	7
Galliformes	Cracidae	*Mitu salvini*	Salvin's Curassow	No	7
Galliformes	Cracidae	*Mitu tomentosum*	Crestless Curassow	No	7
Galliformes	Cracidae	*Mitu tuberosum*	Razor-billed Curassow	No	7
Galliformes	Cracidae	*Nothocrax urumutum*	Nocturnal Curassow	No	7
Galliformes	Cracidae	*Oreophasis derbianus*	Horned Guan	No	7
Galliformes	Cracidae	*Ortalis canicollis*	Chaco Chachalaca	No	7
Galliformes	Cracidae	*Ortalis cinereiceps*	Grey-headed Chachalaca	Yes	7
Galliformes	Cracidae	*Ortalis erythroptera*	Rufous-headed Chachalaca	Yes	7
Galliformes	Cracidae	*Ortalis garrula*	Chestnut-winged Chachalaca	No	7
Galliformes	Cracidae	*Ortalis guttata*	Speckled Chachalaca	No	7
Galliformes	Cracidae	*Ortalis leucogastra*	White-bellied Chachalaca	No	7
Galliformes	Cracidae	*Ortalis motmot*	Little Chachalaca	No	7
Galliformes	Cracidae	*Ortalis poliocephala*	West Mexican Chachalaca	No	7
Galliformes	Cracidae	*Ortalis ruficauda*	Rufous-vented Chachalaca	No	7
Galliformes	Cracidae	*Ortalis superciliaris*	Buff-browed Chachalaca	Yes	7
Galliformes	Cracidae	*Ortalis vetula*	Plain Chachalaca	No	7
Galliformes	Cracidae	*Ortalis wagleri*	Rufous-bellied Chachalaca	Yes	7
Galliformes	Cracidae	*Pauxi pauxi*	Helmeted Curassow	No	7
Galliformes	Cracidae	*Pauxi unicornis*	Horned Curassow	No	7
Galliformes	Cracidae	*Penelope albipennis*	White-winged Guan	Yes	7
Galliformes	Cracidae	*Penelope argyrotis*	Band-tailed Guan	No	7

Order	Family	Scientific name	Common name	Data deficient	Taxonomic reference
Galliformes	Cracidae	*Penelope barbata*	Bearded Guan	No	7
Galliformes	Cracidae	*Penelope dabbenei*	Red-faced Guan	No	7
Galliformes	Cracidae	*Penelope jacquacu*	Spix's Guan	No	7
Galliformes	Cracidae	*Penelope jacucaca*	White-browed Guan	No	7
Galliformes	Cracidae	*Penelope marail*	Marail Guan	Yes	7
Galliformes	Cracidae	*Penelope montagnii*	Andean Guan	No	7
Galliformes	Cracidae	*Penelope obscura*	Dusky-legged Guan	No	7
Galliformes	Cracidae	*Penelope ochrogaster*	Chestnut-bellied Guan	No	7
Galliformes	Cracidae	*Penelope ortoni*	Baudo Guan	No	7
Galliformes	Cracidae	*Penelope perspicax*	Cauca Guan	No	7
Galliformes	Cracidae	*Penelope pileata*	White-crested Guan	No	7
Galliformes	Cracidae	*Penelope purpurascens*	Crested Guan	No	7
Galliformes	Cracidae	*Penelope superciliaris*	Rusty-margined Guan	No	7
Galliformes	Cracidae	*Penelopina nigra*	Highland Guan	No	7
Galliformes	Cracidae	*Pipile cujubi*	Red-throated Piping Guan	No	7
Galliformes	Cracidae	*Pipile cumanensis*	Blue-throated Piping Guan	No	7
Galliformes	Cracidae	*Pipile jacutinga*	Black-fronted Piping Guan	No	7
Galliformes	Cracidae	*Pipile pipile*	Trinidad Piping Guan	No	7
Galliformes	Numididae	*Acryllium vulturinum*	Vulturine Guineafowl	No	11
Galliformes	Numididae	*Agelastes meleagrides*	White-breasted Guineafowl	No	11
Galliformes	Numididae	*Agelastes niger*	Black Guineafowl	Yes	11
Galliformes	Numididae	*Guttera plumifera*	Plumed Guineafowl	No	11
Galliformes	Numididae	*Guttera pucherani*	Crested Guineafowl	No	11
Galliformes	Numididae	*Numida meleagris*	Helmeted Guineafowl	No	11
Galliformes	Odontophoridae	*Callipepla californica*	California Quail	No	4
Galliformes	Odontophoridae	*Callipepla douglasii*	Elegant Quail	No	4
Galliformes	Odontophoridae	*Callipepla gambelii*	Gambel's Quail	No	4
Galliformes	Odontophoridae	*Callipepla squamata*	Scaled Quail	No	4
Galliformes	Odontophoridae	*Colinus cristatus*	Crested Bobwhite	No	4
Galliformes	Odontophoridae	*Colinus leucopogon*	Spot-bellied Bobwhite	Yes	4
Galliformes	Odontophoridae	*Colinus nigrogularis*	Yucatan Bobwhite	Yes	4
Galliformes	Odontophoridae	*Colinus virginianus*	Northern Bobwhite	No	4

Order	Family	Scientific name	Common name	Data deficient	Taxonomic reference
Galliformes	Odontophoridae	*Cyrtonyx montezumae*	Montezuma Quail	No	4
Galliformes	Odontophoridae	*Cyrtonyx ocellatus*	Ocellated Quail	Yes	4
Galliformes	Odontophoridae	*Dactylortyx thoracicus*	Singing Quail	Yes	4
Galliformes	Odontophoridae	*Dendrortyx barbatus*	Bearded Wood Partridge	Yes	4
Galliformes	Odontophoridae	*Dendrortyx leucophrys*	Buffy-crowned Wood Partridge	Yes	4
Galliformes	Odontophoridae	*Dendrortyx macroura*	Long-tailed Wood Partridge	Yes	4
Galliformes	Odontophoridae	*Odontophorus atrifrons*	Black-fronted Wood Quail	Yes	4
Galliformes	Odontophoridae	*Odontophorus balliviani*	Stripe-faced Wood Quail	Yes	4
Galliformes	Odontophoridae	*Odontophorus capueira*	Spot-winged Wood Quail	No	4
Galliformes	Odontophoridae	*Odontophorus columbianus*	Venezuelan Wood Quail	Yes	4
Galliformes	Odontophoridae	*Odontophorus dialeucos*	Tacarcuna Wood Quail	Yes	4
Galliformes	Odontophoridae	*Odontophorus erythrops*	Rufous-fronted Wood Quail	Yes	4
Galliformes	Odontophoridae	*Odontophorus gujanensis*	Marbled Wood Quail	No	4
Galliformes	Odontophoridae	*Odontophorus guttatus*	Spotted Wood Quail	Yes	4
Galliformes	Odontophoridae	*Odontophorus hyperythrus*	Chestnut Wood Quail	Yes	4
Galliformes	Odontophoridae	*Odontophorus leucolaemus*	Black-breasted Wood Quail	No	4
Galliformes	Odontophoridae	*Odontophorus melanonotus*	Dark-backed Wood Quail	Yes	4
Galliformes	Odontophoridae	*Odontophorus melanotis*	Black-eared Wood Quail	Yes	4
Galliformes	Odontophoridae	*Odontophorus speciosus*	Rufous-breasted Wood Quail	No	4
Galliformes	Odontophoridae	*Odontophorus stellatus*	Starred Wood Quail	Yes	4
Galliformes	Odontophoridae	*Odontophorus strophium*	Gorgeted Wood Quail	Yes	4
Galliformes	Odontophoridae	*Oreortyx pictus*	Mountain Quail	No	4
Galliformes	Odontophoridae	*Philortyx fasciatus*	Banded Quail	Yes	4
Galliformes	Odontophoridae	*Ptilopachus nahani*	Nahan's Partridge	No	10
Galliformes	Odontophoridae	*Ptilopachus petrosus*	Stone Partridge	No	10
Galliformes	Odontophoridae	*Rhynchortyx cinctus*	Tawny-faced Quail	Yes	4
Galliformes	Phasianidae	*Afropavo congensis*	Congo Peacock	No	10
Galliformes	Phasianidae	*Alectoris barbara*	Barbary Partridge	No	10
Galliformes	Phasianidae	*Alectoris chukar*	Chukar Partridge	No	10

Order	Family	Scientific name	Common name	Data deficient	Taxonomic reference
Galliformes	Phasianidae	*Alectoris graeca*	Rock Partridge	No	10
Galliformes	Phasianidae	*Alectoris magna*	Przevalski's Partridge	No	10
Galliformes	Phasianidae	*Alectoris melanocephala*	Arabian Partridge	No	10
Galliformes	Phasianidae	*Alectoris philbyi*	Philby's Partridge	No	10
Galliformes	Phasianidae	*Alectoris rufa*	Red-legged Partridge	No	10
Galliformes	Phasianidae	*Ammoperdix griseogularis*	See-see Partridge	Yes	10
Galliformes	Phasianidae	*Ammoperdix heyi*	Sand Partridge	No	10
Galliformes	Phasianidae	*Anurophasis monorthonyx*	Snow Mountains Quail	Yes	10
Galliformes	Phasianidae	*Arborophila ardens*	Hainan Partridge	No	10
Galliformes	Phasianidae	*Arborophila atrogularis*	White-cheeked Partridge	Yes	10
Galliformes	Phasianidae	*Arborophila brunneopectus*	Bar-backed Partridge	Yes	10
Galliformes	Phasianidae	*Arborophila cambodiana*	Chestnut-headed Partridge	Yes	10
Galliformes	Phasianidae	*Arborophila charltonii*	Chestnut-necklaced Partridge	Yes	10
Galliformes	Phasianidae	*Arborophila chloropus*	Green-legged Partridge	Yes	10
Galliformes	Phasianidae	*Arborophila crudigularis*	Taiwan Partridge	Yes	10
Galliformes	Phasianidae	*Arborophila davidi*	Orange-necked Partridge	Yes	10
Galliformes	Phasianidae	*Arborophila gingica*	White-necklaced Partridge	No	10
Galliformes	Phasianidae	*Arborophila hyperythra*	Red-breasted Partridge	Yes	10
Galliformes	Phasianidae	*Arborophila javanica*	Chestnut-bellied Partridge	No	10
Galliformes	Phasianidae	*Arborophila mandellii*	Chestnut-breasted Partridge	Yes	10
Galliformes	Phasianidae	*Arborophila merlini*	Annam Hill Partridge	Yes	10
Galliformes	Phasianidae	*Arborophila orientalis*	Grey-breasted Partridge	Yes	10
Galliformes	Phasianidae	*Arborophila rubrirostris*	Red-billed Partridge	Yes	10
Galliformes	Phasianidae	*Arborophila rufipectus*	Sichuan Partridge	No	10
Galliformes	Phasianidae	*Arborophila rufogularis*	Rufous-throated Partridge	No	10
Galliformes	Phasianidae	*Arborophila sumatrana*	Grey-breasted Partridge	Yes	10
Galliformes	Phasianidae	*Arborophila torqueola*	Hill Partridge	No	10
Galliformes	Phasianidae	*Argusianus argus*	Great Argus	No	10
Galliformes	Phasianidae	*Bambusicola fytchii*	Mountain Bamboo Partridge	No	10

Order	Family	Scientific name	Common name	Data deficient	Taxonomic reference
Galliformes	Phasianidae	*Bambusicola thoracicus*	Chinese Bamboo Partridge	No	10
Galliformes	Phasianidae	*Bonasa umbellus*	Ruffed Grouse	No	6
Galliformes	Phasianidae	*Caloperdix oculeus*	Ferruginous Partridge	No	10
Galliformes	Phasianidae	*Catreus wallichii*	Cheer Pheasant	No	10
Galliformes	Phasianidae	*Centrocercus minimus*	Gunnison Grouse	No	6
Galliformes	Phasianidae	*Centrocercus urophasianus*	Sage Grouse	No	6
Galliformes	Phasianidae	*Chrysolophus amherstiae*	Lady Amherst's Pheasant	No	10
Galliformes	Phasianidae	*Chrysolophus pictus*	Golden Pheasant	No	10
Galliformes	Phasianidae	*Coturnix coromandelica*	Rain Quail	Yes	10
Galliformes	Phasianidae	*Coturnix coturnix*	Common Quail	No	10
Galliformes	Phasianidae	*Coturnix delegorguei*	Harlequin Quail	No	10
Galliformes	Phasianidae	*Coturnix japonica*	Japanese Quail	No	10
Galliformes	Phasianidae	*Coturnix pectoralis*	Stubble Quail	No	10
Galliformes	Phasianidae	*Crossoptilon auritum*	Blue Eared Pheasant	No	10
Galliformes	Phasianidae	*Crossoptilon crossoptilon*	White Eared Pheasant	No	10
Galliformes	Phasianidae	*Crossoptilon harmani*	Tibetan Eared Pheasant	No	14
Galliformes	Phasianidae	*Crossoptilon mantchuricum*	Brown Eared Pheasant	No	10
Galliformes	Phasianidae	*Dendragapus fuliginosus*	Sooty Grouse	No	1
Galliformes	Phasianidae	*Dendragapus obscurus*	Dusky Grouse	No	6
Galliformes	Phasianidae	*Dendroperdix sephaena*	Crested Francolin	No	10
Galliformes	Phasianidae	*Excalfactoria adansonii*	Blue Quail	Yes	10
Galliformes	Phasianidae	*Excalfactoria chinensis*	King Quail	No	10
Galliformes	Phasianidae	*Falcipennis candensis*	Spruce Grouse	No	6
Galliformes	Phasianidae	*Falcipennis falcipennis*	Siberian Grouse	No	6
Galliformes	Phasianidae	*Falcipennis franklinii*	Franklin’s Grouse	No	8
Galliformes	Phasianidae	*Francolinus francolinus*	Black Francolin	No	10
Galliformes	Phasianidae	*Francolinus gularis*	Swamp Francolin	No	10
Galliformes	Phasianidae	*Francolinus pictus*	Painted Francolin	No	10
Galliformes	Phasianidae	*Francolinus pintadeanus*	Chinese Francolin	No	10
Galliformes	Phasianidae	*Francolinus pondicerianus*	Grey Francolin	No	10
Galliformes	Phasianidae	*Galloperdix bicalcarata*	Sri Lanka Spurfowl	Yes	10
Galliformes	Phasianidae	*Galloperdix lunulata*	Painted Spurfowl	Yes	10

Order	Family	Scientific name	Common name	Data deficient	Taxonomic reference
Galliformes	Phasianidae	*Galloperdix spadicea*	Red Spurfowl	Yes	10
Galliformes	Phasianidae	*Gallus gallus*	Red Junglefowl	No	10
Galliformes	Phasianidae	*Gallus lafayetii*	Sri Lanka Junglefowl	No	10
Galliformes	Phasianidae	*Gallus sonneratii*	Grey Junglefowl	No	10
Galliformes	Phasianidae	*Gallus varius*	Green Junglefowl	No	10
Galliformes	Phasianidae	*Haematortyx sanguiniceps*	Crimson-headed Partridge	No	10
Galliformes	Phasianidae	*Ithaginis cruentus*	Blood Pheasant	No	10
Galliformes	Phasianidae	*Lagopus lagopus*	Willow Ptarmigan	No	6
Galliformes	Phasianidae	*Lagopus leucura*	White-tailed Ptarmigan	No	6
Galliformes	Phasianidae	*Lagopus muta*	Rock Ptarmigan	No	6
Galliformes	Phasianidae	*Lagopus scoticus*	Red Grouse	No	8
Galliformes	Phasianidae	*Lerwa lerwa*	Snow Partridge	Yes	10
Galliformes	Phasianidae	*Lophophorus impejanus*	Himalayan Monal	No	10
Galliformes	Phasianidae	*Lophophorus lhuysii*	Chinese Monal	No	10
Galliformes	Phasianidae	*Lophophorus sclateri*	Sclater's Monal	No	10
Galliformes	Phasianidae	*Lophura bulweri*	Bulwer's Pheasant	No	10
Galliformes	Phasianidae	*Lophura diardi*	Siamese Fireback	No	10
Galliformes	Phasianidae	*Lophura edwardsi*	Edwards's Pheasant	No	10
Galliformes	Phasianidae	*Lophura erythrophthalma*	Crestless Fireback	No	10
Galliformes	Phasianidae	*Lophura ignita*	Crested Fireback	No	10
Galliformes	Phasianidae	*Lophura inornata*	Salvadori's Pheasant	No	10
Galliformes	Phasianidae	*Lophura leucomelanos*	Kalij Pheasant	No	10
Galliformes	Phasianidae	*Lophura nycthemera*	Silver Pheasant	No	10
Galliformes	Phasianidae	*Lophura swinhoii*	Swinhoe's Pheasant	No	10
Galliformes	Phasianidae	*Lyrurus mlokosiewiczi*	Caucasian Grouse	No	6
Galliformes	Phasianidae	*Lyrurus tetrix*	Black Grouse	No	6
Galliformes	Phasianidae	*Margaroperdix madagarensis*	Madagascar Partridge	No	10
Galliformes	Phasianidae	*Melanoperdix niger*	Black Partridge	Yes	10
Galliformes	Phasianidae	*Meleagris gallopavo*	Wild Turkey	No	12
Galliformes	Phasianidae	*Meleagris ocellata*	Ocellated Turkey	No	12
Galliformes	Phasianidae	*Pavo cristatus*	Indian Peafowl	No	10

Order	Family	Scientific name	Common name	Data deficient	Taxonomic reference
Galliformes	Phasianidae	*Pavo muticus*	Green Peafowl	No	10
Galliformes	Phasianidae	*Peliperdix albogularis*	White-throated Francolin	No	10
Galliformes	Phasianidae	*Peliperdix coqui*	Coqui Francolin	No	10
Galliformes	Phasianidae	*Peliperdix lathami*	Latham's Francolin	No	10
Galliformes	Phasianidae	*Peliperdix schlegelii*	Schlegel's Francolin	No	10
Galliformes	Phasianidae	*Perdicula argoondah*	Rock Bush Quail	Yes	10
Galliformes	Phasianidae	*Perdicula asiatica*	Jungle Bush Quail	No	10
Galliformes	Phasianidae	*Perdicula erythrorhyncha*	Painted Bush Quail	Yes	10
Galliformes	Phasianidae	*Perdicula manipurensis*	Manipur Bush Quail	Yes	10
Galliformes	Phasianidae	*Perdix dauurica*	Daurian Partridge	No	10
Galliformes	Phasianidae	*Perdix hodgsoniae*	Tibetan Partridge	No	10
Galliformes	Phasianidae	*Perdix perdix*	Grey Partridge	No	10
Galliformes	Phasianidae	*Phasianus colchicus*	Common Pheasant	No	10
Galliformes	Phasianidae	*Phasianus versicolor*	Green Pheasant	No	10
Galliformes	Phasianidae	*Polyplectron bicalcaratum*	Grey Peacock-Pheasant	No	10
Galliformes	Phasianidae	*Polyplectron chalcurum*	Bronze-tailed Peacock-Pheasant	No	10
Galliformes	Phasianidae	*Polyplectron germaini*	Germain's Peacock-Pheasant	No	10
Galliformes	Phasianidae	*Polyplectron inopinatum*	Mountain Peacock-Pheasant	No	10
Galliformes	Phasianidae	*Polyplectron katsumatae*	Hainan Peacock-Pheasant	No	5
Galliformes	Phasianidae	*Polyplectron malacense*	Malayan Peacock-Pheasant	No	10
Galliformes	Phasianidae	*Polyplectron napoleonis*	Palawan Peacock-Pheasant	No	10
Galliformes	Phasianidae	*Polyplectron schleiermacheri*	Bornean Peacock-Pheasant	No	10
Galliformes	Phasianidae	*Pternistis adspersus*	Red-billed Spurfowl	No	10
Galliformes	Phasianidae	*Pternistis afer*	Red-necked Spurfowl	No	10
Galliformes	Phasianidae	*Pternistis ahantensis*	Ahanta Francolin	Yes	10
Galliformes	Phasianidae	*Pternistis bicalcaratus*	Double-spurred Francolin	No	10
Galliformes	Phasianidae	*Pternistis camerunensis*	Mount Cameroon Francolin	No	10

Order	Family	Scientific name	Common name	Data deficient	Taxonomic reference
Galliformes	Phasianidae	*Pternistis capensis*	Cape Spurfowl	No	10
Galliformes	Phasianidae	*Pternistis castaneicollis*	Chestnut-naped Francolin	No	10
Galliformes	Phasianidae	*Pternistis clappertoni*	Clapperton's Francolin	No	10
Galliformes	Phasianidae	*Pternistis erckelii*	Erckel's Francolin	No	10
Galliformes	Phasianidae	*Pternistis griseostriatus*	Grey-striped Francolin	No	10
Galliformes	Phasianidae	*Pternistis hartlaubi*	Hartlaub's Spurfowl	No	10
Galliformes	Phasianidae	*Pternistis harwoodi*	Harwood's Francolin	No	10
Galliformes	Phasianidae	*Pternistis hildebrandti*	Hildebrandt's Francolin	No	10
Galliformes	Phasianidae	*Pternistis icterorhynchus*	Heuglin's Francolin	No	10
Galliformes	Phasianidae	*Pternistis jacksoni*	Jackson's Francolin	No	10
Galliformes	Phasianidae	*Pternistis leucoscepus*	Yellow-necked Spurfowl	No	10
Galliformes	Phasianidae	*Pternistis natalensis*	Natal Spurfowl	No	10
Galliformes	Phasianidae	*Pternistis nobilis*	Handsome Francolin	No	10
Galliformes	Phasianidae	*Pternistis ochropectus*	Djibouti Francolin	No	10
Galliformes	Phasianidae	*Pternistis rufopictus*	Grey-breasted Spurfowl	No	10
Galliformes	Phasianidae	*Pternistis squamatus*	Scaly Francolin	No	10
Galliformes	Phasianidae	*Pternistis swainsonii*	Swainson's Spurfowl	No	10
Galliformes	Phasianidae	*Pternistis swierstrai*	Swierstra's Francolin	No	10
Galliformes	Phasianidae	*Pucrasia macrolopha*	Koklass Pheasant	No	10
Galliformes	Phasianidae	*Rheinardia ocellata*	Crested Argus	No	10
Galliformes	Phasianidae	*Rhizothera longirostris*	Long-billed Partridge	Yes	10
Galliformes	Phasianidae	*Rollulus rouloul*	Crested Partridge	No	10
Galliformes	Phasianidae	*Scleroptila afra*	Grey-winged Francolin	No	10
Galliformes	Phasianidae	*Scleroptila finschi*	Finsch's Francolin	No	10
Galliformes	Phasianidae	*Scleroptila levaillantii*	Red-winged Francolin	No	10
Galliformes	Phasianidae	*Scleroptila levaillantoides*	Orange River Francolin	No	10
Galliformes	Phasianidae	*Scleroptila psilolaemus*	Moorland Francolin	No	10
Galliformes	Phasianidae	*Scleroptila shelleyi*	Shelley's Francolin	No	10
Galliformes	Phasianidae	*Scleroptila streptophora*	Ring-necked Francolin	No	10
Galliformes	Phasianidae	*Synoicus australis*	Brown Quail	No	13
Galliformes	Phasianidae	*Synoicus ypsilophora*	Swamp Quail	No	10
Galliformes	Phasianidae	*Syrmaticus ellioti*	Elliot's Pheasant	No	10

Order	Family	Scientific name	Common name	Data deficient	Taxonomic reference
Galliformes	Phasianidae	*Syrmaticus humiae*	Mrs. Hume's Pheasant	No	10
Galliformes	Phasianidae	*Syrmaticus mikado*	Mikado Pheasant	No	10
Galliformes	Phasianidae	*Syrmaticus reevesii*	Reeves's Pheasant	No	10
Galliformes	Phasianidae	*Syrmaticus soemmerringii*	Copper Pheasant	No	10
Galliformes	Phasianidae	*Tetrao parvirostris*	Black-billed Capercaillie	No	6
Galliformes	Phasianidae	*Tetrao urogallus*	Western Capercaillie	No	6
Galliformes	Phasianidae	*Tetraogallus altaicus*	Altai Snowcock	No	10
Galliformes	Phasianidae	*Tetraogallus caspius*	Caspian Snowcock	No	10
Galliformes	Phasianidae	*Tetraogallus caucasicus*	Caucasian Snowcock	Yes	10
Galliformes	Phasianidae	*Tetraogallus himalayensis*	Himalayan Snowcock	No	10
Galliformes	Phasianidae	*Tetraogallus tibetanus*	Tibetan Snowcock	No	10
Galliformes	Phasianidae	*Tetraophasis obscurus*	Verreaux's Monal-Partridge	No	10
Galliformes	Phasianidae	*Tetraophasis szechenyii*	Szechenyi's Monal-Partridge	No	10
Galliformes	Phasianidae	*Tetrastes bonasia*	Hazel Grouse	No	6
Galliformes	Phasianidae	*Tetrastes sewerzowi*	Chinese Grouse	No	6
Galliformes	Phasianidae	*Tragopan blythii*	Blyth's Tragopan	No	10
Galliformes	Phasianidae	*Tragopan caboti*	Cabot's Tragopan	No	10
Galliformes	Phasianidae	*Tragopan melanocephalus*	Western Tragopan	Yes	10
Galliformes	Phasianidae	*Tragopan satyra*	Satyr Tragopan	No	10
Galliformes	Phasianidae	*Tragopan temminckii*	Temminck's Tragopan	No	10
Galliformes	Phasianidae	*Tympanuchus cupido*	Greater Prairie Chicken	No	6
Galliformes	Phasianidae	*Tympanuchus pallidicinctus*	Lesser Prairie Chicken	No	6
Galliformes	Phasianidae	*Tympanuchus phasianellus*	Sharp-tailed Grouse	No	6
Galliformes	Phasianidae	*Xenoperdix obscurata*	Rubeho Forest Partridge	No	2
Galliformes	Phasianidae	*Xenoperdix udzungwensis*	Udzungwa Forest Partridge	No	10

Appendix 3.2. GenBank and Barcode of Life Data System accession numbers for the mitochondrial and nuclear DNA sequences used in the Bayesian phylochronological reconstructions for the Galliformes.

Species	Mitochondrial									Nuclear				
	Control region	12s rDNA	16s rDNA	CO1	Cyt *b*	ND1	ND2	ND4	ND5	C-MOS	AGRP Intron 1 and 2	Rhodopsin Intron 1	B-Fibrinogen Intron 7	Ovomucoid Intron 7
Aburria aburri	660	619	892	1253	999	0	996	1326	826	586	0	0	899	0
	AF165430	AF165442	AF165454	JN801479	AF165466	-	AY140740	AY141940	AY140754	AY140718	-	-	AY140704	-
	-	-	-	AF165490	-	-	-	-	-	-	-	-	-	-
Acryllium vulturinum	662	617	1347	1545	1137	966	1035	1374	1764	0	1132	0	0	443
	NC014180	NC014180	NC014180	NC014180	NC014180	NC014180	NC014180	NC014180	NC014180	-	EF571219	-	-	DQ832070
Aepypodius arfakianus	0	0	0	0	0	0	1035	0	0	0	0	765	0	0
	-	-	-	-	-	-	AF394617	-	-	-	-	AF394645	-	-
Afropavo congensis	662	0	0	0	1137	0	1035	0	0	0	1118	838	869	434
	AJ309514	-	-	-	AF013760	-	DQ768253	-	-	-	EF571221	EF569434	DQ306959	AF170991
Agelastes meleagrides	0	0	0	0	1137	0	0	0	0	0	0	0	0	0
	-	-	-	-	AM236884	-	-	-	-	-	-	-	-	-
Alectoris barbara	661	619	0	0	1137	0	0	0	0	0	0	0	0	0
	AJ222726	AM944502	-	-	Z48771	-	-	-	-	-	-	-	-	-
Alectoris chukar	661	619	1347	1545	1137	966	1035	1374	1767	586	1086	845	859	431
	FJ752426	FJ752426	FJ752426	FJ752426	FJ752426	FJ752426	FJ752426	FJ752426	FJ752426	GU214234	EF571220	EF569435	DQ306960	AF170987
Alectoris graeca	661	0	0	0	1137	0	0	0	0	0	0	0	0	0
	AM084673	-	-	-	Z48772	-	-	-	-	-	-	-	-	-
Alectoris magna	661	0	0	0	1137	0	1020	0	0	586	0	0	0	0
	AF435554	-	-	-	Z48776	-	EU845744	-	-	GU214236	-	-	-	-
Alectoris melanocephala	661	0	0	694	1137	0	0	0	0	0	0	0	0	0
	AJ222736	-	-	GU951807	Z48773	-	-	-	-	-	-	-	-	-
Alectoris philbyi	661	0	0	694	1137	0	0	0	0	0	0	0	0	0
	AJ222738	-	-	HQ168031	Z48774	-	-	-	-	-	-	-	-	-
Alectoris rufa	661	619	0	670	1137	0	1035	0	0	0	1106	845	869	431
	AJ222739	FN675555	-	GU951807	Z48775	-	DQ307002	-	-	-	EF571223	EF569436	DQ306961	AF170988
Alectura lathami	641	621	1346	1545	1137	966	1035	1374	1770	0	0	765	896	453
	NC007227	NC007227	NC007227	NC007227	NC007227	NC007227	NC007227	NC007227	NC007227	-	-	AF394644	AY952647	AY952767
Ammoperdix heyi	0	0	0	0	669	0	0	0	0	0	0	0	0	0
	-	-	-	-	AM236901	-	-	-	-	-	-	-	-	-

Species	Mitochondrial									Nuclear				
	Control region	12s rDNA	16s rDNA	CO1	Cyt *b*	ND1	ND2	ND4	ND5	C-MOS	AGRP Intron 1 and 2	Rhodopsin Intron 1	B-Fibrinogen Intron 7	Ovomucoid Intron 7
Anhima cornuta	0	619	0	687	999	0	996	0	832	586	0	0	0	0
	-	U83728	-	AY140729	AY140735	-	AY140737	-	AY140751	AY140715	-	-	-	-
Anseranas semipalmata	658	618	1342	1545	1137	963	1035	1374	1770	0	0	880	885	0
	NC005933	NC005933	NC005933	NC005933	NC005933	NC005933	NC005933	NC005933	NC005933	-	-	EU737172	AY695132	-
Arborophila ardens	0	0	0	0	1137	0	1020	0	0	0	0	0	0	0
	-	-	-	-	JQ825234	-	JQ825228	-	-	-	-	-	-	-
Arborophila gingica	662	619	1347	1545	1137	966	1035	1374	1767	0	0	0	0	0
	FJ752425	FJ752425	FJ752425	FJ752425	FJ752425	FJ752425	FJ752425	FJ752425	FJ752425	-	-	-	-	-
Arborophila javanica	0	616	0	0	1137	0	1035	0	0	0	0	0	0	445
	-	DQ832097	-	-	AM236890	-	DQ093804	-	-	-	-	-	-	DQ832074
Arborophila rufipectus	662	618	1347	1545	1137	963	1035	1374	1764	0	0	0	0	0
	NC012453	NC012453	NC012453	NC012453	NC012453	NC012453	NC012453	NC012453	NC012453	-	-	-	-	-
Arborophila rufogularis	662	619	1347	1545	1137	966	1035	1374	1763	0	0	0	0	0
	FJ752424	FJ752424	FJ752424	FJ752424	FJ752424	FJ752424	FJ752424	FJ752424	FJ752424	-	-	-	-	-
Arborophila torqueola	662	0	0	0	1137	0	0	0	0	0	0	0	0	0
	DQ834475	-	-	-	AM236889	-	-	-	-	-	-	-	-	-
Argusianus argus	661	0	0	808	1137	0	0	0	0	0	1112	0	0	432
	AJ309515	-	-	BROMB873-07.COI-5P	AF013761	-	-	-	-	-	EF571222	-	-	AF331954
Bambusicola fytchii	664	619	1347	1545	1137	966	1035	1374	1767	0	0	0	0	0
	FJ752423	FJ752423	FJ752423	FJ752423	FJ752423	FJ752423	FJ752423	FJ752423	FJ752423	-	-	-	-	-
Bambusicola thoracicus	664	618	1347	1545	1137	966	1035	1374	1767	586	0	815	859	429
	NC011816	NC011816	NC011816	NC011816	NC011816	NC011816	NC011816	NC011816	NC011816	GU214270	-	EF569437	DQ306962	AF170978
Bonasa umbellus	659	618	0	788	609	0	1035	0	0	0	0	0	0	0
	AF532417	U83740	-	BROMB631-07.COI-5P	AY509677	-	AF222541	-	-	-	-	-	-	-
Callipepla californica	661	0	0	694	1137	0	300	0	0	0	0	0	0	0
	DQ834473	-	-	JQ174236	AB120131	-	AF028773	-	-	-	-	-	-	-
	-	-	-	AY666478	-	-	-	-	-	-	-	-	-	-
Callipepla douglasii	661	0	0	0	433	0	300	0	0	0	0	0	0	0
	DQ834470	-	-	-	AF028750	-	AF028752	-	-	-	-	-	-	-

Species	Mitochondrial									Nuclear				
	Control region	12s rDNA	16s rDNA	CO1	Cyt *b*	ND1	ND2	ND4	ND5	C-MOS	AGRP Intron 1 and 2	Rhodopsin Intron 1	B-Fibrinogen Intron 7	Ovomucoid Intron 7
Callipepla gambelii	660	617	1347	672	1137	0	300	0	0	0	0	0	875	0
	DQ834472	DQ485791	DQ485829	DQ433415	DQ485889	-	AF028764	-	-	-	-	-	DQ494145	-
Callipepla squamata	659	0	0	652	433	0	300	0	0	0	0	0	0	0
	DQ834471	-	-	DQ432806	AF028753	-	AF028758	-	-	-	-	-	-	-
Caloperdix oculeus	0	0	0	0	367	0	0	0	0	0	0	0	0	0
	-	-	-	-	EF620768	-	-	-	-	-	-	-	-	-
Catreus wallichii	662	0	0	0	1137	0	1035	0	0	0	1111	833	638	428
	DQ834499	-	-	-	AF028792	-	DQ768254	-	-	-	EF571213	EF569438	DQ306963	AF170980
Centrocercus minimus	661	0	0	652	0	0	0	0	0	0	0	0	0	0
	GQ902778	-	-	DQ432832	-	-	-	-	-	-	-	-	-	-
Centrocercus urophasianus	661	617	0	697	609	0	1035	0	0	0	0	0	0	0
	AY569303	AF222573	-	DQ433466	AF230177	-	AF222542	-	-	-	-	-	-	-
Chamaepetes goudotii	660	619	891	1253	999	0	996	1326	826	586	0	0	899	0
	AF165434	AF165443	AF165455	JN801552	AF165467	-	AY140741	AY141941	AY140755	AY140719	-	-	AY140705	-
	-	-	-	AF165491	-	-	-	-	-	-	-	-	-	-
Chamaepetes unicolor	0	0	0	652	660	0	0	0	0	0	0	0	0	0
	-	-	-	JQ174405	AY659796	-	-	-	-	-	-	-	-	-
Chauna torquata	0	619	0	687	1137	966	1035	0	0	586	0	887	891	0
	-	AY140700	-	AY140730	AY274030	AY274053	AY274053	-	-	AY140716	-	EU737192	AY140702	-
Chrysolophus amherstiae	662	619	1347	1545	1137	966	1035	1374	1767	0	0	0	0	419
	FJ752434	FJ752434	FJ752434	FJ752434	FJ752434	FJ752434	FJ752434	FJ752434	FJ752434	-	-	-	-	DQ832080
Chrysolophus pictus	662	619	1347	1545	1136	966	1035	1374	1767	586	0	830	640	427
	FJ752433	FJ752433	FJ752433	FJ752433	FJ752433	FJ752433	FJ752433	FJ752433	FJ752433	GU214249	-	EF569439	DQ306964	DQ307014
Colinus cristatus	0	617	0	652	0	0	1035	0	0	0	0	0	880	0
	-	AF222575	-	JQ174493	-	-	AF222544	-	-	-	-	-	EU739393	-
Colinus virginianus	660	617	0	697	1137	966	1035	0	0	0	0	0	875	427
	DQ834469	AF222576	-	DQ433524	AY952697	EU166949	EU166949	-	-	-	-	-	AY952654	AY952772
Coturnix coturnix	0	0	1198	744	1137	0	0	0	0	586	940	787	832	0
	-	-	AF302070	GQ481649	L08377	-	-	-	-	GU214268	EF571216	EU737202	EU739399	-
	-	-	-	SWEBI018-11.COI-5P	-	-	-	-	-	-	-	-	-	-

Species	Mitochondrial									Nuclear				
	Control region	12s rDNA	16s rDNA	CO1	Cyt *b*	ND1	ND2	ND4	ND5	C-MOS	AGRP Intron 1 and 2	Rhodopsin Intron 1	B-Fibrinogen Intron 7	Ovomucoid Intron 7
Coturnix delegorguei	0	0	0	730	0	0	0	0	0	0	0	0	0	0
	-	-	-	BROMB649-07.COI-5P	-	-	-	-	-	-	-	-	-	-
Coturnix japonica	662	617	1347	1545	1137	966	1035	1374	1767	0	918	787	834	447
	AP003195	AP003195	AP003195	NC003408	AP003195	AP003195	AP003195	AP003195	AP003195	-	EF571215	AY952756	AY952657	AY952773
Coturnix pectoralis	61	0	0	645	598	0	0	0	0	0	0	0	0	0
	GQ150370	-	-	GQ150379	GQ150388	-	-	-	-	-	-	-	-	-
Crax alberti	660	0	0	1488	999	0	996	1326	826	0	0	0	0	0
	AY145304	-	-	AY141910	AY141920	-	AY141930	AY141942	AY141960	-	-	-	-	-
Crax alector	660	0	0	1488	999	0	996	1326	826	0	924	802	899	0
	AY145315	-	-	AY141911	AY141921	-	AY141931	AY141943	AY141961	-	EF571218	EU737204	EU739401	-
Crax blumenbachii	660	619	891	1088	999	0	996	1326	826	586	0	0	899	0
	AF165438	AF165444	AF165456	AF165492	AF165468	-	AY140747	AY141944	AY140761	AY140725	-	-	AY140711	-
Crax daubentoni	660	0	0	1488	999	0	996	1326	826	0	0	0	0	0
	AY145305	-	-	AY141912	AY141922	-	AY141932	AY141945	AY141962	-	-	-	-	-
Crax fasciolata	660	0	0	1488	999	0	996	1326	826	0	0	0	0	0
	AY145306	-	-	AY141913	AY141923	-	AY141933	AY141946	AY141963	-	-	-	-	-
Crax globulosa	660	0	0	1488	999	0	996	1326	826	0	0	0	0	0
	AY145316	-	-	AY141914	AY141924	-	AY141934	AY141947	AY141964	-	-	-	-	-
Crax rubra	660	619	0	1488	1137	963	1035	1326	826	0	0	803	898	458
	AY145307	AY274003	-	AY141915	AY956378	AY274050	AY952746	AY141948	AY141965	-	-	AY952750	AY952650	AY952770
Crossoptilon auritum	662	617	1347	1545	1137	966	1035	1374	1767	0	0	0	0	0
	JF937589	JF937589	JF937589	JF937589	JF937589	JF937589	JF937589	JF937589	JF937589	-	-	-	-	-
Crossoptilon crossoptilon	662	617	1347	1545	1137	966	1035	1374	1767	586	1110	833	642	434
	NC016679	NC016679	NC016679	NC016679	NC016679	NC016679	NC016679	NC016679	NC016679	GU214247	EF571217	EF569440	DQ306965	AF170981
Crossoptilon harmani	662	0	0	0	1137	0	0	0	0	586	0	0	0	0
	AY343521	-	-	-	AY343524	-	-	-	-	GU214248	-	-	-	-
Crossoptilon mantchuricum	662	0	0	0	1137	0	0	0	0	0	1113	0	0	0
	AY343522	-	-	-	AF534553	-	-	-	-	-	EF571214	-	-	-
Cyrtonyx montezumae	660	617	0	665	1137	0	1035	0	0	0	0	0	865	434
	DQ834467	AY952764	-	JN801309	AF068192	-	AY952748	-	-	-	-	-	AY952655	AF170976

Species	Mitochondrial									Nuclear				
	Control region	12s rDNA	16s rDNA	CO1	Cyt *b*	ND1	ND2	ND4	ND5	C-MOS	AGRP Intron 1 and 2	Rhodopsin Intron 1	B-Fibrinogen Intron 7	Ovomucoid Intron 7
Dendragapus fuliginosus	661	0	0	674	0	0	0	0	0	0	0	0	0	0
	AF532429	-	-	DQ433563	-	-	-	-	-	-	-	-	-	-
Dendragapus obscurus	661	617	0	697	609	0	1035	0	0	0	0	803	0	0
	AF532426	AF222580	-	DQ433564	AF230178	-	AF222549	-	-	-	-	AF394638	-	-
Dendrocygna javanica	659	618	1343	1545	1137	966	1035	1374	1770	0	0	0	0	0
	NC012844	NC012844	NC012844	NC012844	NC012844	NC012844	NC012844	NC012844	NC012844	-	-	-	-	-
Dendroperdix sephaena	664	617	0	0	1137	0	1035	0	0	0	0	0	0	420
	DQ834515	DQ832104	-	-	AM236894	-	FR691574	-	-	-	-	-	-	DQ832083
Eulipoa wallacei	0	0	0	0	0	0	1035	0	0	0	0	762	0	0
	-	-	-	-	-	-	AF394623	-	-	-	-	AF394651	-	-
Excalfactoria chinensis	661	616	1347	1545	1137	966	1035	1374	1764	0	0	0	0	0
	AB073301	AB073301	AB073301	NC004575	AB073301	AB073301	AB073301	AB073301	AB073301	-	-	-	-	-
Falcipennis candensis	661	617	0	697	1137	0	1035	0	0	0	0	797	622	429
	AF532452	AF222577	-	DQ432923	AF170992	-	AF222546	-	-	-	-	EF569441	DQ306966	AF170986
Falcipennis falcipennis	661	332	0	0	609	0	1035	0	0	0	0	0	0	0
	AF532456	AF230151	-	-	AF230169	-	AF222547	-	-	-	-	-	-	-
Falcipennis franklinii	661	617	0	570	0	0	1035	0	0	0	0	0	0	0
	AF532453	AF222579	-	GQ375588	-	-	AF222548	-	-	-	-	-	-	-
Francolinus francolinus	663	617	465	687	1137	0	913	0	0	0	0	0	0	0
	DQ834514	FR691548	DQ868944	JF498851	AF013762	-	FR691585	-	-	-	-	-	-	-
Francolinus gularis	664	0	0	0	660	0	0	0	0	0	0	0	0	0
	HE793500	-	-	-	U90649	-	-	-	-	-	-	-	-	-
Francolinus pictus	664	0	0	0	175	0	0	0	0	0	0	0	0	0
	HE793492	-	-	-	FR694142	-	-	-	-	-	-	-	-	-
Francolinus pintadeanus	663	617	1347	1545	1137	966	1035	1371	1767	0	0	0	0	0
	NC011817	NC011817	NC011817	NC011817	NC011817	NC011817	NC011817	NC011817	NC011817	-	-	-	-	-
Francolinus pondicerianus	663	618	0	694	1137	0	1035	0	0	0	1090	0	0	427
	HE793502	DQ832103	-	JF498853	FR691632	-	DQ768279	-	-	-	EF571211	-	-	DQ832081
Galloperdix lunulata	0	0	0	0	661	0	0	0	0	0	0	0	0	0
	-	-	-	-	EF620766	-	-	-	-	-	-	-	-	-

Species	Mitochondrial									Nuclear				
	Control region	12s rDNA	16s rDNA	CO1	Cyt *b*	ND1	ND2	ND4	ND5	C-MOS	AGRP Intron 1 and 2	Rhodopsin Intron 1	B-Fibrinogen Intron 7	Ovomucoid Intron 7
Gallus gallus	664	618	1347	1545	1137	966	1035	1374	1767	586	0	811	863	430
	AP003322	AP003322	AP003322	NC007236	AP003322	AP003322	AP003322	AP003322	AP003322	GU214269	-	AY952757	AY952658	AF170979
Gallus lafayetii	664	618	1347	1545	1137	966	1035	1374	1767	0	0	810	862	430
	AP003325	AP003325	AP003325	NC007239	AP003325	AP003325	AP003325	AP003325	AP003325	-	-	EF569442	EF569462	EF569483
Gallus sonneratii	663	565	1347	1545	1137	966	1035	1374	1767	0	1093	811	860	428
	AP006741	AP006741	AP006741	AP006741	AP006741	AP006741	AP006741	AP006741	AP006741	-	EF571210	EF569443	EF569463	EF569484
Gallus varius	663	618	1347	1545	1137	966	1035	1374	1767	0	0	811	865	430
	AP003324	AP003324	AP003324	NC007238	AP003324	AP003324	AP003324	AP003324	AP003324	-	-	EF569444	EF569464	EF569485
Guttera plumifera	0	0	0	201	1137	0	0	0	0	0	0	0	0	0
	-	-	-	HQ998082	AM236883	-	-	-	-	-	-	-	-	-
Guttera pucherani	0	616	0	0	1137	0	1035	0	0	0	0	829	862	444
	-	AY952763	-	-	AM236882	-	AY952747	-	-	-	-	AY952752	AY952652	AY952771
Haematortyx sanguiniceps	469	617	0	0	1137	0	1026	0	0	0	0	0	0	433
	EU036221	FR691560	-	-	EU036222	-	FR691581	-	-	-	-	-	-	FR691704
Ithaginis cruentus	JF921875	JF921875	JF921875	JF921875	JF921875	JF921875	JF921875	JF921875	JF921875	GU214256	-	-	-	
	662	619	1347	1542	1137	966	1035	1374	1767	586	0	0	0	428
Lagopus lagopus	661	617	0	697	609	0	1035	0	0	0	0	0	0	0
	AF532444	AF222583	-	DQ433710	AF230170	-	AF222552	-	-	-	-	-	-	-
Lagopus leucura	661	617	0	680	609	0	1035	0	0	0	0	0	0	0
	AF532439	AF222584	-	DQ433716	AF230171	-	AF222553	-	-	-	-	-	-	-
Lagopus muta	660	617	0	682	1031	0	1035	0	0	0	0	0	0	0
	AJ297170	AF222585	-	DQ433736	AY156344	-	AF222554	-	-	-	-	-	-	-
Lagopus scoticus	0	0	0	0	1137	0	0	0	0	0	1088	0	0	0
	-	-	-	-	EF571187	-	-	-	-	-	EF571206	-	-	-
Leipoa ocellata	0	621	0	0	1137	0	1035	0	0	0	0	758	905	451
	-	AF222586	-	-	AY952695	-	AF222555	-	-	-	-	AF394647	AY952648	AY952768
Lophophorus impejanus	662	617	0	0	1137	0	1035	0	0	0	1116	841	655	431
	AF230309	DQ832098	-	-	AF028796	-	DQ768259	-	-	-	EF571207	EF569445	DQ306967	DQ832075
Lophophorus lhuysii	662	619	1347	1545	1137	966	1035	1374	1767	586	0	0	0	0
	GQ871234	GQ871234	GQ871234	GQ871234	GQ871234	GQ871234	GQ871234	GQ871234	GQ871234	GU214263	-	-	-	-

Species	Mitochondrial									Nuclear				
	Control region	12s rDNA	16s rDNA	CO1	Cyt *b*	ND1	ND2	ND4	ND5	C-MOS	AGRP Intron 1 and 2	Rhodopsin Intron 1	B-Fibrinogen Intron 7	Ovomucoid Intron 7
Lophophorus sclateri	662	618	1347	1545	1137	966	1035	1374	1767	0	0	0	0	0
	FJ752432	FJ752432	FJ752432	FJ752432	FJ752432	FJ752432	FJ752432	FJ752432	FJ752432	-	-	-	-	-
Lophura bulweri	662	0	0	0	1137	0	0	0	0	0	0	0	0	0
	AJ300146	-	-	-	AF314637	-	-	-	-	-	-	-	-	-
Lophura diardi	662	0	0	698	1137	0	0	0	0	0	1112	0	0	0
	AJ300147	-	-	JN709933	AF028797	-	-	-	-	-	EF571209	-	-	-
Lophura edwardsi	662	0	0	445	1137	0	0	0	0	0	1100	0	0	0
	AJ300148	-	-	JN709935	AF314638	-	-	-	-	-	EF571208	-	-	-
Lophura erythrophthalma	662	0	0	0	1137	0	0	0	0	0	0	0	0	0
	AJ300149	-	-	-	AF314639	-	-	-	-	-	-	-	-	-
Lophura ignita	662	618	1347	1545	1137	966	1035	1374	1767	0	0	0	0	0
	NC010781	NC010781	NC010781	NC010781	NC010781	NC010781	NC010781	NC010781	NC010781	-	-	-	-	-
Lophura inornata	662	0	0	0	1137	0	1035	0	0	0	0	834	637	432
	AJ300152	-	-	-	AF314642	-	DQ768260	-	-	-	-	EF569446	DQ306968	DQ307016
Lophura leucomelanos	662	0	0	0	1137	0	0	0	0	0	0	0	0	0
	AJ300153	-	-	-	AF314643	-	-	-	-	-	-	-	-	-
Lophura nycthemera	662	619	1347	1545	1137	966	1035	1374	1767	586	1092	830	641	434
	NC012895	NC012895	NC012895	NC012895	NC012895	NC012895	NC012895	NC012895	NC012895	GU214252	EF571205	EF569447	DQ306969	DQ307017
Lophura swinhoii	662	0	0	0	1137	0	1035	0	0	0	1106	837	641	434
	AJ300155	-	-	-	AF314644	-	DQ768262	-	-	-	EF571204	EF569448	DQ306970	DQ307018
Lyrurus mlokosiewiczi	660	618	0	0	560	0	1035	0	0	0	0	0	0	0
	AF532461	AF222591	-	-	AF230173	-	AF222562	-	-	-	-	-	-	-
Lyrurus tetrix	660	618	0	749	1137	0	1035	0	0	0	1094	0	0	0
	AF532459	AF222593	-	GU571652	EF571183	-	AF222564	-	-	-	EF571203	-	-	-
Macrocephalon maleo	0	0	0	0	1137	0	1035	0	0	0	0	758	0	0
	-	-	-	-	AM236881	-	AF394621	-	-	-	-	AF394649	-	-
Margaroperdix madagarensis	662	0	0	0	660	0	0	0	0	0	0	0	0	0
	DQ834528	-	-	-	U90640	-	-	-	-	-	-	-	-	-
Megapodius cumingii	0	0	0	0	0	0	1035	0	0	0	0	0	0	0
	-	-	-	-	-	-	AF394624	-	-	-	-	-	-	-

Species	Mitochondrial									Nuclear				
	Control region	12s rDNA	16s rDNA	CO1	Cyt *b*	ND1	ND2	ND4	ND5	C-MOS	AGRP Intron 1 and 2	Rhodopsin Intron 1	B-Fibrinogen Intron 7	Ovomucoid Intron 7
Megapodius decollatus	0	0	0	0	0	0	1035	0	0	0	0	0	0	0
	-	-	-	-	-	-	AF394625	-	-	-	-	-	-	-
Megapodius eremita	0	621	0	0	1137	966	1035	0	0	0	0	769	898	0
	-	AY274005	-	-	AF082065	AY274052	AY274052	-	-	-	-	AF394652	EU739436	-
Megapodius forsteni	0	0	0	0	0	0	1035	0	0	0	0	0	0	0
	-	-	-	-	-	-	AF394630	-	-	-	-	-	-	-
Megapodius freycinet	0	0	0	0	660	0	1035	0	0	0	0	769	0	0
	-	-	-	-	AM236880	-	AF394632	-	-	-	-	AF394654	-	-
Megapodius layardi	0	621	0	0	1137	0	1035	0	0	0	0	769	896	455
	-	AY952761	-	-	AY952696	-	AF394635	-	-	-	-	AF394657	AY952649	AY952769
Megapodius pritchardii	0	0	0	0	0	0	1035	0	0	0	0	769	0	0
	-	-	-	-	-	-	AF394636	-	-	-	-	AF394658	-	-
Megapodius reinwardt	0	621	890	1087	999	0	1035	0	832	583	0	769	900	0
	-	AF165441	AF165453	AF165489	AF165465	-	AF394633	-	AY140753	AY140717	-	AF394655	AY140703	-
Megapodius tenimberensis	0	0	0	0	0	0	1035	0	0	0	0	769	0	0
	-	-	-	-	-	-	AF394637	-	-	-	-	AF394659	-	-
Meleagris gallopavo	662	618	1347	1545	1137	966	1035	1374	1767	0	1111	843	614	429
	NC010195	NC010195	NC010195	NC010195	NC010195	NC010195	NC010195	NC010195	NC010195	-	EF571202	AY952758	AY952660	AF170984
Meleagris ocellata	264	0	0	0	0	0	0	0	0	0	0	0	0	0
	AF487120	-	-	-	-	-	-	-	-	-	-	-	-	-
Mitu mitu	660	0	0	1488	999	0	996	1326	826	0	0	0	0	0
	AY145308	-	-	AY141916	AY141926	-	AY141936	AY141949	AY141966	-	-	-	-	-
Mitu salvini	660	0	0	1473	999	0	996	1326	826	0	0	0	0	0
	AY145309	-	-	AY141917	AY141927	-	AY141937	AY141950	AY141967	-	-	-	-	-
Mitu tomentosum	660	0	0	1488	999	0	996	1326	826	0	0	0	0	0
	AY145310	-	-	AY141918	AY141928	-	AY141938	AY141951	AY141968	-	-	-	-	-
Mitu tuberosum	660	619	892	1253	999	0	996	1326	826	586	0	0	898	0
	AF165437	AF165445	AF165457	EU525439	AF165469	-	AY140748	AY141952	AY140762	AY140726	-	-	AY140712	-
	-	-	-	AF165493	-	-	-	-	-	-	-	-	-	-
Nothocrax urumutum	660	619	890	1088	999	0	996	1326	826	586	0	0	899	0
	AF165440	AF165446	AF165458	AF165494	AF165470	-	AY140749	AY141953	AY140763	AY140727	-	-	AY140713	-

Species	Mitochondrial									Nuclear				
	Control region	12s rDNA	16s rDNA	CO1	Cyt *b*	ND1	ND2	ND4	ND5	C-MOS	AGRP Intron 1 and 2	Rhodopsin Intron 1	B-Fibrinogen Intron 7	Ovomucoid Intron 7
Numida meleagris	662	616	1347	1545	1137	954	1035	1374	1764	586	1125	838	853	444
	AP005595	AP005595	AP005595	AP005595	AP005595	AP005595	AP005595	AP005595	AP005595	U88425	EF571201	EU737246	AY952653	AF170975
Odontophorus capueira	661	617	0	0	1137	0	1032	0	0	0	0	0	0	420
	FR691371	FR691563	-	-	FR694136	-	FR691582	-	-	-	-	-	-	FR691703
Odontophorus gujanensis	659	614	0	730	973	0	1023	0	0	0	0	0	0	420
	FR691372	FR691562	-	JN801876	FR694137	-	FR691583	-	-	-	-	-	-	FR691702
Odontophorus leucolaemus	0	0	0	652	0	0	0	0	0	0	0	0	0	0
	-	-	-	JQ175603	-	-	-	-	-	-	-	-	-	-
Odontophorus speciosus	659	617	0	0	1137	0	1032	0	0	0	0	0	0	420
	FR691373	FR691561	-	-	FR694138	-	FR691584	-	-	-	-	-	-	FR691701
Oreophasis derbianus	659	619	891	1088	999	0	996	0	826	586	0	0	899	0
	AF165435	AF165447	AF165459	AF165495	AF165471	-	AY140745	-	AY140759	AY140723	-	-	AY140709	-
Oreortyx pictus	660	615	0	672	1137	0	1035	0	0	0	0	0	872	434
	DQ834468	AY952765	-	DQ433856	AF252860	-	AY952749	-	-	-	-	-	AY952656	AF170977
Ortalis canicollis	656	619	891	1088	999	0	996	1326	826	586	0	0	899	0
	AF165436	AF165448	AF165460	AF165496	AF165472	-	AY140746	AY141954	AY140760	AY140724	-	-	AY140710	-
Ortalis cinereiceps	0	619	0	652	0	0	1035	0	0	0	0	0	0	0
	-	AF222588	-	JQ175625	-	-	AF222558	-	-	-	-	-	-	-
Ortalis garrula	0	0	0	0	660	0	0	0	0	0	0	0	0	0
	-	-	-	-	AY659780	-	-	-	-	-	-	-	-	-
Ortalis guttata	0	618	1346	0	660	0	0	0	0	0	0	0	0	0
	-	AF173561	AY173561	-	AY659782	-	-	-	-	-	-	-	-	-
Ortalis leucogastra	0	0	0	0	660	0	0	0	0	0	0	0	0	0
	-	-	-	-	AY659779	-	-	-	-	-	-	-	-	-
Ortalis motmot	0	0	0	652	660	0	0	0	0	0	0	0	0	0
	-	-	-	JQ175626	AY659778	-	-	-	-	-	-	-	-	-
Ortalis ruficauda	0	0	0	0	660	0	0	0	0	0	0	0	0	0
	-	-	-	-	AY659781	-	-	-	-	-	-	-	-	-
Ortalis vetula	0	619	0	0	1137	0	1035	0	0	0	0	803	899	460
	-	AY952762	-	-	L08384	-	AF394614	-	-	-	-	AY952751	AY952651	AF170974

Species	Mitochondrial									Nuclear				
	Control region	12s rDNA	16s rDNA	CO1	Cyt *b*	ND1	ND2	ND4	ND5	C-MOS	AGRP Intron 1 and 2	Rhodopsin Intron 1	B-Fibrinogen Intron 7	Ovomucoid Intron 7
Pauxi pauxi	660	619	890	1088	1137	0	996	1326	826	586	0	0	899	460
	AF165439	AF165449	AF165461	AF165497	AF068190	-	AY140750	AY141955	AY140764	AY140728	-	-	AY140714	AF170973
Pauxi unicornis	646	0	0	1488	999	0	996	1326	826	0	0	0	0	0
	AY145317	-	-	AY141919	AY141929	-	AY141939	AY141956	AY141969	-	-	-	-	-
Pavo cristatus	663	618	0	757	1137	0	1035	0	0	0	1110	843	873	434
	AJ309513	AY952766	-	BROMB638-07.COI-5P	L08379	-	AF394612	-	-	-	EF571200	AF394640	AY952659	AF170990
Pavo muticus	662	618	1347	1545	1137	965	1035	1374	1767	0	1111	844	873	434
	NC012897	NC012897	NC012897	NC012897	NC012897	NC012897	NC012897	NC012897	NC012897	-	EF571196	EF569449	EF569465	AF170989
Peliperdix albogularis	0	0	0	298	1137	0	0	0	0	0	0	0	0	0
	-	-	-	HQ997931	FR694148	-	-	-	-	-	-	-	-	-
Peliperdix coqui	664	618	0	100	1137	0	1035	0	0	0	0	0	0	427
	FR691379	DQ832105	-	HQ998369	FR691633	-	DQ768278	-	-	-	-	-	-	DQ832084
Peliperdix lathami	664	614	0	298	1137	0	1035	0	0	0	0	0	0	429
	FR691377	FR691546	-	HQ998417	AM236893	-	DQ768257	-	-	-	-	-	-	DQ832082
Peliperdix schlegelii	0	0	0	0	1137	0	0	0	0	0	0	0	0	0
	-	-	-	-	FR694149	-	-	-	-	-	-	-	-	-
Penelope albipennis	0	0	0	822	0	0	0	0	0	0	0	0	0	0
	-	-	-	JN801889	-	-	-	-	-	-	-	-	-	-
Penelope argyrotis	0	0	0	0	660	0	0	0	0	0	0	0	0	0
	-	-	-	-	AY659803	-	-	-	-	-	-	-	-	-
Penelope barbata	0	0	0	824	0	0	0	0	0	0	0	0	0	0
	-	-	-	JN801892	-	-	-	-	-	-	-	-	-	-
Penelope jacquacu	647	0	0	785	660	0	0	0	0	0	0	0	0	0
	AY145318	-	-	JN801894	AY659801	-	-	-	-	-	-	-	-	-
Penelope marail	0	0	0	652	0	0	0	0	0	0	0	0	0	0
	-	-	-	JQ175711	-	-	-	-	-	-	-	-	-	-
Penelope montagnii	0	0	0	0	660	0	0	0	0	0	0	0	0	0
	-	-	-	-	AY659802	-	-	-	-	-	-	-	-	-
Penelope obscura	660	619	892	1203	999	0	996	1326	826	586	0	0	899	0
	AF165432	AF165450	AF165462	JQ175714	AF165474	-	AY140742	AY141957	AY140756	AY140720	-	-	AY140706	-
	-	-	-	AF165498	-	-	-	-	-	-	-	-	-	-

Species	Mitochondrial									Nuclear				
	Control region	12s rDNA	16s rDNA	CO1	Cyt *b*	ND1	ND2	ND4	ND5	C-MOS	AGRP Intron 1 and 2	Rhodopsin Intron 1	B-Fibrinogen Intron 7	Ovomucoid Intron 7
Penelope ochrogaster	658	0	0	0	696	0	451	0	0	0	0	0	0	0
	AY145311	-	-	-	AY367101	-	AY367089	-	-	-	-	-	-	-
Penelope pileata	0	0	0	651	0	0	0	0	0	0	0	0	0	0
	-	-	-	JQ175717	-	-	-	-	-	-	-	-	-	-
Penelope purpurascens	660	0	0	657	696	0	451	0	0	0	0	0	0	0
	AY145312	-	-	JQ175718	AY367103	-	AY367091	-	-	-	-	-	-	-
Penelope superciliaris	660	0	0	0	696	0	451	0	0	0	0	0	0	0
	AY145313	-	-	-	AY367102	-	AY367090	-	-	-	-	-	-	-
Penelopina nigra	660	619	892	1088	999	0	996	1326	826	586	0	0	897	0
	AF165433	AF165451	AF165463	AF165499	AF165475	-	AY140743	AY141958	AY140757	AY140721	-	-	AY140707	-
Perdicula asiatica	662	0	0	0	1137	0	0	0	0	0	0	0	0	0
	DQ834530	-	-	-	AM236902	-	-	-	-	-	-	-	-	-
Perdix dauurica	661	617	1346	1545	1137	966	1035	1374	1767	586	0	0	0	0
	FJ752431	FJ752431	FJ752431	FJ752431	FJ752431	FJ752431	FJ752431	FJ752431	FJ752431	GU214239	-	-	-	-
Perdix hodgsoniae	0	0	0	0	1137	0	1020	0	0	586	0	0	0	0
	-	-	-	-	EU839470	-	EU845764	-	-	GU214244	-	-	-	-
Perdix perdix	661	616	0	719	1137	0	1035	0	0	586	1112	844	646	434
	AF115405	AF222590	-	GU571528	AF028791	-	AF222560	-	-	GU214241	EF571194	EF569456	DQ306971	AF170982
Phasianus colchicus	662	618	1344	1545	1137	966	1035	1374	1767	583	1099	830	638	432
	FJ752430	FJ752430	FJ752430	FJ752430	FJ752430	FJ752430	FJ752430	FJ752430	FJ752430	GU214254	EF571199	AY952759	AY952661	AY952774
Phasianus versicolor	662	617	1346	1545	1137	966	1035	1374	1767	0	0	0	0	0
	AB164626	AB164626	AB164626	NC010778	AB164626	AB164626	AB164626	AB164626	AB164626	-	-	-	-	-
Pipile cujubi	659	0	0	0	696	0	451	0	0	0	0	0	0	0
	AY145314	-	-	-	AY367104	-	AY367092	-	-	-	-	-	-	-
Pipile cumanensis	659	0	0	0	696	0	451	0	0	0	0	0	0	0
	AY145319	-	-	-	AY367105	-	AY367093	-	-	-	-	-	-	-
Pipile jacutinga	660	619	891	1088	999	0	996	1326	826	586	0	0	899	0
	AF165431	AF165452	AF165464	AF165500	AF165476	-	AY140744	AY141959	AY140758	AY140722	-	-	AY140708	-
Pipile pipile	659	0	0	652	696	0	451	0	0	0	0	0	0	0
	AY145320	-	-	JQ175862	AY367106	-	AY367094	-	-	-	-	-	-	-

Species	Mitochondrial									Nuclear				
	Control region	12s rDNA	16s rDNA	CO1	Cyt *b*	ND1	ND2	ND4	ND5	C-MOS	AGRP Intron 1 and 2	Rhodopsin Intron 1	B-Fibrinogen Intron 7	Ovomucoid Intron 7
Polyplectron bicalcaratum	662	618	1347	1545	1137	966	1035	1374	1767	0	0	834	847	419
	NC012900	NC012900	NC012900	NC012900	NC012900	NC012900	NC012900	NC012900	NC012900	-	-	EF569450	EF569466	AF331959
Polyplectron chalcurum	662	0	0	0	1137	0	1035	0	0	0	0	834	845	420
	AJ295256	-	-	-	AF330061	-	DQ768264	-	-	-	-	EF569451	EF569467	AF331956
Polyplectron germaini	662	0	0	0	1137	0	1035	0	0	0	0	836	848	421
	AJ295257	-	-	-	AF330063	-	DQ768266	-	-	-	-	EF569453	DQ306972	AF331960
Polyplectron inopinatum	662	0	0	0	1137	0	1035	0	0	0	1108	836	848	420
	AJ295258	-	-	-	AF330064	-	DQ768267	-	-	-	EF571197	EF569454	EF569469	AF331958
Polyplectron katsumatae	624	0	0	0	1137	0	1035	0	0	0	0	0	0	0
	EU005263	-	-	-	JQ917217	-	JQ917212	-	-	-	-	-	-	-
Polyplectron malacense	662	0	0	0	1137	0	1035	0	0	0	1108	835	846	427
	AJ295260	-	-	-	AF330065	-	DQ768268	-	-	-	EF571195	EF569455	DQ306973	AF331957
Polyplectron napoleonis	662	0	0	0	1137	0	1035	0	0	0	1104	835	848	434
	AJ295259	-	-	-	AF330062	-	DQ768265	-	-	-	EF571198	EF569452	EF569468	AF331955
Polyplectron schleiermacheri	662	0	0	0	1137	0	0	0	0	0	0	0	0	420
	EU005264	-	-	-	EU005260	-	-	-	-	-	-	-	-	EU005269
Pternistis adspersus	662	617	0	0	1137	0	1035	0	0	0	0	0	0	446
	FR691381	DQ832113	-	-	FR691623	-	DQ768276	-	-	-	-	-	-	DQ832095
Pternistis afer	662	618	0	100	1137	0	1032	0	0	0	0	0	0	440
	DQ834533	DQ832111	-	HQ997925	AM236908	-	FR691579	-	-	-	-	-	-	DQ832092
Pternistis bicalcaratus	662	619	0	0	1137	0	1034	0	0	0	0	0	0	433
	FR691370	FR691551	-	-	FR691624	-	FR691578	-	-	-	-	-	-	FR691690
Pternistis camerunensis	662	617	0	100	1136	0	1032	0	0	0	0	0	0	433
	FR691382	FR691552	-	HQ998367	FR691591	-	FR691577	-	-	-	-	-	-	FR691694
Pternistis capensis	662	617	0	0	1137	0	1035	0	0	0	0	0	0	442
	DQ834534	DQ832112	-	-	AM236909	-	DQ768282	-	-	-	-	-	-	DQ832093
Pternistis castaneicollis	0	0	0	0	1137	0	0	0	0	0	0	0	0	0
	-	-	-	-	AM236903	-	-	-	-	-	-	-	-	-
Pternistis clappertoni	662	619	0	0	1137	0	1034	0	0	0	0	0	0	433
	FR691383	FR716655	-	-	FR691602	-	FR691576	-	-	-	-	-	-	FR691693

Species	Mitochondrial									Nuclear				
	Control region	12s rDNA	16s rDNA	CO1	Cyt *b*	ND1	ND2	ND4	ND5	C-MOS	AGRP Intron 1 and 2	Rhodopsin Intron 1	B-Fibrinogen Intron 7	Ovomucoid Intron 7
Pternistis erckelii	0	619	0	694	1137	0	1031	0	0	0	0	0	0	0
	-	FR691553	-	JF498849	FR691589	-	FR691575	-	-	-	-	-	-	-
Pternistis griseostriatus	662	618	0	0	763	0	1035	0	0	0	0	0	0	434
	FR691384	FR691554	-	-	AM236905	-	DQ768284	-	-	-	-	-	-	DQ832089
Pternistis hartlaubi	662	619	0	0	1137	0	1035	0	0	0	0	0	0	433
	FR716656	FR691555	-	-	FR691618	-	FR691572	-	-	-	-	-	-	FR691692
Pternistis harwoodi	0	0	0	0	1137	0	0	0	0	0	0	0	0	0
	-	-	-	-	FR691600	-	-	-	-	-	-	-	-	-
Pternistis hildebrandti	662	0	0	0	1137	0	0	0	0	0	0	0	0	433
	FR691386	-	-	-	FR691595	-	-	-	-	-	-	-	-	FR691691
Pternistis icterorhynchus	0	0	0	0	1137	0	0	0	0	0	0	0	0	0
	-	-	-	-	FR691601	-	-	-	-	-	-	-	-	-
Pternistis jacksoni	0	0	0	0	1137	0	0	0	0	0	0	0	0	0
	-	-	-	-	FR691594	-	-	-	-	-	-	-	-	-
Pternistis leucoscepus	662	619	0	0	1137	0	1032	0	0	0	0	0	0	407
	FR691387	FR691556	-	-	AM236906	-	DQ768283	-	-	-	-	-	-	DQ832090
Pternistis natalensis	662	618	0	0	1137	0	1035	0	0	0	0	0	0	440
	DQ834536	FR691557	-	-	AM236911	-	DQ768285	-	-	-	-	-	-	DQ832094
Pternistis nobilis	0	0	0	100	1137	0	0	0	0	0	0	0	0	0
	-	-	-	HQ998110	FR691592	-	-	-	-	-	-	-	-	-
Pternistis ochropectus	0	0	0	0	1137	0	0	0	0	0	0	0	0	0
	-	-	-	-	FR691590	-	-	-	-	-	-	-	-	-
Pternistis rufopictus	0	0	0	0	1137	0	0	0	0	0	0	0	0	0
	-	-	-	-	FR691588	-	-	-	-	-	-	-	-	-
Pternistis squamatus	662	618	0	298	1133	0	1035	0	0	0	0	0	0	442
	FR691388	DQ832109	-	HQ998353	AM236904	-	DQ768286	-	-	-	-	-	-	DQ832088
Pternistis swainsonii	662	618	0	0	1137	0	1035	0	0	0	0	0	0	443
	DQ834532	DQ832110	-	-	AM236907	-	DQ768287	-	-	-	-	-	-	DQ832091
Pternistis swierstrai	0	0	0	0	1137	0	0	0	0	0	0	0	0	433
	-	-	-	-	FR691593	-	-	-	-	-	-	-	-	FR839020

Species	Mitochondrial									Nuclear				
	Control region	12s rDNA	16s rDNA	CO1	Cyt *b*	ND1	ND2	ND4	ND5	C-MOS	AGRP Intron 1 and 2	Rhodopsin Intron 1	B-Fibrinogen Intron 7	Ovomucoid Intron 7
Ptilopachus nahani	661	616	0	100	1137	0	1035	0	0	0	0	0	0	433
	FR691374	FR691545	-	HQ998326	AM236885	-	DQ768288	-	-	-	-	-	-	DQ832071
Ptilopachus petrosus	659	616	0	0	1134	0	1035	0	0	0	0	0	0	433
	FR691375	FR691544	-	-	AM236886	-	DQ768289	-	-	-	-	-	-	DQ832072
Pucrasia macrolopha	662	619	1347	1545	1137	966	1035	1374	1767	586	0	845	655	429
	FJ752429	FJ752429	FJ752429	FJ752429	FJ752429	FJ752429	FJ752429	FJ752429	FJ752429	GU214266	-	EF569457	DQ306974	AF170983
Rheinardia ocellata	661	0	0	0	1137	0	0	0	0	0	0	0	0	0
	AJ309517	-	-	-	AF330060	-	-	-	-	-	-	-	-	-
Rhizothera longirostris	0	0	0	0	704	0	0	0	0	0	0	0	0	0
	-	-	-	-	EF620767	-	-	-	-	-	-	-	-	-
Rollulus rouloul	0	0	0	0	1134	0	0	0	0	0	1102	778	824	0
	-	-	-	-	AM236888	-	-	-	-	-	EF571193	EU737280	EU739482	-
Scleroptila afra	664	618	0	0	1137	0	1035	0	0	0	0	0	0	427
	DQ834517	AF222581	-	-	AM236897	-	AF222550	-	-	-	-	-	-	DQ832086
Scleroptila finschi	0	0	0	0	1137	0	700	0	0	0	0	0	0	0
	-	-	-	-	FR691607	-	DQ768290	-	-	-	-	-	-	-
Scleroptila levaillantii	664	618	0	298	1137	0	1035	0	0	0	1067	0	0	429
	DQ834516	DQ832106	-	HQ997933	AM236913	-	DQ768291	-	-	-	EF571212	-	-	DQ832085
Scleroptila levaillantoides	664	616	0	0	1137	0	1035	0	0	0	0	0	0	425
	DQ834519	DQ832108	-	-	AM236900	-	DQ768292	-	-	-	-	-	-	FR691698
Scleroptila psilolaemus	0	0	0	0	1137	0	0	0	0	0	0	0	0	0
	-	-	-	-	FR691614	-	-	-	-	-	-	-	-	-
Scleroptila shelleyi	664	618	0	100	1137	0	1035	0	0	0	0	0	0	429
	DQ834518	DQ832107	-	HQ997926	AM236898	-	FR691580	-	-	-	-	-	-	DQ832087
Scleroptila streptophora	663	618	0	0	1137	0	1035	0	0	0	0	0	0	0
	FR691380	FR691550	-	-	FR691617	-	FR691573	-	-	-	-	-	-	-
Synoicus australis	61	617	0	645	598	0	1035	0	0	0	0	0	0	0
	GQ150356	AF222574	-	GQ150384	GQ150393	-	AF222543	-	-	-	-	-	-	-
Synoicus ypsilophora	61	0	0	645	598	0	0	0	0	0	0	0	0	0
	GQ150374	-	-	GQ150383	GQ105394	-	-	-	-	-	-	-	-	-

Species	Mitochondrial									Nuclear				
	Control region	12s rDNA	16s rDNA	CO1	Cyt *b*	ND1	ND2	ND4	ND5	C-MOS	AGRP Intron 1 and 2	Rhodopsin Intron 1	B-Fibrinogen Intron 7	Ovomucoid Intron 7
Syrmaticus ellioti	664	616	1345	1545	1137	966	1035	1374	1767	586	0	829	645	430
	AB164624	AB164624	AB164624	NC010771	AB164624	AB164624	AB164624	AB164624	AB164624	GU214251	-	EF569458	DQ306975	DQ832078
Syrmaticus humiae	664	616	1344	1545	1137	966	1035	1374	1767	0	0	0	0	430
	AB164625	AB164625	AB164625	NC010774	AB164625	AB164625	AB164625	AB164625	AB164625	-	-	-	-	DQ832077
Syrmaticus mikado	663	614	0	0	1137	0	1034	0	0	0	0	0	0	431
	AY368070	DQ832101	-	-	AY368056	-	DQ768294	-	-	-	-	-	-	DQ832079
Syrmaticus reevesii	663	617	1347	1545	1137	966	1035	1374	1767	0	1108	829	645	433
	AB164623	AB164623	AB164623	NC010770	AB164623	AB164623	AB164623	AB164623	AB164623	-	EF571192	EF569459	DQ306976	DQ307020
Syrmaticus soemmerringii	664	617	1347	1545	1137	966	1035	1374	1767	0	0	0	0	0
	AB164622	AB164622	AB164622	NC010767	AB164622	AB164622	AB164622	AB164622	AB164622	-	-	-	-	-
Talegalla fuscirostris	0	0	0	0	0	0	1035	0	0	0	0	764	0	0
	-	-	-	-	-	-	AF394620	-	-	-	-	AF394648	-	-
Tetrao parvirostris	660	618	0	694	557	0	1035	0	0	0	0	0	0	0
	AF532464	AF222592	-	GQ482765	DQ352131	-	AF222563	-	-	-	-	-	-	-
Tetrao urogallus	660	618	0	723	1137	0	1035	0	0	0	1096	0	0	0
	AJ297154	AF222594	-	GU571653	AB120132	-	AF222565	-	-	-	EF571189	-	-	-
Tetraogallus altaicus	0	0	0	694	535	0	0	0	0	0	0	0	0	0
	-	-	-	GQ482760	AY563127	-	-	-	-	-	-	-	-	-
Tetraogallus caspius	0	0	0	0	465	0	0	0	0	0	0	0	0	0
	-	-	-	-	EU106676	-	-	-	-	-	-	-	-	-
Tetraogallus himalayensis	662	0	0	826	1137	0	1020	0	0	586	0	0	0	0
	GQ343513	-	-	BROMB617-07.COI-5P	AY678108	-	EU845748	-	-	GU214275	-	-	-	-
Tetraogallus tibetanus	662	0	0	0	1137	0	1020	0	0	586	0	0	0	0
	GQ343551	-	-	-	EU839456	-	EU845746	-	-	GU214274	-	-	-	-
Tetraophasis obscurus	662	619	1346	1545	1137	966	1035	1374	1767	586	0	0	0	434
	JF921876	JF921876	JF921876	JF921876	JF921876	JF921876	JF921876	JF921876	JF921876	GU214260	-	-	-	EU049331
Tetraophasis szechenyii	662	619	1347	1545	1137	966	1035	1374	1767	586	0	0	0	434
	FJ752428	FJ752428	FJ752428	FJ752428	FJ752428	FJ752428	FJ752428	FJ752428	FJ752428	GU214259	-	-	-	EU049330
Tetrastes bonasia	660	618	1345	1545	1137	966	1035	1374	1767	0	0	805	0	0
	FJ752435	FJ752435	FJ752435	FJ752435	FJ752435	FJ752435	FJ752435	FJ752435	FJ752435	-	-	AF394639	-	-

Species	Mitochondrial									Nuclear				
	Control region	12s rDNA	16s rDNA	CO1	Cyt *b*	ND1	ND2	ND4	ND5	C-MOS	AGRP Intron 1 and 2	Rhodopsin Intron 1	B-Fibrinogen Intron 7	Ovomucoid Intron 7
Tetrastes sewerzowi	660	617	0	0	609	0	1035	0	0	0	0	0	0	0
	AF532422	AF222572	-	-	AF230166	-	AF222540	-	-	-	-	-	-	-
Tragopan blythii	662	0	0	788	1137	0	1035	0	0	0	0	846	652	433
	AF230300	-	-	BROMB311-06.COI-5P	AF200722	-	DQ768272	-	-	-	-	EF569460	DQ306977	DQ307021
Tragopan caboti	662	619	1346	1545	1137	966	1035	1374	1767	0	0	0	0	0
	NC013619	NC013619	NC013619	NC013619	NC013619	NC013619	NC013619	NC013619	NC013619	-	-	-	-	-
Tragopan melanocephalus	184	0	0	0	0	0	0	0	0	0	0	0	0	0
	AF230311	-	-	-	-	-	-	-	-	-	-	-	-	-
Tragopan satyra	662	0	0	798	1137	0	0	0	0	0	1074	0	0	0
	AF532413	-	-	BROMB526-07.COI-5P	AF229837	-	-	-	-	-	EF571191	-	-	-
Tragopan temminckii	662	619	1346	1545	1137	966	1035	1374	1767	586	1076	841	650	424
	FJ752427	FJ752427	FJ752427	FJ752427	FJ752427	FJ752427	FJ752427	FJ752427	FJ752427	GU214265	EF571190	AY952760	EF552789	AY952775
Tympanuchus cupido	661	617	0	668	609	0	1035	0	0	0	0	0	0	0
	AY569305	AF222596	-	AY666333	AF230179	-	AF222567	-	-	-	-	-	-	-
Tympanuchus pallidicinctus	661	617	0	674	609	0	1035	0	0	0	0	0	0	0
	AF532434	AF222597	-	DQ434202	AF230180	-	AF222568	-	-	-	-	-	-	-
Tympanuchus phasianellus	661	617	0	807	1137	0	1035	0	0	0	0	806	621	429
	AF532435	AF222598	-	BROMB254-06.COI-5P	AF068191	-	AF222569	-	-	-	-	EF569461	DQ306978	AF170985
Xenoperdix obscurata	0	0	0	0	0	0	1035	0	0	0	0	0	0	0
	-	-	-	-	-	-	DQ093803	-	-	-	-	-	-	-
Xenoperdix udzungwensis	660	615	0	0	1137	0	1035	0	0	0	0	0	0	431
	DQ834474	DQ832096	-	-	AM236887	-	DQ093800	-	-	-	-	-	-	DQ832073

Chapter 4.

Male competition is not sufficient to explain sexual-size dimorphism in Galliform birds

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R.W. Stein designed the study, assembled the trait data, conducted the phylogenetic comparative analyses and ancestral character state reconstructions, and drafted the manuscript.

Abstract

The Galliformes has been recognized as a model system of sexual selection for over a century. A novel form of evolved weaponry, the tarsal spur, implicated in male-male competition in the Galliformes (landfowl) provides a rare opportunity to separate the effects of intrasexual selection and mode of parental care on sexual-size dimorphism (SSD). Using phylogenetic comparative methods, I test predictions regarding the roles of intrasexual selection and divergent reproductive roles in generating SSD. Ancestral character state reconstruction demonstrated that tarsal spurs are derived in the Galliformes and that spurless taxa either belong to lineages that never evolved tarsal spurs or to lineages that lost tarsal spurs secondarily. I used these reconstructions to assign taxa to tarsal-spur – parental-care groups encompassing variation in intrasexual selection and reproductive roles, which I then used to explain SSD. I predicted and demonstrated that increased intrasexual selection, as indicated by the presence of tarsal spurs in males, was associated with increased SSD when mode of parental care was held constant. I further predicted and demonstrated that divergence in reproductive

roles, as reflected by mode of parental care, was associated with a further increase in SSD when the presence of tarsal spurs in males was held constant. The allometry of SSD varied markedly among tarsal-spur – parental-care groups, such that four of the five groups were characterized by a slope very close to isometry, while the remaining group, which included taxa from lineages that lost tarsal spurs secondarily and had female-only parental care, exhibited pronounced positive allometry suggesting sexually antagonistic selection on body size. It appears that sexual selection alone is not sufficient to account for SSD in the Galliformes.

Introduction

SSD is common in animals whenever the reproductive roles of males and females differ (reviewed in Fairbairn et al. 2007). The most prevalent form of SSD, where females are the larger sex (female-biased SSD), is typical of taxa with indeterminate growth (many arthropods, fish, amphibians and reptiles; Abouheif and Fairbairn 1997). In these groups, female-biased SSD has been attributed to the fecundity gains associated with larger female size (e.g. Head 1995; Prenter et al. 1999; but see Fairbairn et al. 2007). In contrast, males are typically the larger sex (male-biased SSD) in taxa with determinate growth (mammals and birds; Abouheif and Fairbairn 1997); here, male-biased SSD has been attributed to male-male competition over access to females (hereafter, intrasexual selection; Lindenfors, Gittleman and Jones 2007; Székely, Lislevand and Figuerola 2007). Both of these hypotheses lack an explanation for the evolution of smaller body size in one sex, respectively. This is an important omission because SSD does not evolve directly (Price 1984). SSD is a by-product of different body-size optima for males and females that arise as a consequence of natural selection, including sexual selection and sexually antagonistic selection, acting differently on the sexes (Price 1984; Arnqvist and Rowe 2005). Implicit in these hypotheses proposed to explain female-biased and male-biased SSD is the notion that large body size is costly and that natural selection opposes the evolution of large size unless the costs are offset by fecundity gains.

In his original treatise on evolution by sexual selection, Darwin (1871) proposed intrasexual selection as an explanation for why the male is the larger sex in many bird

species and recent large-scale phylogenetic comparative analyses of SSD in birds offer support to his assertion (see, e.g., Dale et al. 2007; Székely, Lislevand and Figuerola 2007). It is important to note, however, that social mating system, classified as polyandry, monogamy or polygyny (see Dunn et al. 2001 for a more detailed classification scheme), was used as proxy for strength of sexual selection in the comparative analyses cited above. It has been suggested that classification schemes for social mating system reflect patterns of parental care rather than strength of sexual selection (Bennett and Owens 2000), and this could be problematic for at least three reasons. First, paternity analyses have revealed important inconsistencies between social mating systems and genetic mating systems. For example, extra-pair paternity is more common in socially monogamous, than in socially polygynous, passerine bird species (Hasselquist and Sherman 2001). Second, mating system is assigned at the species level even though males and females can have different mating systems. For example, paternity analyses in the lekking black grouse (*Lyrurus tetrix*) have demonstrated that males are genetically polygynous and that females are genetically monogamous (Lebigre et al. 2007). Third, and perhaps most importantly, simple classification schemes for social mating system combine male-based intrasexual selection (classic sexual selection) with mode of parental care (Bennett and Owens 2002). Parental care is the arena where sexual conflict over divergent reproductive interests (seeking additional mating opportunities vs. investing in offspring) is resolved (Arnqvist and Rowe 2005; Lessels 2012). Indeed, it has been suggested that sexually antagonistic selection associated with conflict over parental investment could contribute to SSD independent of sexual selection (Gavrilets, Arnqvist and Friberg 2001; Arnqvist and Rowe 2005). As a consequence, social mating system may not be sufficient to capture important sources of variation in the reproductive roles of males and females necessary to provide a process-based explanation of SSD (see below).

Male-based intrasexual selection is frequently associated with the evolution of morphological structures specifically associated with fighting, i.e. weapons (Andersson 1994; Fairbairn 1997; Emlen 2008). In a broad review of animal weaponry, Emlen (2008) concluded that (i) weapons typically evolve in circumstances where males defend spatially restricted resources (ii) weapons tend to be the most variable morphological structures among males, and (iii) variation in the morphology of weapons typically

reflects individual variation in age, body size and/or quality. Birds were not included in Emlen's (2008) review of animal weaponry, likely because specialized weapons are not common in this group (Davison 1985). Although uncommon, two distinct forms of weaponry, wing spurs and tarsal spurs, have evolved in birds (Davison 1985). Both types of spurs are permanent, unelaborated structures derived from bone (Stettenheim 2000). Strong positive relationships between fitness and tarsal spur length and between fitness and tarsal spur symmetry have been demonstrated in *Meleagris gallopavo* (wild turkey; Badyaev et al. 1998). This suggests that avian tarsal spurs evolved in similar contexts and serve similar functions as those attributed to other animal weapons (see Badyaev et al. 1998; Emlen 2008). Tarsal spurs are the most prevalent form of weaponry in birds and they occur predominantly in the order Galliformes (Davison 1985). There is good evidence from the Phasianidae, where tarsal spurs are common, that tarsal spurs play an important role in intrasexual selection (Wittzell 1991; Badyaev et al. 1998). In clades where weaponry evolved in the context of intrasexual selection (such as tarsal spurs), the presence vs. absence of weaponry may provide a proxy for strength of intrasexual selection. If this is the case, then evolved weaponry may also provide the opportunity to distinguish the contributions of intrasexual selection and divergent reproductive roles to SSD. This is the topic of this chapter.

Tarsal spurs are common but not ubiquitous in the Galliformes (Davison 1985); consequently, tarsal spurs provide a rare opportunity to distinguish the effects of intrasexual selection and divergent reproductive roles on SSD. Parental care is the arena where the reproductive roles of males and females diverge most sharply; this is because there is sexual conflict over seeking additional mating opportunities vs. investing in offspring is resolved (Arnqvist and Rowe 2005; Lessels 2012). Thus, mode of parental care integrates female-based intersexual selection and sexually antagonistic selection stemming from conflicts over parental care. In the present study, I first ask the simple question whether SSD (using wing cord as an index of body size) in Galliformes can be predicted by both the presence (vs. absence) of tarsal spurs and mode of parental care (female-only vs. biparental), as predicted by Darwin (1871) and Trivers (1972), respectively. I allow for additive and interactive effects of intrasexual selection and divergence in reproductive roles on the allometry of SSD by modeling tarsal spurs and mode of parental care simultaneously and in conjunction with their interactions with

the size covariate, female wing cord length. The improved performance of a model that includes tarsal spurs and its interaction with female wing chord over a model that includes tarsal spurs and wing cord motivated the investigation of lineage history on the prediction of SSD by tarsal spurs. At the species level, tarsal spurs can be absent in both sexes, present in males only, or present in males and females (Davison 1985). Species that lack tarsal spurs can be classed into two evolutionarily distinct groups: those that belong to lineages that never evolved spurs (spurs are derived within the order, see below) and those that belong to lineages where spurs were lost secondarily. These different histories may impose important selective constraints on the form of SSD, especially in the context of different modes of parental care. As a consequence, I reconstructed the evolutionary history of tarsal spurs on a time-calibrated molecular phylogeny of Galliformes. I used these reconstructions to assign taxa to tarsal-spur – parental-care groups, which encompass variation in intrasexual selection and in the reproductive roles of males and females. I used these groupings to test two directional predictions regarding the contributions of intrasexual selection and divergent reproductive roles to SSD in the Galliformes. First, using tarsal spurs as proxy for intrasexual selection, I predicted that presence of tarsal spurs in males would be associated with increased SSD when mode of parental care was held constant. Second, I predicted that the transition from biparental care to female-only parental care would be associated with an additional increase in SSD when the presence of tarsal spurs in males was held constant. Here, there is also the implicit assumption that large size is costly and that natural selection opposes the evolution of large size in females.

Methods

The Galliformes (landfowl) is composed of five families (Megapodiidae (22 spp.), Cracidae (50 spp.), Numididae (6 spp.), Odontophoridae (32 spp.) and Phasianidae (181 spp.)), 84 genera and 291 species, which are distributed across all of the continents except for Antarctica (del Hoyo, Elliot and Sargatal 1994). All of the taxa within the Megapodiidae, Cracidae and Odontophoridae lack tarsal spurs, and taxa within the Numididae and Phasianidae may or may not have tarsal spurs (Davison 1985). The Megapodiidae is characterized by a completely novel reproductive system among birds; they use extrinsic, abiotic heat sources to incubate their eggs and they do not provide

post-hatching parental care. All of the species within the Numididae and Odontophoridae have biparental care. The majority of the species within Cracidae have biparental care (92%); four taxa within the Cracidae have female-only parental care. In the Phasianidae species exhibit either female-only parental care or biparental care. Due to the complete absence of post-hatching parental care in the Megapodiidae, I excluded all 22 species from analyses of SSD. However, as the sister group to all other Galliformes, taxa within the Megapodiidae were included in ancestral character state reconstructions for tarsal spurs (see below).

I recorded data from the literature for the 223 Galliform taxa and 4 Anseriform outgroup taxa (Appendix 4.1) represented in the time-calibrated molecular phylogeny of Galliformes used in these analyses (see Chapter 3). Specifically, I compiled data for mean male wing-chord length and sample size (n = 195; mm), mean female wing-chord length and sample size (n = 195; mm), mode of post-hatching parental care (n = 223) and tarsal-spur complement (n = 223). For tarsal spurs to be coded as present in females they had to be at least 25% of the length of those present in the male of the same species; this precludes minor protrusions ("raised leg scales") from being coded as weaponry (Hall 1963, Davison 1988).

Parental-care data were taken from Cockburn (2006), who reported known and inferred modes of post-hatch parental care at the species level. These data were subsequently verified, corrected where necessary, and updated through a comprehensive literature review (Appendix 4.1). Among the 223 species of Galliformes and 4 species of Anseriformes (Outgroup taxa) represented in the molecular phylogeny used in these analyses, I was unable to verify mode of parental care for 20 species reported as "known" by Cockburn (2006). These 20 cases were accepted as coded by Cockburn (2006). Eight cases were identified where species with "known" mode of parental care was assessed to be incorrect, and these eight cases were corrected. Five additional cases were identified where species with "inferred" mode of parental care was also assessed to be incorrect; these five cases were also corrected. After excluding the Megapodiidae (n = 22), mode of parental care for the remaining 201 ingroup taxa had the following verification status: for 133 taxa parental care was reported as "known" by Cockburn (2006) and verified as correct, for 20 taxa mode of parental care was reported as "known" by Cockburn (2006) but unverified and for the remaining 48 taxa parental

care was “inferred” by Cockburn (2006) and unverified. Of the 48 taxa where mode of parental care was “inferred” by Cockburn (2006) and unverified, 25% (n = 12) were inferred to have female-only parental care and 75% (n = 36) were inferred to have biparental care.

Wing cord is commonly used as proxy for body size in comparative studies of SSD (Székely et al. 2004; Székely et al. 2007; Dale et al. 2007). In the present study, wing cord was used as the sole proxy for body size (over body mass or tarsometatarus length) because wing cord is much less variable diurnally and seasonally than body mass (Kruger 2005) and because in the Galliformes spurred taxa have longer tarsometatarsus lengths, relative to wing-cord length, than spurless taxa (Davison 1985). Hereafter, “size” refers specifically to wing-cord length. One species, the great argus pheasant (*Argusianus argus*), was excluded from analyses of SSD because the wing feathers of males (primaries, secondaries and tertials) have evolved as an ornament used in sexual displays, much like the upper tail coverts of the peacock’s (*Pavo* spp.) train (Davison 1981). As a consequence, the wing cord of male *Argusianus argus* does not reliably reflect body size. Six ingroup taxa lacked adequate sample sizes (sex-specific n < 2) for wing cord, so these taxa had to be excluded from analyses of SSD. Of the 390 (= 195 spp. × 2 sexes) wing-cord measurements, 84.9% (n = 331) are means with known sample sizes, and of these 331 measurements 0.6% (n = 2) are based on a sample of two individuals. Of the remaining 59 wing-cord measurements, 35.6% (n = 21) are based on the midpoint value of a range with known sample size, 39.0% (n = 23) are based on a mean value with unknown sample size and 25.4% (n = 15) are based on the midpoint value of a range with unknown sample size (Appendix 1).

It has been suggested (e.g. by Fairbairn 1997) that body-size allometry should be characterized with reduced major axis (RMA), rather than ordinary least squares (OLS), regression models because male and female size are subject to measurement error. However, Webb and Freckleton (2007) point out that the difference between the slope parameter (B) estimated under RMA and OLS regression approaches 0 as the correlation between male size and female size approaches 1.0 ($B_{RMA} = B_{OLS} \times r^{-1}$). In birds, a high correlation ($r > 0.9$) is expected between male and female size (Dale et al. 2007; Webb and Freckleton 2007); so the difference between these methods should be negligible. In the present study I use phylogenetic generalized least squares (PGLS;

Pagel 1999, Freckleton et al. 2002) regression models, which are an extension of the OLS regression models (see below). So, first I compare B_{RMA}, B_{OLS} and B_{PGLS} from regressions of ln(male size) on ln(female size). Webb and Freckleton (2007) also point out that the results of OLS, and by extension PGLS, regression of ln(male size) on ln(female size) are statistically identical to those from regression of SSD (ln(male size) – ln(female size)) on ln(female size). SSD is the primary response variable in the present study so I also verify this assertion for PGLS before analyzing SSD.

PGLS is analogous to OLS in that the error distribution is Gaussian; however, the assumption of independence is relaxed in PGLS (Freckleton et al. 2002). The lack of independence among taxa is accounted for in PGLS by using phylogenetic covariance to specify the expected residual correlation structure (Freckleton et al. 2002) and I used a well-supported, dated molecular phylogeny of the Galliformes to specify the expected residual correlation structure (see Chapter 3). To model error variance, I used Pagel's (1999) correlation structure, which assumes constant variance through time. Pagel's (1999) methodology uses maximum likelihood to estimate phylogenetic autocorrelation, lambda (λ), which specifies the optimal branch length transformation for correlated characters (Freckleton et al. 2002). When $\lambda = 0$ character evolution is independent of phylogeny and when $\lambda = 1$ character evolution conforms exactly to Brownian motion. When $0 < \lambda < 1$ the influence of phylogeny is weaker than strict Brownian motion (Freckleton et al. 2002).

I used PGLS regression models, AIC model selection criteria and a set of candidate models (Burnham and Anderson 1998) to characterize SSD in the Galliformes. The PGLS analyses proceeded in two stages. In the first stage, I coded tarsal spurs as a two-state character (presence vs. absence). Here, I competed a set of eight candidate models to explain SSD that included tarsal spurs (proxy for intrasexual selection), mode of parental care (proxy for divergence in reproductive roles), a size covariate (ln(female size) and their interactions. The apparent poor fit between the best models of the candidate set and the data (figure 4.2) suggested that presence vs. absence might be an inadequate characterization of tarsal-spur character states, so I used maximum likelihood to reconstruct the ancestral character states (Pagel 1994) for tarsal spurs (see Chapter 3). Here, I coded tarsal spurs as a discrete character with three states: absent in males and females, present in males and absent in females, and

present in males and females. The model of evolution in ancestral character state reconstruction is determined by the number of character states and specified by a transition rate matrix. I inferred ancestral character states under three nested models ("equal rates", "symmetric rates" and "all rates different") and used likelihood-ratio tests to identify the best-fit model. Ancestral character state reconstruction revealed that tarsal spurs would be more appropriately modeled as a four-state character. Tarsal spurs are derived in the Galliformes, which means that some lineages never evolved spurs and that other lineages lost tarsal spurs secondarily. There are eight possible combinations of tarsal spur and parental care (tarsal-spur – parental-care groups), but three of these groups (never evolved spurs – female-only parental care (n = 4), spurs present in male and females – biparental care (n = 10) and spurs present in males and females – female-only parental care (n = 2)) lacked adequate sample sizes to allow inference from regression models.

In the second stage of PGLS analyses, I included the five tarsal-spur – parental-care groups with sufficient sample sizes (never evolved spurs – biparental care (n = 55), spurs present in males only – biparental care (n = 55), spurs present in males only – female-only parental care (n = 38), spurs lost secondarily – biparental care (n = 17), and spurs lost secondarily – female-only parental care (n = 20)), the size covariate and their interactions. I had two primary goals for these analyses: the first was identifying the best-fit model (or set of models) that explain SSD and the second was testing two *a priori* predictions regarding the roles of intrasexual selection and divergence in reproductive roles on SSD. Identifying the best-fit model (or set of models) requires a flexible modeling approach, so I used "dummy variables," coded as 0 or 1, to assign a different intercept to each of the five tarsal-spur – parental-care groups. Next, I constructed and competed a set of 16 candidate models to explain SSD; the full model included all of the dummy variables, the size covariate and all possible interactions between the dummy variables and the size covariate. Finally, I tested predictions regarding the contributions of intrasexual selection and divergence in reproductive roles to SSD in Galliformes. Testing these predictions required reparameterization of the best-fit model such that the tarsal-spur – parental-care groups were specified as a five-level factor. First, I predicted that the presence of tarsal spurs in males would be associated with an increase in SSD when mode of parental care was held constant. Second, I predicted that the transition

from biparental care to female-only parental care would be associated with an additional increase in SSD when the presence of tarsal spurs in males was held constant. I generated least-squares means within the PGLS model and evaluated these directional predictions with one-tailed tests. Parameter estimates are reported with standard errors. All analyses were conducted in APE v3.0-8 (Paradis et al. 2004; R Development Core Team 2011).

Results

First, I characterized body-size allometry (regression of ln(male size) on ln(female size)) under the OLS, RMA and PGLS regression. OLS characterized positive allometry ($B_{\ln(\text{female size})}$ = 1.056 ± 0.009, t_{199} = 112.9, p < 0.0001, 95% CI = 1.038 – 1.074) with r > 0.99. From the OLS results I calculated the RMA slope ($B_{\ln(\text{female size})}$ = 1.056 × 0.99^{-1} = 1.065 ± 0.009, 95% CI = 1.047 – 1.083), which was very similar to the OLS slope because of the high correlations between male and female size. PGLS similarly characterized positive allometry (Figure 4.1, $B_{\ln(\text{female size})}$ = 1.101 ± 0.015, t_{199} = 72.7, p < 0.0001, 95% CI = 1.072 – 1.131, λ = 0.85). Second, I validated the assertion that regression of SSD, (ln(male size) – ln(female size)), on ln(female size) is statistically equivalent to regression of ln(male size) on ln(female size) in the PGLS framework. PGLS regression of SSD on ln(female size) yielded $B_{\ln(\text{female size})}$ = 0.101 ± 0.015 and λ = 0.85. The B estimates under these PGLS regression models differed by exactly 1.000, the standard errors of the B estimates were identical (0.015, in both cases) and λ = 0.85 in both analyses. These results confirm that these PGLS regression models are statistically identical. In subsequent analyses I focus exclusively on explaining SSD using PGLS.

In the first set of analyses explaining SSD, tarsal spur complement was coded as presence (vs. absence). Including tarsal spur complement improved model fit relative to a reduced model including mode of parental care and the size covariate (Table 4.1, model 2 vs. 4, ΔAIC = 2.9). In addition, tarsal spur complement was included in the two top-ranked models (Table 4.1, models 4 and 6). Model 6 included tarsal spur complement, the size covariate and their interaction, but not mode of parental care (Figure 4.2). Taken together, these results indicate that intrasexual selection is important

in explaining SSD independent of mode of parental care. The importance of tarsal spur complement in explaining SSD in combination with the apparently poor fit of the best models to the data (Figure 4.2; Table 4.1) suggested that the evolutionary history of tarsal spurs warranted investigation.

I conducted maximum likelihood ancestral character state reconstructions with tarsal spur complement coded as a discrete, three-state character (absent in males and females, present in males and absent in females, and present in males and females). The equal-rates model estimates a single transition rate; the symmetric-rates model estimates three rates, and the all-rates-different model estimates six rates (Table 4.2). Two of the transition rates in the all-rates-different model were unidentifiable (Table 4.2), so I constrained them to be 0. When I mapped the ancestral character states inferred by the all-rates-different model (with unidentifiable parameters constrained) back onto the phylogeny that they were inferred from the reconstructions were unrealistic (not shown). As a consequence, I did not consider the reconstructions from the all-rates-different model to be realistic. A likelihood-ratio test indicated that the symmetric-rates model was preferred to the equal rates model ($p = 0.014$). The reconstruction based on symmetric rates appeared to be realistic (Figure 4.3), indicating that there were two classes of taxa that lack tarsal spurs – those that retain the spurless-ancestral condition and those that have lost tarsal spurs secondarily.

In the second stage of analyses explaining SSD, the evolutionary history of tarsal spurs was taken into account by specifying different intercepts for the five tarsal-spur – parental-care groups with sufficient sample sizes. Two sets of models were identified by ΔAIC, those that include an interaction between the size covariate and the tarsal-spur – parental-care group characterized by secondary spur loss – female-only parental care (dummy variable three) and those that do not include this interaction (Table 4.3). Two of the eight models that included this interaction have AIC values > 2 units larger than the AIC of the top models (Table 4.3, models 1 and 2). The six remaining models that include this interaction (Table 4.3, models 4,5,7,9,11,14) can be viewed as equally good fits to the data. I use the simplest of the six top models (Table 4.3, model 14) to characterize allometry of SSD (Figure 4.4; Table 4.4). There was pronounced among-group heterogeneity of slope, such that four of the five tarsal-spur – parental-care groups exhibited a slope very close to isometry ($B_{\ln(\text{female size})} = 0.026 \pm 0.011$; $t_{178} = 2.3$, $p =$

0.024, 95% CI = 0.004 – 0.048) while the tarsal-spur – parental-care group characterized by secondary-spur loss and female-only parental care exhibited pronounced positive allometry ($B_{\ln(\text{female size})}$ = 0.319 ± 0.068, t_{178} = 9.2, p < 0.0001, 95% CI = 0.251 – 0.387). Finally, I generated least-squares means from the PGLS model to test the predictions concerning the contribution of intrasexual selection and divergence in reproductive roles to SSD. As predicted, when mode of parental care was held constant the presence of tarsal spurs in males was associated with increased SSD (difference between least-squares means = 0.035 ± 0.013, t_{178} = 2.7, one-tailed p = 0.0034). Similarly, when the presence of tarsal spurs in males was held constant, the transition from bi-parental care to female-only parental care was associated with a further increase in SSD (difference between least-squares means = 0.044 ± 0.007, t_{178} = 6.0, one-tailed p < 0.0001), as predicted.

Discussion

Using phylogenetic comparative methods and a new molecular phylogenetic hypothesis for the Galliformes, I demonstrate that SSD in the Galliformes is correlated with intrasexual selection and divergence in the reproductive roles of males and females. These results were in accord with predictions. SSD increased in association with the acquisition of tarsal spurs in males, and there was a further increase in SSD in association with the transition from biparental care to female-only parental care. Once the effects of intrasexual selection and mode of parental care were accounted for, there was striking among-group heterogeneity in the allometry of SSD. The tarsal-spur – parental-care group characterized by secondary loss of tarsal spurs and female-only parental care exhibited pronounced positive allometry for SSD (95% CI = 0.25 – 0.39), while the four remaining tarsal-spur – parental-care groups were nearly isometric (95% CI = 0.00 – 0.05). These results are likely robust to ambiguities in tarsal-spur – parental-care group membership associated with ancestral character state reconstruction, which were local and not global reconstructions. The reason for this assertion is that the only ambiguities in tarsal-spur – parental-care group membership were associated with spurless taxa (never evolved spurs vs. secondary spur loss) with biparental care, and allometry of SSD did not differ between these groups (Table 4.4; Figure 4.4). These

results are also likely robust to uncertainties in the trait data because 85% of the wing-cord measurements are means with known sample sizes and because > 75% of the mode of parental care data was verified to be correct. The remainder of the mode of parental care data, inferred to be 75% biparental care and 25% female-only parental care for 48 taxa, is in reasonably close agreement with a recent assessment of 80% biparental care across all birds (Cockburn 2006). It appears that in the Galliformes, SSD has evolved incrementally and dynamically in response to divergence in the reproductive roles of males and females and that intrasexual sexual alone is not sufficient to account for SSD in this order.

Differences in the reproductive interests of males and females arise from strong, persistent selection against inbreeding (members of a pair are typically unrelated) and from anisogamy (sex-dependent differences in reproductive investment; Trivers 1972). As a consequence, the reproductive roles of males and females also differ. Reproductive roles diverge in response to selection from demographic factors (e.g. differential mortality rates and sex-ratio bias) and from the complex social ecology inherent to reproduction (Kokko and Jennions 2008). Differences in the reproductive roles of males and females are associated with selection toward different sex-specific body-size optima and SSD arises as a by product (Fairbairn 1997). Reproductive bouts can be broken down into at least three stages where selection can act on body size: intrasexual competition for access to mates (typically among males), mate choice (typically by females), and parental care (typically by females and males in birds). Intrasexual contest competition among males for access to females is expected to favour large males (Darwin 1871). Female choice operates subsequently on the subset of highly competitive males in the population (Darwin 1871). Working under the assumption that large body size is costly and that natural selection tends to opposes large size in females, female choice can decrease or increase the effect that intrasexual selection can have on SSD. For example, by selecting on traits not directly associated with male size (e.g. age or agility), female choice can decrease the effect of intrasexual selection on SSD. Alternatively, by selecting for extreme forms of condition-dependent ornaments, which are costly, female choice can strengthen the effect of intrasexual selection on SSD and lead to runaway selection (Rowe and Houle 1996; Gavriltes Arnqvist and Friberg 2001). Parental care, a component of parental investment (*sensu* Trivers 1972),

evolves under the trade off between current and future reproduction (Trivers 1972; Kokko and Jennions 2008; Lessels 2012). Parental care enhances offspring fitness at a cost to the fitness of the parent that provides care (Lessels 2012). The fitness of both parents is influenced by the total amount of parental care that their offspring receive, such that each parent would have higher fitness if the other parent provided more of the care (Lessels 2012). Consequently, sexual conflict over parental care is an inescapable component of sexual reproduction (Arnqvist and Rowe 2005; Lessels 2012).

I used the construct outlined above to derive predictions about how intrasexual selection and conflict over reproductive interests should influence SSD. Ancestral character state reconstruction for tarsal spurs provided a unique opportunity to separate the contributions of intrasexual selection and divergent reproductive interests to SSD. Using the presence of tarsal spurs in males as proxy for the strength of intrasexual selection, I predicted that SSD would be higher in taxa with spurred males relative to taxa with spurless males (from lineages that never evolved spurs). Consistent with this first prediction, taxa with spurred males exhibited higher SSD than taxa with spurless males, when parental care was held constant. However, variation in SSD within tarsal-spur – parental-care groups with spurred males was high, suggesting that tarsal spurs may not always be correlated with increases in male size (Figure 4.4). I further predicted that SSD would be higher in taxa with female-only parental care relative to taxa with biparental care. Consistent with this second prediction, taxa with female-only parental care exhibited higher SSD than taxa with biparental care, when the presence of tarsal spurs in males was held constant. These results suggest that a substantial portion of SSD could be generated by intrasexual selection and selection stemming from divergent reproductive interests between males and females. These results suggest that social mating system provides an incomplete characterization of the processes generating SSD.

It was relatively straightforward to generate testable predictions associated with the acquisition of traits indicative of increases in intrasexual selection and changes in mode of parental care; however, generating similar predictions concerning the loss and or reversal of these same traits was less obvious. The reason for this is, at least in part, because male-based intrasexual selection, female choice and sexual conflict over divergent reproductive interests can interact dynamically, via either positive or negative

feedback (Crespi 2004; Kokko and Jennions 2008). There were two scenarios where the loss of tarsal spurs was inferred. When tarsal spurs were lost in taxa with biparental care, SSD became indistinguishable from that of taxa that had never evolved spurs. In this circumstance, it could be that survival selection acting on females during reproduction may have favored increased parental care in males. This may have favoured reduced investment in mating competition in males, smaller male size and a decrease in SSD (Kokko and Jennions 2008). When tarsal spurs were lost in taxa with female-only parental care, allometry of SSD became distinct and pronounced through an interaction with female size. This "interaction" group includes all of the lekking grouse, the dispersed, promiscuous forest grouse and two monotypic genera (*Argusianus argus* and *Rheinartia ocellata*) from the subfamily Pavoninae (also in the Phasianidae). Most grouse have north-temperate distributions (Wiley 1974). The two monotypic genera from the Pavoninae are confined to the tropical rainforests of southeast Asia; however, they have social ecologies that are analogous to those of the disperse, promiscuous forest grouse (Wiley 1974; Davison 1981). The ancestral character state reconstructions for tarsal spurs indicate that the pronounced SSD exhibited within the interaction group is not consistent with the hypothesis that a lekking social system led to the evolution of male-biased SSD in the Phasianidae (Lislevand et al. 2009). Lekking is derived within the grouse and SSD is pronounced across the Phasianidae, such that some of the smaller spurred taxa with female-only parental care (e.g. *Polyplectron* and *Gallus* spp.) exhibit pronounced SSD (Figure 4.4). Alternatively, the presence of multiple elaborate and presumably condition-dependent ornaments in the males of many species characterized by secondary spur loss and female-only parental care coupled with a strong interactive effect of female size on SSD suggest that sexually antagonistic selection on body size may have lead to the distinct and marked SSD allometry characteristic of with female-only parental care that lost tarsal spurs secondarily. If female size was under selection to remain small, due to increased mortality associated with uniparental care, and male size was under selection to become large via male-male competition and or female choice for the extreme forms of condition-dependent ornaments, then pronounced male-biased SSD could have resulted from sexually antagonistic selection, and possibly to sexually antagonistic coevolution, for body size (Locke and Houle 1996; Gavriltes Arnqvist and Friberg 2001; Crespi 2004).

The majority of the recent studies attempting to explain SSD in birds have used social mating system as proxy for the strength of intrasexual competition for mates (Dale et al. 2007; Székely et al. 2007; Lislevand et al. 2009) even though social mating system largely reflects mode of parental care (Bennett and Owens 2002). If social mating system does predominantly reflect mode of parental care then social mating system might be a better proxy for the extent of divergence in reproductive roles, rather than sexual selection, *per se*. Changes in parental care regime are driven by complex interactions between costs of care, male genetic mating system (sexual selection) and female genetic mating system (female choice), sex ratio and other demographic processes (Kokko and Jennions 2008), as mortality bias between males and females (ln(adult male mortality) – ln(adult female mortality)) is positively correlated with male-male competition and with the extent of post-hatching paternal care (Liker and Székely 2005). In the Galliformes, SSD appears to be associated with the acquisition or loss of weapons and divergence in the reproductive interests of males and females (Darwin 1871; Trivers 1972; Arnqvist and Rowe 2005; Lessels 2012). Novel, evolved weaponry, such as tarsal spurs, provides a rare opportunity to separate the effects of intrasexual selection from divergence in reproductive roles.

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Tables and Figures

Table 4.1. AIC model comparison across a candidate set of eight models explaining sexual-size dimorphism in the Galliformes. Models are fit with phylogenetic generalized least squares regression and include a female size covariate (FS), male-based intrasexual (IS), mode of parental care (PC) and their interactions.

Model	**λ**	**AIC**	**∆AIC**
1. FS	0.85	-754.9	8.9
2. FS + PC	0.80	-758.8	5.0
3. FS + IS	0.84	-756.9	6.9
4. FS + PC + IS	0.76	-761.7	2.1
5. FS + PC + FS×PC	0.82	-759.2	4.5
6. FS + IS + FS×IS	0.84	-763.8	0
7. FS + PC + FS×PC + IS + FS×IS	0.82	-733.2	30.6
8. FS + PC + IS + FS×PC + FS×IS + FS×PC×IS	0.78	-725.9	37.8

Notes. PC is coded as female-only vs. biparental; IS is coded as tarsal spur presence vs. absence.

Table 4.2. State transition models used in local ancestral character state reconstructions for tarsal-spur complement in the Galliformes. Tarsal spur complement was coded as a discrete three state character (absent in males and females, present in males and absent in females, and present in males and females). Although the all-rates-different model was preferred, it provided an unrealistic reconstruction (see text for details).

Model	Parameter	Transition rate	Standard error	Log-likelihood
Equal rates	q_{01},q_{02},q_{10},q_{12},q_{20},q_{21}	0.0040	0.0007	-82.97
Symmetric rates	q_{01},q_{10}	0.0032	0.0009	-79.95
	q_{02},q_{20}	0.0019	0.0009	
	q_{12},q_{21}	0.0089	0.0023	
All rates different	q_{10}	0.0036	0.0034	-71.17
	q_{20}	0.0454	0.0128	
	q_{01}	0.0000	--	
	q_{21}	0.0082	0.0054	
	q_{02}	0.0000	--	
	q_{12}	0.0139	0.0029	
All rates different	q_{10}	0.0036	0.0034	-71.17
(parameters constrained)	q_{20}	0.0454	0.0225	
	q_{21}	0.0082	0.0069	
	q_{12}	0.0139	0.0050	

Notes. By convention, transition rate parameters are abbreviated by "*q*." Transition rate parameter subscripts are as follows: "0" corresponds to the state where tarsal spurs are absent in males and females, "1" corresponds to the state where tarsal spurs are present in males and absent in females and "2" corresponds to the state where tarsal spurs are present in males and females. The transition rate parameter "q_{01}" represents the transition rate from tarsal spurs absent in males and females to tarsal spurs present in males and absent in females.

Table 4.3. AIC model comparison across a candidate set of 16 models explaining sexual-size dimorphism in the Galliformes. Models are fit with phylogenetic generalized least squares regression and include five tarsal-spur – parental care groups.

Model	**λ**	**AIC**	**∆AIC**
1. FS + DVs(1 + 2 + 3 + 4) + FS×DVs(1 + 2 + 3 + 4)	0.26	-762.6	3.3
2. FS + DVs(1 + 2 + 3 + 4) + FS×DVs(1 + 2 + 3)	0.33	-763.6	2.3
3. FS + DVs(1 + 2 + 3 + 4) + FS×DVs(1 + 2 + 4)	0.71	-703.7	62.2
4. FS + DVs(1 + 2 + 3 + 4) + FS×DVs(1 + 3 + 4)	0.24	-764.4	1.5
5. FS + DVs(1 + 2 + 3 + 4) + FS×DVs(2 + 3 + 4)	0.24	-763.9	2.0
6. FS + DVs(1 + 2 + 3 + 4) + FS×DVs(1 + 2)	0.68	-694.9	71.0
7. FS + DVs(1 + 2 + 3 + 4) + FS×DVs(1 + 3)	0.32	-764.7	1.2
8. FS + DVs(1 + 2 + 3 + 4) + FS×DVs(1 + 4)	0.63	-699.0	66.9
9. FS + DVs(1 + 2 + 3 + 4) + FS×DVs(2 + 3)	0.38	-764.5	1.4
10. FS + DVs(1 + 2 + 3 + 4) + FS×DVs(2 + 4)	0.65	-702.4	63.5
11. FS + DVs(1 + 2 + 3 + 4) + FS×DVs(3 + 4)	0.31	-765.9	0
12. FS + DVs(1 + 2 + 3 + 4) + FS×DV1	0.65	-695.4	70.5
13. FS + DVs(1 + 2 + 3 + 4) + FS×DV2	0.65	-696.3	69.6
14. FS + DVs(1 + 2 + 3 + 4) + FS×DV3	0.37	-765.9	0
15. FS + DVs(1 + 2 + 3 + 4) + FS×DV4	0.59	-700.3	65.6
16. FS + DVs(1 + 2 + 3 + 4)	0.63	-697.2	68.7

Notes. FS is an abbreviation for female size. DV is an abbreviation for dummy variable. All four dummy variables are included in each model (indicated by DVs(1 + 2 + 3 + 4)). Interactions between the size covariate and the dummy variables are indicated by FS×DV, with FS×DVs(1 + 2 + 3 + 4) indicating inclusion of all individual interactions between the size covariate and the dummy variables. Taxa in the never evolved spurs – biparental care group are represented by the model intercept. Taxa in the secondary-spur loss – biparental care group are represented by DV1. Taxa in the male-only spurs – biparental care group are represented by DV2. Taxa in the secondary-spur loss – female-only care group are represented by DV3. Taxa in the male-only spurs – female-only care group are represented by DV4.

Table 4.4. The simplest phylogenetic generalized least-squares regression model (Table 4.3, model 14) explaining sexual-size dimorphism in the Galliformes based on five tarsal-spur – parental-care groups.

Variable	Parameter estimate	Standard error	t_{178}	*p*-value
FS	0.026	0.011	2.28	0.024
Never evolved spurs – biparental care	-0.102	0.060	-1.69	0.093
Male-only spurs – biparental care	0.035	0.013	2.74	0.007
Male-only spurs – female-only care	0.079	0.013	5.91	<0.001
Secondary-spur loss – biparental care	-0.002	0.014	-1.33	0.185
Secondary-spur loss – female-only care	-1.669	0.186	-8.97	<0.001
Secondary-spur loss – female-only care × FS	0.319	0.035	9.21	<0.001

Notes. FS is an abbreviation for the female size covariate.

Figure 4.1. Phylogenetic generalized least squares regression model describing positive body-size allometry in the Galliformes. Wing cord is used as proxy for male and female size. The solid black line is the fitted regression model and the solid gray line represents isometry. In one species, *Argusianus argus*, the wing feathers of the male have evolved as an ornament used in sexual displays and wing cord in not an accurate measure of body size; this species is excluded from the regression analyses but it is included for reference as an unfilled black diamond.

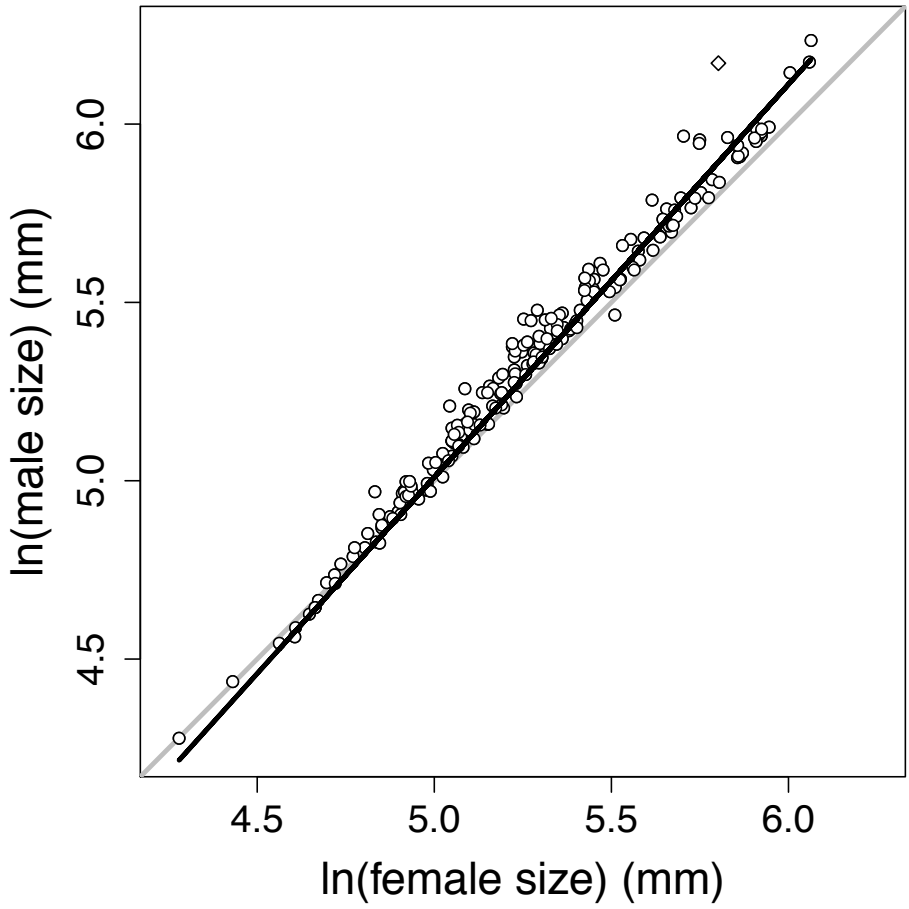


Figure 4.2. Phylogenetic generalized least squares regression model explaining allometry of sexual-size dimorphism in Galliformes based on tarsal spur presence vs. absence and mode of parental care (Table 4.1, model 6). Wing cord is used as proxy for male and female size. The unfilled black circles and black line represent spurless taxa. The filled black triangles and gray line represent spurred taxa. The horizontal gray line represents isometry. In one species, *Argusianus argus*, the wing feathers of the male have evolved as an ornament used in sexual displays and wing cord in not an accurate measure of body size; this species is excluded from the regression analyses but it is included for reference as an unfilled black diamond.

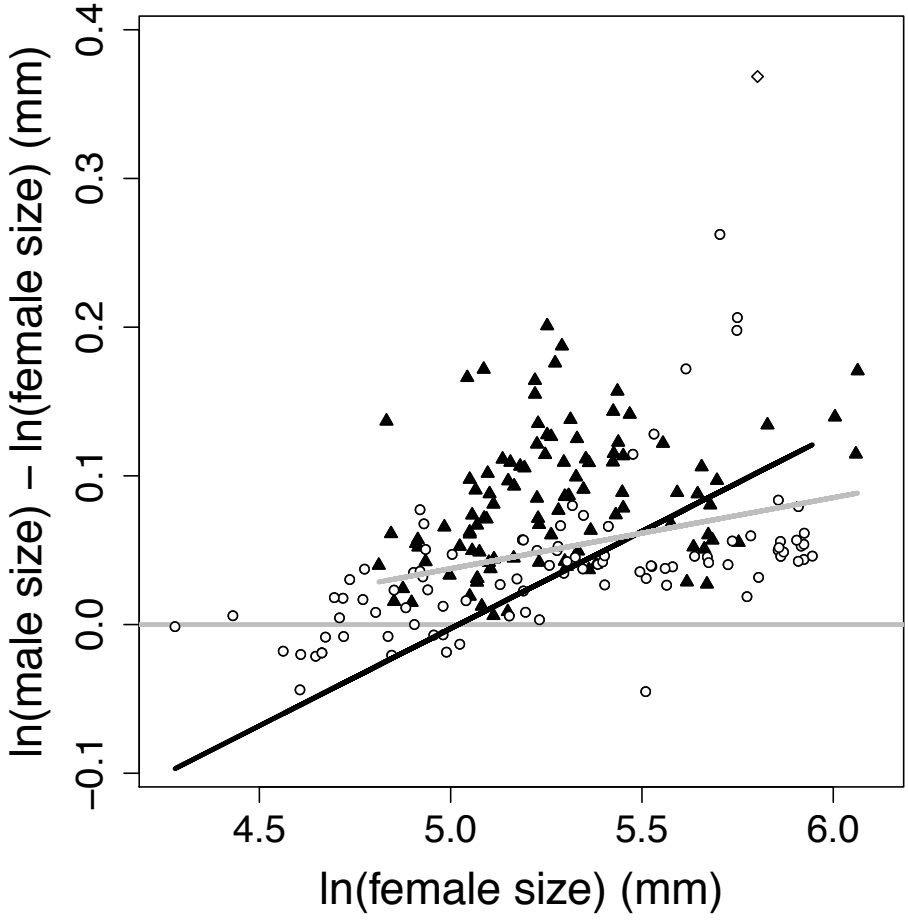


Figure 4.3. Local maximum likelihood ancestral character state reconstruction of tarsal spurs inferred under a symmetric-rates model and represented on a time-calibrated molecular phylogeny of the Galliformes. For the tip states and ancestral reconstructions at nodes white represents tarsal spurs absent in males and females, gray represents tarsal spurs present in males and absent in females, and black represents tarsal spurs present in males and females.

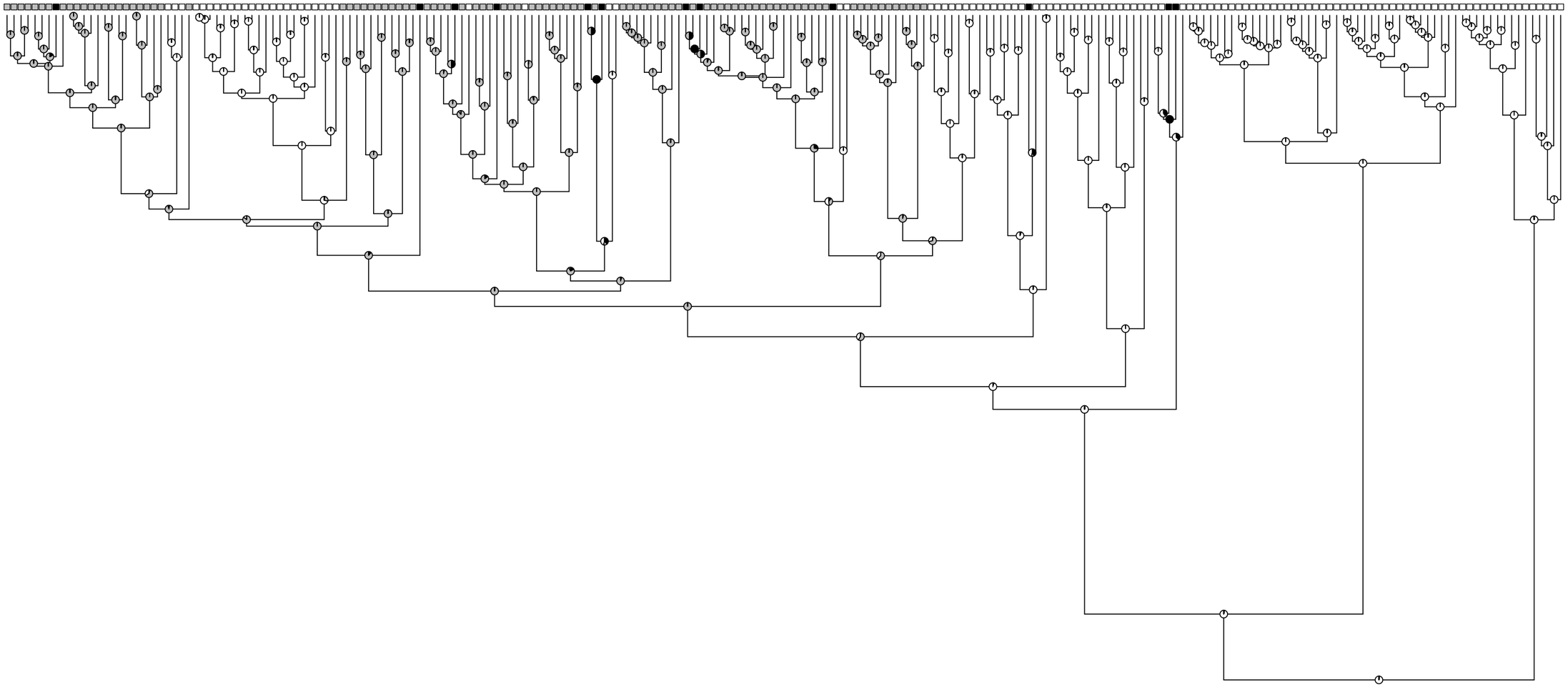
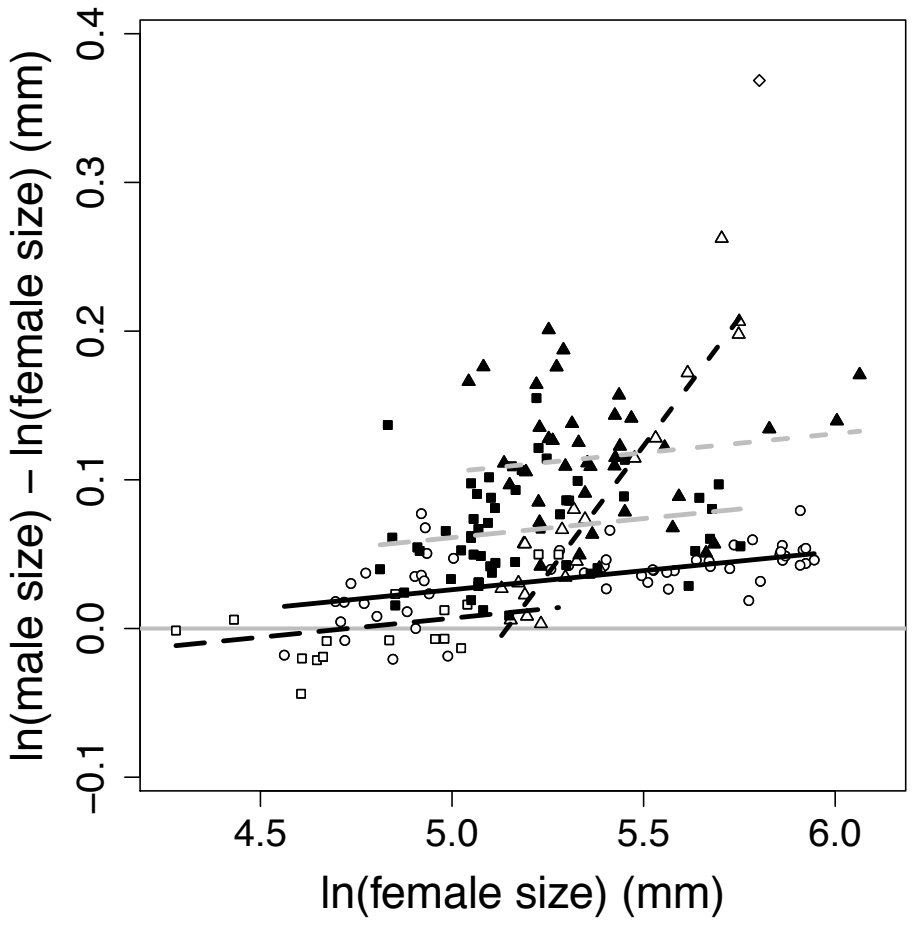


Figure 4.4. Phylogenetic generalized least-squares regression model explaining allometry of sexual-size dimorphism in the Galliformes that incorporates the evolutionary history of tarsal spurs (Table 4.3, model 14). Wing cord is used as proxy for male and female size. Spurless taxa are represented by unfilled symbols and black lines. The unfilled circles and solid black line represent taxa that never evolved spurs and have biparental care. The filled black squares and long-dash gray line represent male-spurred taxa with biparental care. The unfilled black squares and long-dash black line represent spurless taxa (secondary loss in males) with biparental care. The unfilled black triangles and short-dash gray line represent male-spurred taxa with female-only parental care. The filled black triangles and short-dash black line represent spurless taxa (due to secondary loss in males) with female-only parental care. The solid horizontal gray line represents isometry. In one species, *Argusianus argus*, with secondary loss of tarsal spurs the wing feathers of the male have evolved as an ornament used in sexual displays and wing cord in not an accurate measure of body size; this species is excluded from the regression analyses but it is included for reference (unfilled black diamond in upper right corner).



Appendices

Appendix 4.1. Traits values used in ancestral character state reconstruction and phylogenetic comparative analyses of sexual-size dimorphism in the Galliformes.

Species	Male wing cord		Female wing cord		Tarsal spur	Parental care
	(mm)	(n)	(mm)	(n)		
Aburria aburri	371.6	28[91]	351.4	20[91]	0[23]	2[17]
Acryllium vulturinum	298.0	31[89]	290.0	20[89]	2[23]	2[89]
Aepypodius arfakianus	--	--	--	--	0[23]	0[17]
Afropavo congensis	318.0	32[89]	286.0	14[89]	2[23]	2[89]
Agelastes meleagrides	209.0	R7[89]	201.0	R4[89]	2[23]	2[17]
Alectoris barbara	166.0	15[21]	156.0	8[21]	1[23]	2[21]
Alectoris chukar	165.8	20[92]	156.0	25[92]	1[23]	2[21]
Alectoris graeca	160.2	56[92]	152.0	49[92]	1[23]	2[21]
Alectoris magna	173.8	10[15]	172.3	10[50]	1[23]	2[21]
Alectoris melanocephala	193.5	R[45]	173.5	R[45]	1[23]	2[17]
Alectoris philbyi	172.0	M[45]	156.0	M[45]	1[23]	2[17]
Alectoris rufa	165.0	14[21]	157.0	9[21]	1[23]	2[21]
Alectura lathami	--	--	--	--	0[23]	0[17]
Ammoperdix heyi	131.0	12[21]	128.0	11[21]	0[23]	2[21]
*Anhima cornuta**	--	--	--	--	0[23]	2[17]
*Anseranas semipalmata**	--	--	--	--	0[23]	2[17]
Arborophila ardens	120.0	8[49]	118.0	8[49]	0[23]	2[17]
Arborophila gingica	144.1	5[105]	146.8	6[105]	0[23]	2[17]
Arborophila javanica	142.0	M[23]	137.0	M[45]	0[23]	2[17]
Arborophila rufipectus	146.2	26[48]	139.0	27[48]	0[23]	2[5]
Arborophila rufogularis	148.1	R[44]	138.4	R[44]	0[23]	2[3]
Arborophila torqueola	148.0	10[15]	137.0	R8[15]	0[23]	2[3]
Argusianus argus	478.5	R15[93]	331.0	R8[93]	0[23]	1[93]
Bambusicola fytchii	143.7	10[15]	136.4	10[15]	1[23]	2[66]
Bambusicola thoracicus	130.0	23[50]	128.0	10[50]	1[23]	2[17]
Bonasa umbellus	182.0	100[70]	176.5	100[70]	0[23]	1[73]
Callipepla californica	111.5	100[70]	111.0	100[70]	0[23]	2[13]
Callipepla douglasii	111.3	10[71]	112.2	5[71]	0[23]	2[17]

Species	Male wing cord		Female wing cord		Tarsal spur	Parental care
	(mm)	(n)	(mm)	(n)		
Callipepla gambelii	114.0	70[70]	112.0	70[70]	0[23]	2[12]
Callipepla squamata	117.5	80[70]	114.0	80[70]	0[23]	2[22]
Caloperdix oculeus	145.0	R11[93]	139.0	R9[93]	2[23]	2[17]
Catreus wallichii	254	R[44]	232.41	R[44]	1[23]	2[44]
Centrocercus minimus	287.0	100[70]	252.5	100[70]	0[23]	1[76]
Centrocercus urophasianus	326.0	100[70]	274.5	100[70]	0[23]	1[76]
Chamaepetes goudotii	260.7	35[91]	250.6	27[91]	0[23]	2[17]
Chamaepetes unicolor	303.7	21[91]	291.3	11[91]	0[23]	2[25]
*Chauna torquata**	--	--	--	--	0[23]	2[17]
Chrysolophus amherstiae	219.0	15[50]	193.0	6[50]	1[23]	1[97]
Chrysolophus pictus	195.0	R[21]	187.0	R[21]	1[23]	1[4]
Colinus cristatus	94.1	12[94]	95.8	9[94]	0[23]	2[75]
Colinus virginianus	111.5	100[70]	109.5	100[70]	0[23]	2[10]
Coturnix coturnix	104.0	89[21]	106.0	48[21]	0[23]	2[42]
Coturnix delegorguei	95.8	45[42]	100.1	36[42]	0[23]	2[42]
Coturnix japonica	104.0	6[21]	106.0	10[21]	0[23]	2[17]
Coturnix pectoralis	102.1	9[67]	104.3	12[67]	0[23]	2[67]
Crax alberti	390.3	13[91]	373.6	13[91]	0[23]	2[25]
Crax alector	384.2	38[91]	368.2	31[91]	0[23]	2[25]
Crax blumenbachii	391.1	3[91]	371.0	3[91]	0[23]	2[25]
Crax daubentoni	397.7	7[91]	374.0	11[91]	0[23]	1[25]
Crax fasciolata	368.2	16[91]	351.7	20[91]	0[23]	2[25]
Crax globulosa	380.0	14[91]	349.5	15[91]	0[23]	1[17]
Crax rubra	400.0	46[91]	382.0	63[91]	0[23]	2[51]
Crossoptilon auritum	309.1	23[56]	291.0	11[56]	1[23]	2[14]
Crossoptilon crossoptilon	317.0	R5[50]	292.5	R5[50]	1[23]	2[101]
Crossoptilon harmani	295.0	R8[50]	280.0	R4[50]	1[61]	2[62]
Crossoptilon mantchuricum	283.2	46[54]	275.2	46[54]	1[23]	2[54]
Cyrtonyx montezumae	123.0	90[70]	118.5	70[70]	0[23]	2[84]
Dendragapus fuliginosus	221.0	90[70]	204.0	80[70]	0[23]	1[106]
Dendragapus obscurus	226.0	45[70]	210.0	50[70]	0[23]	1[106]

Species	Male wing cord		Female wing cord		Tarsal spur	Parental care
	(mm)	(n)	(mm)	(n)		
*Dendrocygna javanica**	--	--	--	--	0[23]	2[17]
Dendroperdix sephaena	155.9	61[42]	146.0	65[42]	1[23]	2[42]
Eulipoa wallacei	--	--	--	--	0[23]	0[17]
Excalfactoria chinensis	72.1	17[67]	72.2	6[67]	0[23]	2[67]
Falcipennis canadensis	182.0	16[8]	180.5	15[8]	0[23]	1[8]
Falcipennis falcipennis	187.8	18[2]	187.2	18[2]	0[23]	1[27]
Falcipennis franklinii	183.6	11[8]	179.5	8[8]	0[23]	1[8]
Francolinus francolinus	173.7	27[27]	166.2	24[27]	1[23]	2[21]
Francolinus gularis	183.0	M[23]	175.0	M[23]	1[23]	2[66]
Francolinus pictus	144.5	5[1]	145.5	5[1]	0[23]	2[3]
Francolinus pintadeanus	143.9	10[14,15]	125.5	R6[15,35]	1[23]	2[3]
Francolinus pondicerianus	143.2	31[48]	135.6	29[48]	1[23]	2[3]
Gallus gallus	222.5	R22[93]	199.5	R13[93]	1[23]	1[93]
Gallus lafayetii	233.5	8[1]	191.0	3[1]	1[23]	1[17]
Gallus sonneratii	239.4	11[48]	198.5	11[48]	1[23]	1[17]
Gallus varius	232.5	R[26]	195	M[26]	1[23]	1[17]
Guttera plumifera	228.0	15[89]	222.0	11[89]	0[23]	2[89]
Guttera pucherani	268.0	55[42]	261.0	34[42]	0[23]	2[42]
Haematortyx sanguiniceps	168.0	M[23]	160.0	M[23]	1[23]	2[17]
Ithaginis cruentus	205.0	78[98]	193.0	36[98]	1[23]	2[58]
Lagopus lagopus	195.5	100[70]	186.0	100[70]	0[23]	2[21]
Lagopus leucura	189.5	100[70]	179.0	100[70]	0[23]	1[9]
Lagopus muta	190.0	100[70]	179.5	100[70]	0[23]	1[69]
Lagopus scoticus	206.0	15[21]	196.0	16[21]	0[23]	2[21]
Leipoa ocellata	--	--	--	--	0[23]	0[17]
Lophophorus impejanus	293.2	16[49]	268.3	15[49]	1[23]	1[44]
Lophophorus lhuysii	332.9	13[49]	315.0	3[49]	1[23]	2[40,72]
Lophophorus sclateri	309.0	13[49]	283.0	9[49]	1[23]	2[64]
Lophura bulweri	260.0	M[4]	230.0	M[4]	1[23]	1[17]
Lophura diardi	255.0	2[83]	227.3	3[83]	1[23]	1[17]
Lophura edwardsi	228.0	22[41]	214.0	8[41]	1[23]	1[17]

Species	Male wing cord		Female wing cord		Tarsal spur	Parental care
	(mm)	(n)	(mm)	(n)		
Lophura erythrophthalma	246.0	R7[93]	228.5	R9[93]	2[23]	1[93]
Lophura ignita	292.0	R14[93]	258.5	R16[93]	1[23]	1[93]
Lophura inornata	221.0	3[82]	213.0	5[82]	1[23]	2[5]
Lophura leucomelanos	227.5	7[1]	206.0	7[1]	1[23]	2[44]
Lophura nycthemera	252.0	15[41]	233.0	10[41]	1[23]	1[14]
Lophura swinhoii	262.0	7[15,50]	227.0	3[15,50]	1[23]	1[77]
Lyrurus mlokosiewiczi	211.4	30[47]	197.8	7[47]	0[23]	1[21]
Lyrurus tetrix	268.0	68[27]	239.0	59[27]	0[23]	1[21]
Macrocephalon maleo	--	--	--	--	0[23]	0[17]
Margaroperdix madagarensis	125.0	M[32]	126.0	M[32]	0[23]	2[17]
Megapodius cumingii	--	--	--	--	0[23]	0[17]
Megapodius decollatus	--	--	--	--	0[23]	0[17]
Megapodius eremita	--	--	--	--	0[23]	0[17]
Megapodius forsteni	--	--	--	--	0[23]	0[17]
Megapodius freycinet	--	--	--	--	0[23]	0[17]
Megapodius layardi	--	--	--	--	0[23]	0[17]
Megapodius pritchardii	--	--	--	--	0[23]	0[17]
Megapodius reinwardt	--	--	--	--	0[23]	0[17]
Megapodius tenimberensis	--	--	--	--	0[23]	0[17]
Meleagris gallopavo	510.0	47[70]	430.0	36[70]	1[23]	1[29]
Meleagris ocellata	388.5	8[71]	339.7	6[71]	1[23]	1[51]
Mitu mitu	--	--	--	--	0[23]	2[25]
Mitu salvini	372.0	15[91]	354.3	9[91]	0[23]	2[25]
Mitu tomentosum	367.2	12[91]	349.3	10[91]	0[23]	2[17]
Mitu tuberosum	398.7	41[91]	368.3	30[91]	0[23]	2[25]
Nothocrax urumutum	294.0	12[91]	280.8	8[91]	0[23]	2[17]
Numida meleagris	270.0	213[42]	260.0	123[42]	0[23]	2[42]
Odontophorus capueira	156.2	10[6]	149.0	7[6]	0[23]	2[24,31]
Odontophorus gujanensis	143.1	17[70]	139.8	10[70]	0[23]	2[79,80]
Odontophorus leucolaemus	124.6	10[94]	127.2	7[94]	0[23]	2[38,81]
Odontophorus speciosus	139.5	9[6]	134.7	10[6]	0[23]	2[17]

Species	Male wing cord		Female wing cord		Tarsal spur	Parental care
	(mm)	(n)	(mm)	(n)		
Oreophasis derbianus	388.0	21[91]	366.6	9[91]	0[23]	1[25]
Oreortyx pictus	133.5	100[70]	132.0	100[70]	0[23]	2[36]
Ortalis canicollis	232.4	23[91]	221.9	21[91]	0[23]	2[17]
Ortalis garrula	239.4	19[91]	224.1	21[91]	0[23]	2[17]
Ortalis guttata	199.9	76[91]	192.1	61[91]	0[23]	2[17]
Ortalis leucogastra	217.6	26[91]	209.6	31[91]	0[23]	2[17]
Ortalis motmot	207.1	44[91]	196.5	32[91]	0[23]	2[17]
Ortalis ruficauda	230.3	14[91]	220.8	18[91]	0[23]	2[17]
Ortalis vetula	209.9	46[91]	201.2	30[91]	0[23]	2[25]
Pauxi pauxi	394.3	8[91]	373.6	6[91]	0[23]	2[19]
Pauxi unicornis	--	--	--	--	0[23]	1[19]
Pavo cristatus	465.9	11[68]	405.2	9[68]	1[23]	1[96]
Pavo muticus	480.0	4[15,59]	428.0	3[14,15]	2[23]	1[17]
Peliperdix albogularis	135.0	4[89]	127.0	4[89]	1[23]	2[17]
Peliperdix coqui	134.2	221[42]	131.0	139[42]	1[23]	2[17]
Peliperdix lathami	136.0	24[89]	134.0	39[89]	2[23]	2[89]
Peliperdix schlegelii	128.0	10[89]	123.0	8[89]	1[23]	2[17]
Penelope argyrotis	275.6	21[91]	265.1	20[91]	0[23]	2[17]
Penelope barbata	261.1	6[91]	251.1	6[91]	0[23]	2[17]
Penelope jacquacu	303.8	67[91]	290.3	37[91]	0[23]	2[17]
Penelope montagnii	255.2	40[91]	247.4	23[91]	0[23]	2[17]
Penelope obscura	328.1	19[91]	322.0	9[91]	0[23]	2[17]
Penelope ochrogaster	--	--	--	--	0[23]	2[17]
Penelope pileata	319.0	13[91]	306.4	9[91]	0[23]	2[17]
Penelope purpurascens	368.7	30[91]	350.1	18[91]	0[23]	2[34]
Penelope superciliaris	252.2	23[91]	243.4	21[91]	0[23]	2[17]
Penelopina nigra	236.2	29[91]	247.1	15[91]	0[23]	1[25]
Perdicula asiatica	84.5	R[1]	84.0	R[1]	0[23]	2[95]
Perdix dauurica	147.3	12[27]	145.5	13[27]	0[23]	2[104]
Perdix hodgsoniae	141.0	14[15]	142.0	12[15]	0[23]	2[60]
Perdix perdix	157.0	25[27]	154.5	19[27]	0[23]	2[21]

Species	Male wing cord		Female wing cord		Tarsal spur	Parental care
	(mm)	(n)	(mm)	(n)		
Phasianus colchicus	253.0	24[27]	226.8	10[27]	1[23]	1[27]
Phasianus versicolor	234.0	M[87]	206.5	M[87]	1[23]	1[4]
Pipile cujubi	345.2	17[91]	325.2	7[91]	0[23]	2[17]
Pipile cumanensis	327.7	55[91]	309.8	44[91]	0[23]	2[17]
Pipile jacutinga	342.6	5[91]	331.9	14[91]	0[23]	2[25]
Pipile pipile	--	--	--	--	0[23]	2[17]
Polyplectron bicalcaratum	218	9[20]	185	3[20]	1[23]	1[44]
Polyplectron chalcurum	183.0	M[4]	155.0	M[4]	1[23]	1[17]
Polyplectron germaini	190.0	R[26]	172.5	R[26]	1[23]	1[17]
Polyplectron inopinatum	202.5	R9[93]	186.0	R9[93]	1[23]	1[2]
Polyplectron katsumatae	192.1	13[15,33,35,55]	161.1	8[15,33,35,55]	1[23]	1[17]
Polyplectron malacense	213.5	R7[93]	186.5	R5[93]	1[23]	1[93]
Polyplectron napoleonis	190.0	M[4]	170.0	M[4]	1[23]	1[17]
Polyplectron schleiermacheri	200	M[4]	180	M[4]	1[23]	1[17]
Pternistis adspersus	179.4	147[42]	164.3	148[42]	1[23]	2[17]
Pternistis afer	200.3	26[42]	186.5	13[42]	1[23]	1[90]
Pternistis bicalcaratus	170.0	40[89]	159.0	34[89]	1[23]	2[17]
Pternistis camerunensis	171.0	3[89]	164.0	7[89]	1[23]	2[17]
Pternistis capensis	220.4	32[42]	202.1	45[42]	2[39]	2[42]
Pternistis castaneicollis	210.0	31[89]	186.0	30[89]	1[23]	2[17]
Pternistis clappertoni	180.0	24[89]	166.0	18[89]	1[23]	2[17]
Pternistis erckelii	216.0	13[89]	185.0	11[89]	1[23]	2[17]
Pternistis griseostriatus	153.0	5[89]	148.0	8[89]	1[23]	2[17]
Pternistis hartlaubi	144.2	42[42]	136.2	35[42]	2[39]	2[42]
Pternistis harwoodi	181.0	5[89]	163.5	2[89]	1[23]	2[17]
Pternistis hildebrandti	174.0	30[89]	162.0	29[89]	2[23]	2[17]
Pternistis icterorhynchus	169.0	33[89]	157.0	19[89]	1[23]	2[17]
Pternistis jacksoni	218.0	13[89]	200.0	8[89]	1[23]	2[89]
Pternistis leucoscepus	200.0	54[89]	187.0	38[89]	1[23]	2[89]
Pternistis natalensis	173.4	78[42]	158.4	47[42]	1[23]	2[42]
Pternistis nobilis	198.0	11[89]	178.0	13[89]	1[23]	2[17]

Species	Male wing cord		Female wing cord		Tarsal spur	Parental care
	(mm)	(n)	(mm)	(n)		
Pternistis ochropectus	208.7	6[7]	200.0	4[7]	1[23]	2[17]
Pternistis rufopictus	213.0	18[89]	190.0	24[89]	1[23]	2[89]
Pternistis squamatus	175.0	47[89]	163.0	60[89]	1[23]	2[17]
Pternistis swainsonii	192.3	81[42]	175.2	46[42]	1[23]	2[17]
Pternistis swierstrai	171.0	7[89]	164.7	3[89]	1[23]	2[17]
Ptilopachus nahani	135.0	9[89]	135.0	13[89]	0[23]	2[74]
Ptilopachus petrosus	123.0	12[89]	122.0	13[89]	0[23]	2[17]
Pucrasia macrolopha	212.4	10[14]	196.7	10[14]	1[23]	2[4]
Rheinardia ocellata	386.0	3[83,93]	314.0	3[83,93]	0[23]	1[93]
Rollulus rouloul	142.5	R17[93]	138.0	R8[93]	0[23]	2[93]
Scleroptila afra	159.0	47[42]	156.0	37[42]	1[23]	2[42]
Scleroptila finschi	167.0	4[89]	166.0	9[89]	2[23]	2[17]
Scleroptila levaillantii	164.2	29[42]	159.2	20[42]	1[23]	2[42]
Scleroptila levaillantoides	163.0	65[42]	161.0	45[42]	1[23]	2[42]
Scleroptila psilolaemus	164.0	17[89]	159.0	11[89]	1[23]	2[89]
Scleroptila shelleyi	163.6	89[42]	159.0	73[42]	1[39]	2[17]
Scleroptila streptophora	150.0	16[89]	152.0	9[89]	0[23]	2[17]
Synoicus australis	98.3	16[67]	100.3	17[67]	0[23]	2[67]
Synoicus ypsilophora	106.1	15[67]	107.0	9[67]	0[23]	2[67]
Syrmaticus ellioti	233.0	4[15,16]	203.0	4[15,16]	1[23]	1[5]
Syrmaticus humiae	230	M[4]	210	M[4]	1[23]	1[5]
Syrmaticus mikado	217.0	14[11]	191.0	19[11]	1[23]	1[11]
Syrmaticus reevesii	273.0	5[52]	237.0	19[52]	1[23]	1[102]
Syrmaticus soemmerringii	217.5	R[87]	207.0	R[87]	1[23]	1[4]
Talegalla fuscirostris	--	--	--	--	0[23]	0[17]
Tetrao parvirostris	382.2	138[88]	313.6	22[88]	0[23]	1[27]
Tetrao urogallus	390.0	9[21]	300.0	10[21]	0[23]	1[21]
Tetraogallus altaicus	311.4	7[27]	294.2	9[27]	1[23]	1[63]
Tetraogallus caspius	303.0	3[21]	288.0	3[21]	1[23]	1[21]
Tetraogallus himalayensis	328.0	6[27]	297.7	11[27]	1[23]	2[65]
Tetraogallus tibetanus	282.5	R[27]	264.0	R[27]	1[23]	1[1]

Species	Male wing cord		Female wing cord		Tarsal spur	Parental care
	(mm)	(n)	(mm)	(n)		
Tetraophasis obscurus	219.6	7[15,50]	201.5	4[15,50]	1[23]	2[17]
Tetraophasis szechenyii	226.0	4[99]	217.0	4[99]	1[23]	2[100]
Tetrastes bonasia	174.0	75[27]	173.0	54[27]	0[23]	1[21]
Tetrastes sewerzowi	173.5	42[85]	168.9	42[85]	0[23]	1[86]
Tragopan blythii	261.0	M[4]	233.0	M[4]	1[23]	2[5]
Tragopan caboti	236.2	5[50]	211.3	3[50]	1[23]	1[103]
Tragopan satyra	268.5	15[1]	229.5	7[1]	1[23]	1[66]
Tragopan temminckii	237.5	47[53]	213.0	34[53]	1[23]	1[66]
Tympanuchus cupido	227.0	53[70]	218.0	46[70]	0[23]	1[46]
Tympanuchus pallidicinctus	215.0	100[70]	205.5	100[70]	0[23]	1[37]
Tympanuchus phasianellus	206.5	100[70]	199.5	100[70]	0[23]	1[18]
Xenoperdix obscurata	--	--	--	--	0[30]	2[17]
Xenoperdix udzungwensis	--	--	--	--	0[28]	2[17]

SOURCES. [1]Ali and Ripley 1981; [2]Andreev and Hafner 1998; [3]Baker 1935; [4]Beebe 1990; [5]BirdLife International 2001; [6]Blake 1977; [7]Blot 1985; [8]Boag and Schroeder 1992; [9]Braun, Martin and Robb 1993; [10]Brennan 1999; [11]Bridgman 2002; [12]Brown, Hagelin, Taylor and Galloway 1998; [13]Calkins, Hagelin and Lott 1999; [14]Cheng 1967; [15]Cheng 1978; [16]Cheng 1993; [17]Cockburn 2006; [18]Connelly, Gratson and Reese 1998; [19]Collar 1992; [20]Collar 2013; [21]Cramp and Simmons 1980; [22]Dabbert, Pleasant and Schemnitz 2009; [23]Davison 1985; [24]de Azara 1805; [25]del Hoyo and Motis 2004; [26]Delacour 1977; [27]Dement'ev and Gladkov 1967; [28]Dinesen, Lemberg, Svendsen, Hansen and Fjeldså 1994; [29]Eaton 1992; [30]Fjeldså and Kiure 2003; [31]Fleig 1970; [32]Frost 1975; [33]Gao and Yu 1990; [34]Gilbert 2012; [35]Guangdong Institute of Entomology and Zhongshan University 1983; [36]Gutiérrez and Delehanty 1999; [37]Hagen and Giesen 2005; [38]Hale 2006; [39]Hall 1963; [40]He, Lu and Cui 1988; [41]Hennache, Rasmussen, Lucchini, Rimondi and Randi 2003; [42]Hockey, Dean and Ryan 2005; [43]Hume and Davison 1878; [44]Hume and Marshall 1880; [45]Johnsgard 1988; [46]Johnson, Schroeder and Robb 2011; [47]Klaus, Bergman, Marti, Müller, Vitovic and Wiesner 1990; [48]Koelz 1954; [49]Kumar and Singh 2004; [50]Lei and Lu 2004; [51]Leopold 1959; [52]Li 2008; [53]Li 1991; [54]Li and Liu 1993; [55]Liang 2008; [56]Liao 1984; [57]Liu 1992; [58]Liu 2000; [59]Lu 1991; [60]Lu, Gong and Ren 2003; [61]Lu and Wu 2003; [62]Lu and Zheng 2003; [63]Lukianov 1992; [64]Luo and Han 2010; [65]Ma 1992; [66]Madge and McGowan 2002; [67]Marchant and Higgins 1993; [68]Marien 1951; [69]Montgomerie and Holder 2008; [70]Pyle 2008; [71]Ridgway and Friedman 1946; [72]Rimlinger, Landel, Cheng and Guo 2000; [73]Rusch, Destefano, Reynolds and Lauten 2000; [74]Sande, Dranzoa, Wegge and Carroll 2009; [75]Sandoval 2011; [76]Schroeder, Young and Braun 1999; [77]Severinghaus 1996; [78]Silveira, Olmos and Long 2004; [79]Skutch 1947; [80]Skutch 1983; [81]Skutch 1999; [82]Sozer, Shepherd and Darjono 2006; [83]Stepanyan 1995; [84]Stromberg 2009; [85]Sun, Fang, Swenson, Klaus and Zheng 2005; [86]Sun, Swenson, Fang, Klaus and Scherzinger 2003; [87]Taka-Tsukasa 1967; [88]Tirskii 2009; [89]Urban, Fry and Keith 1986; [90]van Niekerk, Barendse and Mare 2000; [91]Vuarie 1968; [92]Watson 1961; [93]Wells 1999; [94]Wetmore 1965; [95]Whistler 1926; [96]Whistler and Kinnear 1949; [97]Yang 1990; [98]Yang, Wen and Yang 1994; [99]Yang 2010; [100]Yang, Zhang, Lloyd, Ran, Xu, Du, Yue, Wang and Klaus 2011; [101]Yi and Peng 1996; [102]Zhang 2004; [103]Zhang 2010; [104]Zhao, Zhang and Feng 1992; [105]Zhou and Jiang 2008; [106]Zwickel and Bendell 2005.

Notes. Male and female wing cord measurements are means (mm) with sample sizes (n) except as follows: “M” denotes means with unknown sample size; “R” denotes midpoint of a range with unknown sample size, and “R” followed by a number indicates midpoint of a range with sample size equal to the number.
Tarsal spur codes: “0” means tarsal spurs absent in males and absent in females, “1” means tarsal spurs present in males and absent in females, and “2” means tarsal spurs present in males and present in females.
Parental care codes: “1” means female-only parental care, and “2” means biparental care.

Chapter 5.

Disproportionate bill-length dimorphism and niche differentiation in the western sandpiper (*Calidris mauri*)

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R.W. Stein designed the study, assembled the data sets, conducted the analyses and drafted the manuscript.

Abstract

The western sandpiper (*Calidris mauri*) is a small-bodied differential migrant that exhibits slight female-biased sexual-size dimorphism (~5%) and disproportionate bill-length dimorphism (~16%). Bill length is a functional feeding character and disproportionate bill-length dimorphism is consistent with niche differentiation. We test two predictions of the niche differentiation hypothesis to assess whether intersexual competition on the wintering grounds can account for this marked asymmetry in bill length. To do this, we develop a null model of bill-length dimorphism, which is corrected for structural size using tarsus length and intended to represent bill-length dimorphism in the absence of competition. First, we test whether bill-length dimorphism is larger, relative to the null model (12%), at two wintering sites with uniform sandpiper densities and strongly male-biased sex ratios. Bill-length dimorphism was significantly larger at the large site (Santa Maŕia: 13.4%) but not at the small site (Punta Banda: 12.7%). Second, using sex ratio as an index of intersexual competition, we test whether bill-length dimorphism increased as sex ratio approached 1. Although the between site difference

in sex ratio was small (5%), there was a marginal increase in bill-length dimorphism in the predicted direction. We suggest that sexual-size dimorphism in western sandpipers resulted from sexual selection favouring different body-size optima for males and females and that intersexual competition on the wintering grounds promoted further divergence in bill length.

Introduction

Sexual-size dimorphism (SSD) is widespread in animals, despite strong positive genetic correlations between the sexes for traits that determine body size (Lande 1980). SSD reflects sex-specific body-size optima and arises as a consequence of sexual selection and (or) natural selection (Price 1984). Comparative phylogenetic analyses demonstrate that sexual selection has been an important evolutionary driver of SSD in Charadriiformes (alcids, gulls, and shorebirds), an order that encompasses almost the entire range of SSD in birds (Székely et al. 2000, 2004). Once SSD has arisen, further divergence in bill morphology can occur in response to disruptive selection acting through intersexual competition for food (Price 1984). This results in niche differentiation, which is characterized by disproportionate bill-length dimorphism and sex-dependent differences in foraging behavior and resource use (Durell 2000). In Charadriiformes, bill-length dimorphism is only weakly associated with indices of sexual selection that explain SSD, suggesting that additional selection pressures have acted on bill length (Székely et al. 2004).

Bill length is a functionally selected trait in shorebirds (Scolopacidae) and pronounced bill-length dimorphism is common in species that feed on invertebrates in soft sediments (Jehl and Murray 1986). In shorebirds, bill length tends to be more dimorphic than tarsometatarsus and wing-chord lengths (Jehl and Murray 1986), and pronounced bill-length dimorphism is often associated with sex-dependent differences in foraging behavior and resource use (Durell 2000). This is consistent with niche differentiation, suggesting that intersexual competition for food may have influenced shorebird bill-length dimorphism routinely. It is unclear, however, when and where in the annual cycle disruptive selection might act on bill length. There is no support for intersexual competition on the breeding grounds as a general process explaining bill-

length dimorphism in shorebirds (Székely et al. 2000, 2004); here, we consider this same mechanism from a wintering grounds perspective (Elner and Seaman 2003).

The western sandpiper (*Calidris mauri* (Cabanis, 1857)) is a small-bodied (22-35 g) differential migrant that breeds in western Alaska and eastern Siberia and tends to use large coastal stopover sites during migration (Warnock and Bishop 1998). Their winter distribution extends along the Pacific coast of the Americas, primarily between California and Peru (Wilson 1994). Males predominate at northern, and females at southern, wintering sites, respectively; this distributional sex-bias is reflected in a latitudinal gradient in sex ratio across the wintering grounds (Nebel et al. 2002). Intrasexual latitudinal clines in body size are also evident: the smallest individuals of each sex occur at the northernmost and the largest at the southernmost wintering sites (O'Hara et al. 2006). The western sandpiper exhibits slight female-biased SSD (6% for tarsus and 5% for wing chord) but disproportionate bill-length dimorphism (16%; Jehl and Murray 1986). Western sandpipers feed on and in soft sediments as well as in the water column (Sutherland et al. 2000; Rubega 2002). Surface feeding predominates in both sexes; however, females probe in sediments more than males (Mathot and Elner 2004; Mathot et al. 2007).

The western sandpiper provides an opportunity to test predictions of niche differentiation specific to the wintering grounds because there is extensive overlap in the winter distribution of males and females (Wilson 1994) and because intersexual competition is likely. We test two predictions of the niche differentiation hypothesis to determine whether intersexual competition on the wintering grounds can explain disproportionate bill-length dimorphism in western sandpipers. To do this, we develop a null model of bill-length dimorphism (based on a large sample of migrants) that is taken to represent dimorphism in the absence of intersexual competition. If there is morphological subpopulation structuring on the wintering grounds resulting from intersexual competition for food then we would predict a higher level of bill-length dimorphism among wintering subpopulations, relative to the null model. Using sex ratio as an index of intersexual competition at wintering sites, we further predict that bill-length dimorphism should increase as sex ratio approaches 1. We use linear models (LM) to test these predictions by contrasting samples of sex-assigned birds from two wintering

sites in Mexico against the null model of bill-length dimorphism and, subsequently, evaluating between-site differences in bill-length dimorphism in relation to sex ratio.

Methods

Data Collection, Compilation and Assessment

The present study is based entirely on pre-existing samples collected between 1995-2003 (Fernández et al. 2003; Guglielmo and Williams 2003; Elner et al. 2005; Stein et al. 2005; Fernández and Lank 2006; Stein and Williams 2006). Bird-handling protocols for the original research projects were approved by the Simon Fraser University Animal Care Committee (permit nos. 529B, 552B, and 671B), conformed to the guidelines of the Canadian Committee for Animal Care and were in accordance with permits from Environment Canada and the Dirección General de Vida Silvestre – Mexico. None of the anatomically sexed migrants (n = 380) were sacrificed for the purpose of this study; these birds were originally collected for investigations into phenotypic flexibility of body composition (Guglielmo and Williams 2003), age-dependent differences in digestive physiology (Stein et al. 2005; Stein and Williams 2006), and functional morphology of a novel foraging mode (Elner et al. 2005). Migrants were studied at two adjacent coastal stopover sites in southwest British Columbia, Canada: Boundary Bay (49°03' N, 123°01' W) and Roberts Bank (49°05' N, 123°12' W). Adults and juveniles were collected at Boundary Bay during northward (20 April – 10 May) and southward (1 July – 30 August) migrations from 1995 to 2000, and at Roberts Bank during northward (20 April – 10 May) migration in 2003. Migrants were sampled during the entire stopover period, ensuring an inclusive sample from the range of wintering sites. Wintering western sandpipers were studied at two sites separated by 1600 km on the Pacific coast of Mexico: Estero de Punta Banda (‘‘Punta Banda’’; 31°52' N, 116°37' W), a small estuary on the northwest coast of Baja California, and Bahia Santa Mańia (‘‘Santa Mańia’’; 25°02' N, 108°18' W), a large wetland complex on the Sinaloa coast. Studies of wintering birds were conducted at Punta Banda from 1 October to 28 or 29 February of 1995–1996 and 1996–1997, and at Santa Mańia from 1 November to 28 or 29 February of 1999–2000, 2000–2001 and 2001–2002.

All of these studies included a standard morphological examination at capture. Age (adult or juvenile) was assigned on the basis of plumage color for migrants, and on the bases of plumage color and primary feather wear for wintering birds (Page et al. 1972; O'Hara et al. 2002). Bill and tarsus lengths were measured with calipers calibrated to 0.05 mm and rounded to 0.1 mm. Exposed culmen (bill) length was measured from the tip of the bill to its base, as demarcated by a fleshy protuberance at the base of the ramphotheca. For migrants, tarsometatarsus (tarsus) was measured according to Prater et al. (1987) and included joint articulations (maintained at 90° to the tarsus). For wintering birds, tarsus was measured according to Pyle et al. (1987) and excluded joint articulations. For migrants, the sex of each individual was verified anatomically at dissection, when sternum length was also measured (from the posterior end to the point of fusion with the ferculum) using calipers calibrated to 0.05 mm and rounded to 0.1 mm. For wintering birds, sex was assigned tentatively using the exposed culmen criterion of Page and Fearis (1971): males < 24.2 mm, females > 24.8 mm and intermediate individuals were classified as unknowns.

Morphometric measurements were made by three individuals, R.W. Stein (RWS), C.G. Guglielmo (CGG) and G. Fernández (GF). So, we assessed observer bias, which we controlled statistically where appropriate. Anatomically sexed migrants were measured by RWS and CGG, and here we assessed observer bias by first confirming that there were no differences in the way the morphometric measurements were made and then by testing for differences within each of the four age-by-sex categories. Only one of 12 comparisons, adult male sternum length (t_{113} = 2.65, p = 0.014), differed between observers. This difference was small (1.2% of the overall mean) and since there was no consistent pattern in these tests, which were conducted on independent samples of birds, we pooled measurements. Subsequent analyses verified that juveniles had completed structural growth: bill, tarsus, and sternum lengths were independent of age for males (p = 0.13-0.99) and females (p = 0.06-0.98). So, we pooled age classes also. Wintering birds were measured exclusively by GF who used a different measurement methodology for tarsus than RWS and CGG. Here, RWS and GF measured a common set of birds (n = 56) to assess observer bias. Their bill measurements were identical (mean = 26.8, sem = 0.1); however, due to the difference in measurement methodology, GF's tarsus (mean = 23.4, sem = 0.1) measurements

were consistently smaller than RWS's (mean = 26.2, sem = 0.1). So, we used linear regression, based on the repeated measurements of these 56 individuals (adjusted r^2 = 0.84), to adjust GF's tarsus measurements so they were comparable with RWS's.

Morphometric sex assignment and statistical analyses

We used discriminant function analysis, performed on the sample of anatomically sexed migrants, to assign sex to individual western sandpipers on the basis of bill and tarsus lengths (Figure 5.1). We validated the accuracy of this sex-assignment criterion by reassigning sex to the anatomically sexed migrants and determining the percentage of misclassified individuals. Under ideal circumstances the prior probability of classification (priors) should be derived from an independent but representative sample; this was not possible here and we chose to use informative priors because the sample of migrants, which the discriminant function was generated from, was female-biased while the sample of wintering birds was male-biased. An emphasis on females in the original sampling protocols caused the migrant sex ratio to be female-biased (58% females, χ^2_1 = 9.47, $p < 0.002$), so we set the priors equal to the proportion of each sex in the sample, which was determined anatomically. Subsequently, 11 individuals (7 males and 4 females) were classified incorrectly (2.9%) and none were classified as "unknown." To facilitate comparisons between migrants and wintering birds, we used this sex-assignment criterion to assign sex to wintering birds. In an attempt to minimize bias in sex assignment at the two winter sites, we assigned sex to all of the wintering birds as a single group. Here, we expected the sex ratio to be strongly male-biased (Nebel et al. 2002), so we used the exposed culmen criterion of Page and Fearis (1971) to estimate sex ratio (1378 males, 569 females, and 79 unknowns). After excluding the 79 unknowns, the apparent sex ratio was male-biased (71% males, χ^2_1 = 336.15, $p < 0.0001$), so again we set the priors equal to the proportion of each sex. We also assigned sex using uninformative priors and this did not influence our results.

Western sandpipers exhibit female-biased SSD (Jehl and Murray 1986), as well as latitudinal clines in structural size (O'Hara et al. 2006) and sex ratio (Nebel et al. 2002) across the wintering grounds. These characterizations provide a predictive framework for our analyses, so we use one-tailed tests to characterize dimorphism, for site comparisons and for changes in dimorphism associated with sex ratio. SSD is

presented according to Storer's (1966) index: $\{(x - y) \times [(x + y) \times 0.5]^{-1}\} \times 100$, with x and y representing either the mean or the least-squares mean for females and males, respectively. To test specifically whether intersexual competition on the wintering grounds can explain bill-length dimorphism, we compare sex-assigned wintering western sandpipers from Mexico to the migrant-based null model of bill-length dimorphism, and subsequently evaluate site differences in bill-length dimorphism in relation to sex ratio. To do this, we first construct a two-factor LM to explain variation in bill length. The first factor is assigned sex and the second factor, ''stage and site'', consolidates stage and site into three groups: migrants, Punta Banda and Santa Maŕia. We use tarsus as a covariate to control for structural size and assess heterogeneity of slope for both factors and their interaction. Subsequently, we consolidate these two factors into one factor with six levels and use contrast statements to make specific, preplanned comparisons within the LM. Statistical analyses were conducted in SAS release 6.03 (SAS Institute Inc. 1990).

Results

Migrants

As expected, anatomically sexed females had longer bill (t_{378} = 37.52, 1-tailed p < 0.0001), tarsus (t_{378} = 22.75, 1-tailed p < 0.0001), and sternum (t_{346} = 14.18, 1-tailed p < 0.0001) lengths than anatomically sexed males, and there was a marked asymmetry in dimorphism between bill (15.7%), tarsus (6.7%) and sternum (4.3%) lengths (Figure 5.2). The sex × tarsus interaction term indicated a common slope ($F_{1,376}$ = 0.44, p = 0.51) for males and females, and sternum length did not explain a significant amount of residual variation ($F_{1,344}$ = 0.78, p = 0.37). Size-corrected bill length dimorphism was 11.5% (Table 5.1, model 1).

Similarly, sex-assigned females had longer bill (t_{378} = 40.27, 1-tailed p < 0.0001), tarsus (t_{378} = 23.52, 1-tailed p < 0.0001), and sternum (t_{346} = 13.84, 1-tailed p < 0.0001; mean ± sem; females: 24.62 ± 0.05 mm; males: 23.60 ± 0.05 mm; dimorphism = 4.3%) lengths than sex-assigned males (Table 5.2). There were only minute changes in the mean trait values (mean change = -0.02 mm) after sex assignment; consequently, the

asymmetry in dimorphism was practically identical for the anatomically sexed and sex-assigned groupings of migrants. The sex × tarsus interaction term indicated a common slope ($F_{1,376}$ = 0.02, p = 0.88) for males and females, and sternum length did not explain a significant amount of residual variation ($F_{1,344}$ = 1.77, p = 0.18). Size-corrected bill-length dimorphism was 12.2% (Table 5.1, model 2).

Wintering sites

Punta Banda (463 ha; Alfaro et al. 2000) and Santa Maŕia (41383 ha; Fuente de León and Carrera 2005) support subpopulations of 4000 (Buenorostro et al. 1999) and 350000 (Engilis et al. 1998) over-wintering western sandpipers, respectively. Despite the difference in site size, western sandpiper densities were uniform at the two sites (Punta Banda: 8.64 individuals × ha^{-1}; Santa Maŕia: 8.46 individuals × ha^{-1}). As expected, sex ratio was male-biased at both sites (Punta Banda: 77% males, χ^2_1 = 58.58, 1-tailed p < 0.0001; Santa Maŕia: 72% males, χ^2_1 = 360.79, 1-tailed p < 0.0001). Sex-assigned females had longer bill (Punta Banda: t_{201} = 21.65, 1-tailed p < 0.0001; Santa Maŕia: t_{1821} = 81.83, 1-tailed p < 0.0001) and tarsus (Punta Banda: t_{201} = 11.50, 1-tailed p < 0.0001; Santa Maŕia: t_{1821} = 43.94, 1-tailed p < 0.0001) lengths than sex-assigned males at both sites (Table 5.2).

The difference in sex ratio between sites was in the direction predicted by the latitudinal cline; however, the change was small (5%, χ^2_1 = 1.95, 1-tailed p = 0.16). As predicted, bill lengths from Santa Maŕia, the southern site, were longer than those from Punta Banda (males: t_{1471} = –4.04, 1-tailed p < 0.0001; females: t_{551} = –2.97, 1-tailed p < 0.01). However, contrary to expectations, tarsus lengths from Santa Maŕia were consistently shorter than those from Punta Banda (males: t_{1471} = 5.70, p < 0.0001; females: t_{551} = 4.19, p < 0.0001).

Bill-length dimorphism

All of the sex-assigned migrant and wintering western sandpipers were included in a two-factor LM explaining variation in bill length and each of the interaction terms indicated a common slope for tarsus across groups (tarsus × ‘‘stage and site’’ × sex: $F_{4,2394}$ = 0.50, p = 0.74; tarsus × ‘‘stage and site’’: $F_{2,2394}$ = 0.44, p = 0.65; tarsus × sex:

$F_{1,2398}$ = 0.16, p = 0.68). After sequentially removing these interactions, both factors were highly significant (sex: $F_{1,2399}$ = 1711.9, p < 0.0001; ‘‘site and stage’’: $F_{2,2399}$ = 38.38, p < 0.0001), and there was a significant sex × ‘‘stage and site’’ interaction ($F_{2,2399}$ = 6.95, p < 0.001), indicating at least one difference in bill-length dimorphism among groups.

To determine whether a difference between males or a difference between females accounted for the difference in bill-length dimorphism, we first tested for intrasexual differences in bill length and then tested for a difference in bill-length dimorphism. Males from Punta Banda, the smaller site, had shorter bills than migrant males (Figure 5.3A; $F_{1,2399}$ = 15.25, p < 0.0001); however, bill length was uniform among females ($F_{1,2399}$ = 2.85, p = 0.09) and there was no difference in bill-length dimorphism (Table 5.2, $F_{1,2399}$ = 0.78, 1-tailed p = 0.10). In contrast, females from Santa Maŕia, the larger site, had longer bills than migrant females (Figure 5.3B; $F_{1,2399}$ = 41.89, p < 0.0001), and bill length was uniform among males ($F_{1,2399}$ = 1.41, p = 0.24). Bill-length dimorphism (13.4%) was significantly larger at Santa Maŕia ($F_{1,2399}$ = 13.0, 1-tailed p < 0.0001). Despite a relatively small change in sex ratio (5%), bill lengths of males (Figure 5.4; $F_{1,2399}$ = 40.90, p < 0.0001) and females ($F_{1,2399}$ = 26.94, p < 0.0001) from Punta Banda were shorter than those from Santa Maŕia, and there was a marginal increase in bill-length dimorphism ($F_{1,2399}$ = 2.05, 1-tailed p = 0.076) in the direction predicted by sex ratio.

Discussion

The western sandpiper exhibits an extreme form of bill-length dimorphism among calidrid sandpipers (Jehl and Murray 1986) and our analyses characterize a pronounced morphological asymmetry in this species: bill length is more than twice as dimorphic as tarsus length and more than three times as dimorphic as sternum length. If tarsus length is a good index of structural body size, as we suggest, then the disproportionate bill-length dimorphism characterized here suggests that western sandpipers differs more in shape than in size and this may have implications for sex-dependent differences in foraging mode. We test two predictions of the niche differentiation hypothesis to assess whether intersexual competition on the wintering grounds can explain the disproportionate bill-length dimorphism in the western sandpiper. Both wintering sites

differed qualitatively from the null model of bill-length dimorphism, and bill-length dimorphism was significantly larger at the large site (Santa Maŕia: 13.4%) but not at the small site (Punta Banda: 12.7%). If this is a general result and bill-length dimorphism tends to be larger at large sites, but not at small sites, then intersexual competition on the wintering grounds could still be an important process maintaining bill-length dimorphism. Although the between site difference in sex ratio was relatively small (5%), there was a marginal increase in bill-length dimorphism in the direction predicted by sex ratio. Though not definitive, these tests reveal consistent fine-scale differences in bill-length dimorphism that are associated with an index of intersexual competition at wintering sites. This provides evidence for an ecological mechanism that may be maintaining disproportionate bill-length dimorphism in the western sandpiper and other sexually size-dimorphic shorebirds, but further work is warranted.

Latitudinal clines: pattern and process

The western sandpiper is thought to exhibit latitudinal clines in body size in each sex that are associated with migration distance (O'Hara et al. 2006). Our results for bill length are consistent with a latitudinal cline; however, tarsus length exhibited the opposite pattern in both sexes. Taken together, these results are not consistent with a latitudinal cline for body size; instead, they suggest that size and shape may vary across the winter distribution of the western sandpiper. One reason for this inconsistency is that, in addition to migration distance, the morphological traits that determine size and shape (bill, tarsus and wing-chord lengths) might covary in relation to factors associated with winter site size and (or) quality, such as predation danger, interspecific or intersexual competition and (or) latitudinal variation in prey distribution (Pomeroy 2006). Consistent with this, intrasexual comparisons with the null model revealed contrasting site differences: the small site, Punta Banda, was characterized by relatively short-billed males while the large site, Santa Maŕia, was characterized by relatively long-billed females. These contrasting differences suggests that site size, or associated characteristics, may be important determinants of variation in body size (tarsus length) and body shape (relative bill length) across the winter distribution of western sandpipers.

Latitudinal variation in body size and shape in the western sandpiper could be shaped, in part, by latitudinal variation in predation risk from falcons (Nebel and

Ydenberg 2005) and (or) interspecific competition with congeners (Cartar 1984). Swaddle and Lockwood (1998) showed that rounded wing tips and short femora, relative to tarsus length, were associated with lower predation risk in forest passerines hunted by Eurasian sparrowhawk (*Accipiter nisus*). While these specific shape attributes would not apply to shorebirds, which typically use more open coastal habitats, we might expect equivalent operating rules. In addition, the winter distributions of the western sandpiper and its sister species, the semipalmated sandpiper (*Calidris pusilla*; Gibson and Baker 2012), overlap on the west coast of Central America (Wilson 1994; Hicklin and Gratto-Trevor 2010). Bill length is shorter and less dimorphic (7.4 vs 16.0%) in the semipalmated sandpiper (Jehl and Murray 1986), and it has been suggested that the semipalmated sandpipers might be an important source of interspecific competition where the two species are sympatric (Cartar 1984). If this is the case, then interspecific competition might contribute to the latitudinal cline in bill length reported by O'Hara et al. (2006) for male western sandpipers.

Male and female western sandpipers forage predominantly on the sediment surface (Mathot and Elner 2004; Mathot et al. 2007); this suggests that intersexual competition could be an important factor influencing bill-length dimorphism on the wintering grounds. Mathot et al. (2007) reported a latitudinal cline in invertebrate burial depth, from epifaunal in the north to infaunal in the south, across a substantial portion of the western sandpiper's winter range. This is an important finding because females probe into the sediment more than males (10%–15% vs. 1%; Mathot et al. 2007), and infaunal invertebrates could provide an opportunity to escape intraspecific competition. Consistent with this hypothesis, our data suggest a latitudinal cline in bill-length dimorphism that mirrors the latitudinal cline in infaunal burial depth, and latitudinal variation in tarsus and bill lengths of wintering subpopulations of western sandpiper may be structured by functional feeding considerations (Elner and Seaman 2003). Intersexual competition on the wintering grounds may be the underlying evolutionary driver for disproportionate bill-length dimorphism, such that competition results in disruptive survival or redistribution and promotes sex-dependent divergence in bill length, foraging mode and resource use.

Bill length dimorphism: origin and maintenance

Previous research has demonstrated that sexual selection has been an important evolutionary driver of SSD in Charadriiformes (Székely et al.2000, 2004). Sexual selection favouring small males, associated with agility in aerial courtship displays (Blomqvist et al. 1997; Lanctot et al. 2000), could have lead to parallel reductions in body size for males and females (Lande 1980). However, natural selection, associated with differences in migration distance (O'Hara et al. 2006) or energetic demands of reproduction, may have favoured different body-size optima for males and females and resulted in female-biased SSD. This suggests that sexual selection acting in combination with natural selection may have generated the combination of small body size and female-biased SSD. Once SSD was established, sex-dependent differences in bill length could then be accentuated by disruptive selection acting through intersexual competition and lead to niche differentiation. The two-stage scenario that we propose to explain the evolution of disproportionate bill-length dimorphism in the western sandpiper invokes interactions between processes occurring on the breeding grounds or during migration and processes occurring on the wintering grounds.

In shorebirds, there is a general correspondence between bill-length dimorphism and intersexual differences in foraging mode (Durell 2000). Consequently, and as we suggest, intersexual competition could be a factor driving the evolution of shorebird bill-length dimorphism (Cartar 1984; Székely et al. 2000; Sandercock 2001). The western sandpiper provides an important test case because the winter distributions of males and females overlap (Nebel et al. 2002) and because intersexual competition is likely (Mathot and Elner 2004; Mathot et al. 2007). The latitudinal cline in invertebrate burial depth reported by Mathot et al (2007) offers an opportunity to escape intersexual competition that could promote intersexual divergence in bill length for species exhibiting SSD (Mathot et al. 2007). Comparative phylogenetic analyses in shorebirds indicate that bill-length dimorphism tends to be consistently larger than SSD; however, these analyses provide no support for niche differentiation on the breeding grounds as a mechanism explaining disproportionate bill-length dimorphism (Székely et al. 2000, 2004). Rather, Székely et al. (2000) suggest that intra- and inter-specific competition throughout the year are probably more important than competition within a pair during the breeding season, and we provide supporting evidence for intersexual competition on the wintering

grounds as a mechanism promoting disproportionate bill-length dimorphism in western sandpipers.

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Tables and Figures

Table 5.1. Linear models explaining variation in bill length for anatomically sexed migrant (males: n = 160 and females: n = 220) and for sex-assigned migrant (males: n = 157 and females: n = 223) and wintering western sandpipers (*Calidris mauri*) from two sites in Mexico, Punta Banda (males: n = 156 and females: n = 47) and Santa Maŕia (males: n = 1317 and females: n = 506).

Models and parameters	**Coefficient ± sem**	***p*-value**	**Partial r^2**
1. Anatomically sexed migrants			
Intercept	7.83 ± 1.57	<0.0001	82.9*
Sex	2.84 ± 0.14	<0.0001	78.8
Tarsus	0.60 ± 0.06	<0.0001	4.1
2. Sex-assigned migrants			
Intercept	9.45 ± 1.55	<0.0001	84.2*
Sex	3.00 ± 0.14	<0.0001	81.1
Tarsus	0.53 ± 0.06	<0.0001	3.1
3. Sex-assigned migrant and wintering birds			
Punta Banda males	7.90 ± 0.74•	<0.0001	82.1*
Migrant males	0.40 ± 0.10	<0.0001	0.1
Santa Maŕia males	0.49 ± 0.08	<0.0001	0.2
Punta Banda females	3.07 ± 0.15	<0.0001	2.7
Migrant females	3.32 ± 0.10	<0.0001	8.3
Santa Maŕia females	3.78 ± 0.09	<0.0001	13.9
Tarsus	0.58 ± 0.03	<0.0001	57.0

Note: In models 1 and 2, sex was modeled as a dummy variable, male = 0 and female = 1. In model 3, dummy variables were created to distinguish each of the six groups of sex-assigned migrant and wintering western sandpipers.

* r^2 of the full model.

• Intercept of the full model.

Table 5.2. Morphometric measurements of sex-assigned migrant and wintering western sandpipers (*Calidris mauri*).

Parameter	Migrants	Punta Banda	Santa Maŕia
Sample size			
Female	223	47	506
Male	157	156	1317
Tarsus (mm)			
Female (mean ± sem)	26.50 ± 0.04	26.64 ± 0.09	26.24 ± 0.02
Male (mean ± sem)	24.76 ± 0.05	25.18 ± 0.05	24.89 ± 0.02
Dimorphism (%)	6.8	5.6	5.3
Bill (mm)			
Female (mean ± sem)	26.55 ± 0.07	26.39 ± 0.14	26.86 ± 0.05
Male (mean ± sem)	22.63 ± 0.07	22.47 ± 0.09	22.79 ± 0.03
Dimorphism (%)	15.9	16.0	16.4
Size-corrected bill (mm)			
Female (LS mean ± sem)	25.89 ± 0.07	25.65 ± 0.14	26.36 ± 0.05
Male (LS mean ± sem)	22.97 ± 0.07	22.58 ± 0.07	23.06 ± 0.03
Dimorphism (%)	12.0	12.7	13.4

Note: Dimorphism is represented according to Storer's (1966) index: $\{(y - x) \times [(y + x) \times 0.5]^{-1}\} \times 100$. Size-corrected bill lengths are least-squares means (LS means) from Table 5.1, model 3.

Figure 5.1. Morphometric differentiation of anatomically sexed male (○; n = 160) and female (●; n = 220) western sandpipers (*Calidris mauri*) by a derived bivariate discriminant function (solid black line; Y = (0.903 × culmen) + (0.286 × tarsus) – 29.865; accuracy > 97%). If Y < 0 there is a higher probability that the individual is male; if Y > 0 there is a higher probability that the individual is female; if Y = 0 there is an equal probability that the individual is male or female. The lower gray line (culmen = 24.2) is 93% accurate in classifying males and the upper gray line (culmen = 24.8) is 90% accurate in classifying females (Page and Fearis 1971).

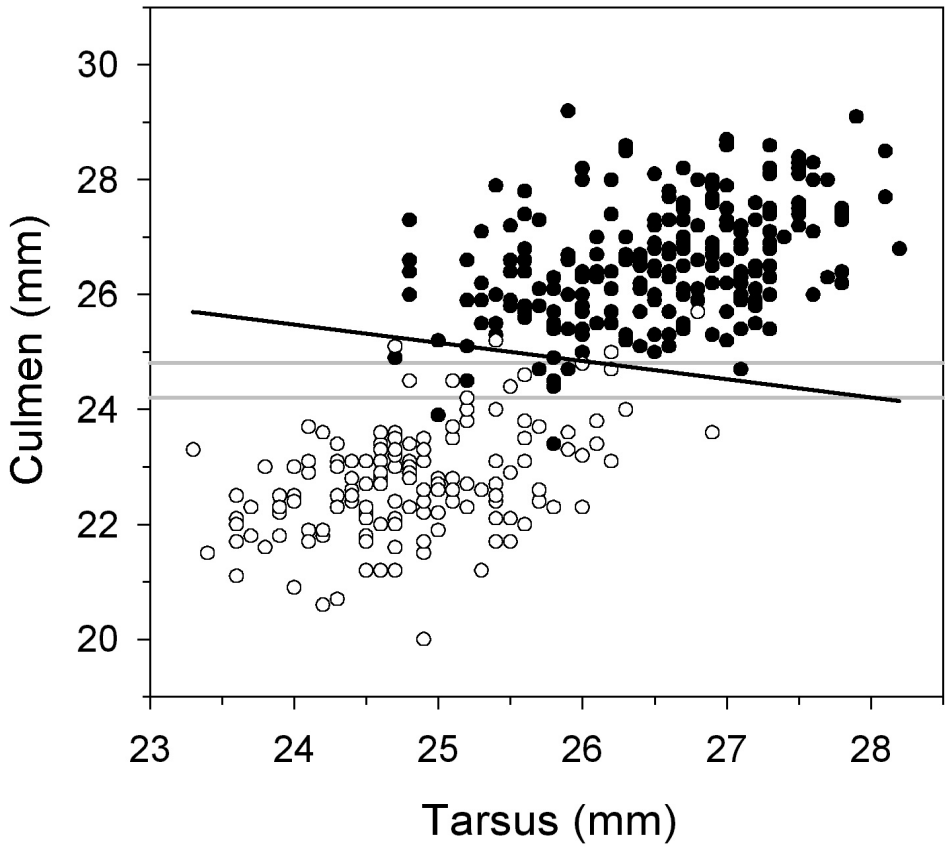


Figure 5.2. Morphometric measurements (mean ± sem) of anatomically sexed western sandpipers (*Calidris mauri*). Disproportionate dimorphism for bill length demonstrates that the sexes differ in size and shape.

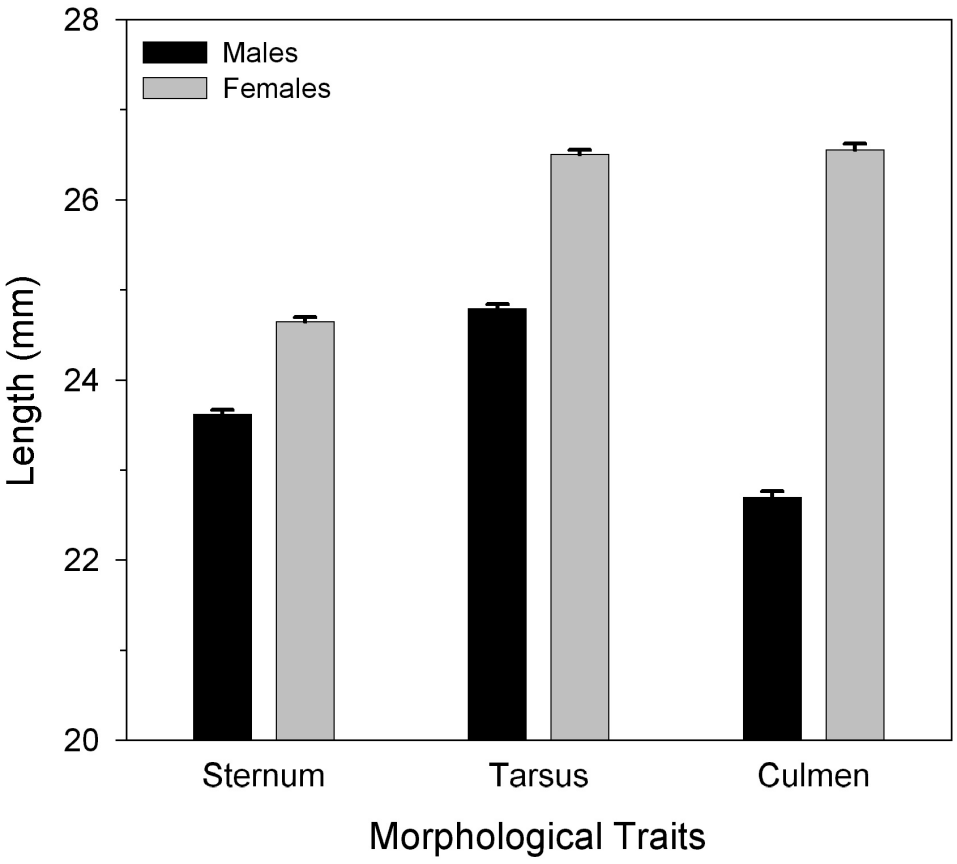


Figure 5.3. For sex-assigned western sandpipers (*Calidris mauri*), sexual dimorphism of size-corrected culmen length is larger at wintering sites than in a random sample of migrants. (A) Comparison between migrants and birds wintering at Punta Banda, a small estuary. (B) Comparison between migrants and birds wintering at Santa Maŕia, a large wetland complex. Migrants are represented by black lines (▲, males (n = 157); ●, females (n = 223)), and wintering birds are represented by gray lines (panel A: ∆, males (n = 47); ○, females (n = 156); panel B: ∆, males (n = 506); ○, females (n = 1317)).

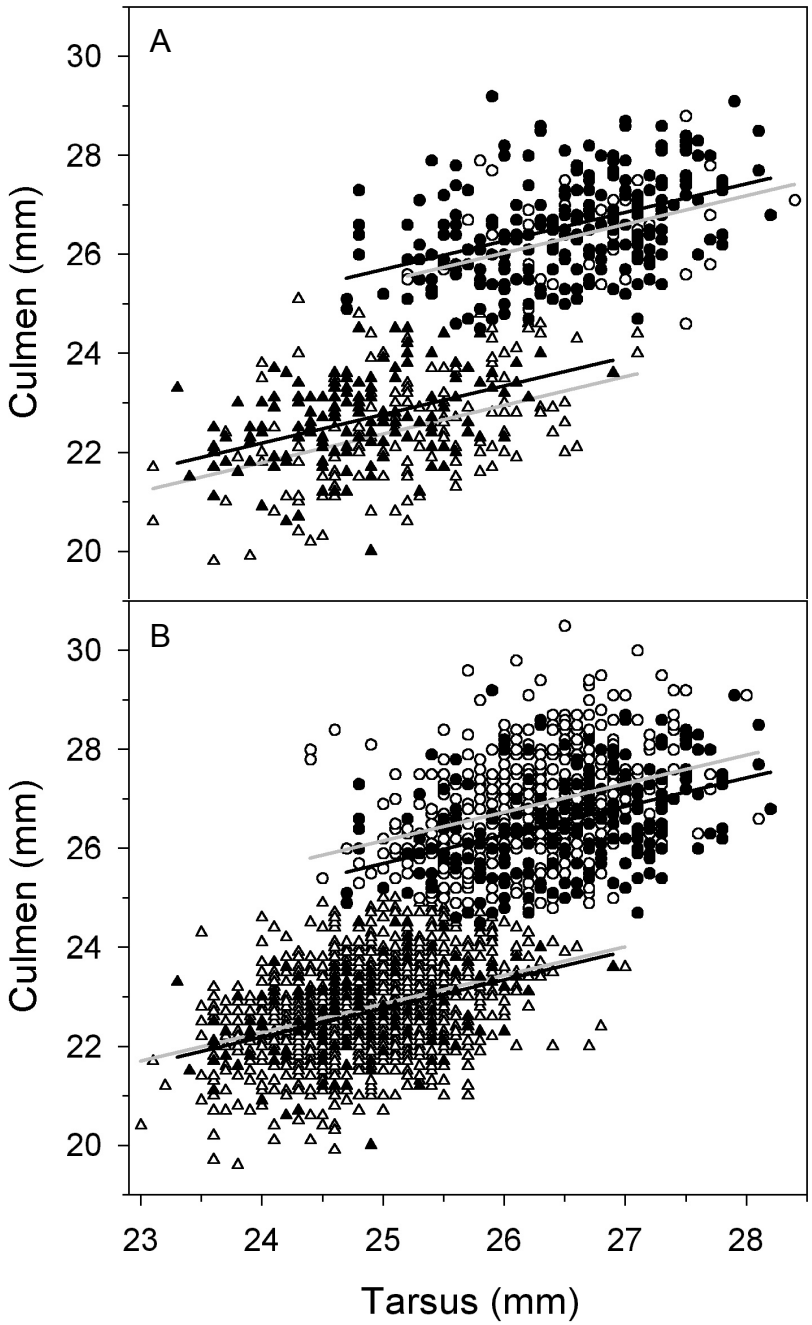
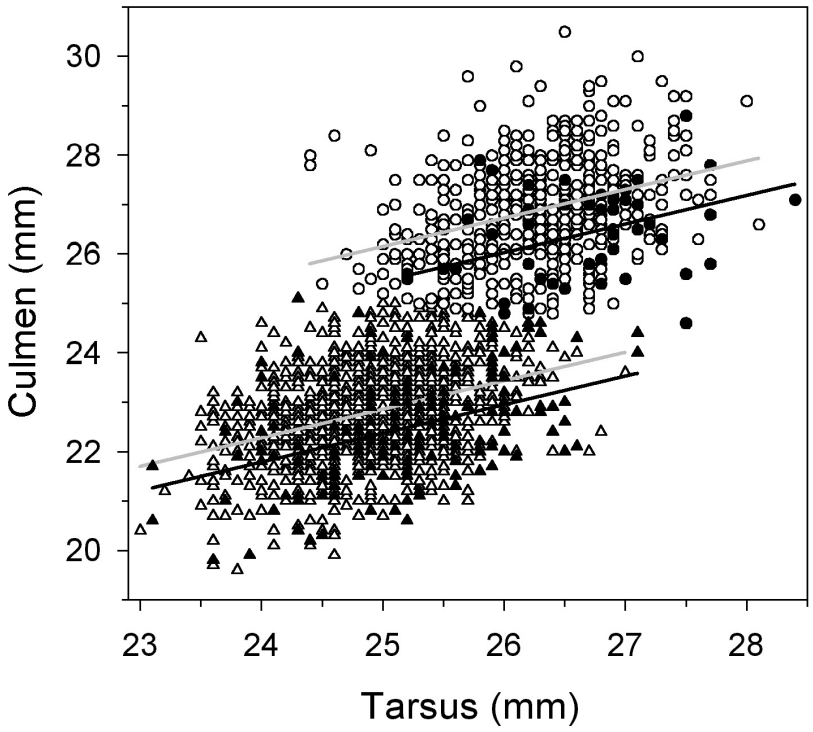


Figure 5.4. Sexual dimorphism of size-corrected culmen length increases as sex ratio becomes more uniform in sex-assigned wintering western sandpipers (*Calidris mauri*). Birds from Punta Banda are represented by black lines (▲, males (n = 47); ●, females (n = 156)), and birds from Santa Maŕia are represented by gray lines (Δ, males (n = 506); ○, females (n = 1317)).



Chapter 6.

Extreme intraclutch egg-size dimorphism in *Eudyptes* penguins, an evolutionary response to clutch-size maladaptation

Stein, R.W., and T.D. Williams. 2013. Extreme intraclutch egg-size dimorphism in *Eudyptes* penguins, an evolutionary response to clutch-size maladaptation. The American Naturalist 182(2):260-270.

R.W. Stein designed the study helped assemble the trait data, conducted the analyses and drafted the manuscript. T.D. Williams assembled the majority of the trait data and contributed to the manuscript through several rounds of editing.

Abstract

Eudyptes penguins (6 spp.) are uniquely characterized by a 2-egg clutch with extreme intraclutch egg-size dimorphism (ESD): the first-laid A-egg is 17.5 – 56.9% smaller than the B-egg. Although A-eggs are viable, they almost never produce fledged chicks (genus average < 1%). Using classical life-history theory and phylogenetic comparative methods we demonstrate a marked slowdown in the life history of *Eudyptes*: age of first reproduction is 52% later and annual fecundity 48% lower compared to other 2-egg clutch penguin species. All 6 *Eudyptes* spp. have retained a 2-egg clutch despite this pronounced life-history slowdown; this suggests evolutionary mismatch between clutch size and chicks fledged per clutch. Consistent with this, we show that *Eudyptes* fledge 43% fewer chicks per clutch than other 2-egg clutch penguin species. Extreme intraclutch ESD in *Eudyptes* is associated primarily with a uniform (5%) increase in relative B-egg size and B-egg size has evolved in accord with life

history. We further show that intraclutch ESD is positively correlated with age of first reproduction in *Eudyptes* but not in other 2-egg clutch penguin species. We argue that *Eudyptes*' persistent failure to evolve a 1-egg clutch constitutes a unique genus-wide evolutionary maladaptation and that extreme intraclutch ESD evolved as a correlated response to selection favouring a slower life history imposed by their extreme pelagic over-wintering and migration ecology.

Introduction

Life histories encompass the major demographic traits associated with fitness and describe variation in schedules for growth, survival and reproduction (Stearns 1992). Life history trajectories evolve in response to age-specific mortality schedules, but are constrained by trade-offs and evolutionary history (Stearns 1992; Charlesworth 1994; Roff 2002). Classical life history theory assumes that traits with clear, direct links to fitness, such as clutch size, are optimized by natural selection (Charlesworth 1994; Roff 2002). However, a lack of standing phenotypic variation (for example, most pelagic seabird species have small, invariant clutch sizes of 1 or 2 eggs; Hamer et al. 2002) could impede trait optimization (Crespi 2000). Evolutionary stasis, including clutch-size invariance in pelagic seabirds, has been explained by persistent stabilizing selection leading to "constraints" mediated by selection (Stearns 1986). "Selective constraints" are considered weak forms of evolutionary constraint because "constraint" is enforced by selection, not by the non-random production of variants (Schwenk 1995). "Constraints" maintained by relatively weak stabilizing selection relax when selection pressures shift; however, strong and persistent selection can lead to trait canalisation (Charlesworth et al. 1982; Stearns 1986). If the clutch-size invariance typical of pelagic seabirds resulted from canalisation then this could constrain clutch-size evolution and lead to maladaptation (Crespi 2000).

Pelagic seabirds have slow life histories (small invariant clutch sizes, low annual fecundity and deferred reproduction) that are shaped, in part, by the large incremental costs associated with provisioning chicks (Weimerskirch 2002). This cost of reproduction can limit annual fecundity by negatively impacting adult survival and by exerting strong, persistent stabilizing selection on clutch size (Weimerskirch 2002). Penguins

(Spheniscidae) have the slow life histories typical of pelagic seabirds even though most penguin species are inshore forgers (Williams 1995). Part of the reason for this is that penguins are flightless, and flipper-propelled swimming is an expensive means of transport. Despite these higher relative transportation costs, *Eudyptes* penguins (6 ssp.) evolved a novel pelagic over-wintering behaviour that involves a temporally and energetically demanding pre-breeding migration (Bost et al. 2009, Green et al. 2009). *Eudyptes* penguins are further characterized by a form of intraclutch egg-size dimorphism (ESD) that is unique (first-laid A-egg is smaller) and extreme (range: 17.5–56.9%) among birds (Slagsvold et al. 1984; Williams 1995). In *Eudyptes*, extreme intraclutch ESD is coupled with systematic loss of the A-egg, which is viable (Williams 1990; Davis and Renner 2003; Poisbleau et al. 2008) but almost always fails to produce a fledged chick (genus average < 1%; Williams 1995). Early, systematic loss of the A-egg or A-chick is assured by obligate clutch and brood reduction tactics that favour the B-egg (St. Clair 1992; St. Clair 1996; St. Clair et al. 1995). In *Eudyptes schlegeli* (royal penguin), the A-egg is lost at or before the time the B-egg is laid; this systematic, early loss of the A-egg has been attributed to maternal egg ejection and interpreted as maternal infanticide (St. Clair et al. 1995). As a consequence, *Eudyptes* penguins appear to sacrifice the time and energy invested in A-egg production, and this suggests that their 2-egg clutch is maladaptive.

Extreme intraclutch ESD in *Eudyptes* has defied explanation despite more than 50 years of research (Lack 1968; Johnson et al. 1987; Williams 1990; St. Clair 1992; St. Clair 1998; St. Clair et al. 1995). The many hypotheses advanced in explanation have focused on adaptive functions for the smaller A-egg; however, the primary candidate hypotheses, brood reduction (Lack 1954) and insurance against B-egg loss (Dorward 1962), have not received empirical support (St. Clair et al. 1995; Slagsvold et al. 1984). These hypotheses emphasize intraclutch ESD rather than clutch size. We propose the life-history slowdown hypothesis as a novel alternative explanation for the evolution of extreme intraclutch ESD in *Eudyptes*. The life-history slowdown hypothesis proposes that extreme intraclutch ESD evolved as a correlated response to selection favouring a slower life history imposed by *Eudyptes*' unique pelagic over-wintering and migration ecology. If life history has slowed down in *Eudyptes*, then we would expect clutch size to decrease from 2 eggs to 1 egg, as seen in the *Aptenodytes* penguins (Williams 1995).

However, all 6 spp. in *Eudyptes* have retained a 2-egg clutch. Here, we use classical life history theory and phylogenetic comparative methods to demonstrate that life history has slowed down in *Eudyptes* and their 2-egg clutch is maladaptive. Retention of a 2-egg clutch despite a slowdown in life history suggests an evolutionary mismatch between clutch size and realised fecundity (number of chicks fledged per 2-egg clutch) in *Eudyptes*. If clutch size has become "stuck" at 2 eggs in *Eudyptes*, this raises a question, how should egg size evolve when clutch size is fixed and life history slows down? So, we use allometry to determine whether extreme ESD in *Eudyptes* is the result of a relative decrease in A-egg size, a relative increase in B-egg size or a combination of the two. Selection favouring a slower life history in combination with a fixed 2-egg clutch raises the possibility that extreme intraclutch ESD in *Eudyptes* resulted from an interaction between these processes; consistent with this, we show that intraclutch ESD is correlated with deferred onset of reproduction (mean age of first reproduction) in *Eudyptes* but not in other 2-egg clutch penguin species.

Our analysis provides support for the interpretation that *Eudyptes*' 2-egg clutch is maladaptive (*sensu* Crespi 2000). This raises a second question, what might be precluding clutch-size optimization? Constraint-based explanations are subject to criticism because it is always possible to postulate a rare selective regime where a putatively "maladaptive" trait could be adaptive (Schwenk 1995). For example, it is plausible that an invariant 2-egg clutch coupled with extreme intraclutch ESD could be advantageous during infrequent periods of super-abundant resources associated with a long-term (decadal or greater) environmental cycle. Acknowledging this possibility, we suggest that constraint on clutch-size reduction might be related to an interaction between the physiology of follicle (yolk) development and *Eudyptes*' unique pelagic non-breeding and migration ecology (see Discussion).

Methods

The Spheniscidae includes the 6 genera and 18 species of extant penguins (Baker et al. 2006; Figure 6.1), which exhibit substantial variation in body mass and life history characteristics (1 – 24 kg; Williams 1995). The 2 largest species (*Aptenodytes*; 10 – 24 kg) first reproduce at 5 – 6 yrs, do so once a year or once every other year, and

have a 1-egg clutch (Williams 1995). The 2 smallest species (*Eudyptula*; 1 kg) first reproduce at 2 – 3 yrs, do so once or twice a year and have a 2-egg clutch with nearly equal-sized eggs (Williams 1995). The 14 species of intermediate-sized penguins (*Pygoscelis, Spheniscus, Megadyptes* and *Eudyptes*; 2 – 6 kg) first breed at 2 – 8 yrs, typically breed once a year and have a 2-egg clutch with either nearly equal-sized eggs (all non-*Eudyptes*) or extremely size-dimorphic eggs (*Eudyptes*). In *Pygoscelis, Spheniscus* and *Megadyptes*, intraclutch ESD (mean = 2.1%, n = 8, calculated as: $100 \times [|A - B|] \times [(A + B) \times 0.5]^{-1}$; Table 6.1) is typical of other non-passerine birds with altricial development (mean = 3.6%; Slagsvold et al. 1984). In *Eudyptes*, intraclutch ESD (mean = 36.8%, n = 6) is 17.5-times larger, on average, than that of other intermediate-sized penguins.

We assembled data for the 16 species of extant penguins with 2-egg clutches (Table 6.1). Specifically, we compiled species-specific mean values for adult female mass (n = 16), A-egg mass (n = 12), B-egg mass (n = 12), age of first reproduction (n = 14), chicks fledged per clutch (n = 14) and annual fecundity (n = 14). There is extensive interspecific variation in the duration of pre-laying and incubation-related fasts, so adult female mass was taken from the chick-rearing period when birds are lean. Age of first reproduction was averaged across males and females (females begin reproducing 0.5 – 1 years earlier than males) and was not adjusted for within-cohort mortality. *Eudyptula minor* (little penguin) and *S. humboldti* can successfully reproduce twice a year, so we distinguish between chicks fledged per clutch and chicks fledged annually. We do not consider adult or juvenile survival because most published survival estimates were generated with flipper tags, which can induce mortality in penguins (Saraux et al. 2011). We collected comparable data for *Aptenodytes forsteri* (emperor penguin), the only penguin species with a 1-egg clutch and an annual reproductive cycle (Williams 1995); however, we restrict formal analyses to 2-egg clutch species.

There are no published data for fresh egg-mass of *Spheniscus humboldti* (Peruvian penguin), *Spheniscus mendiculus* (Galapagos penguin), *Eudyptula albosignata* (white-flippered penguin) and *Eudyptes robustus* (Snares penguin). So, we estimated fresh egg mass for these species. To do this, we assembled species-specific (n = 10) mean length, breadth and mass measurements taken from the same set of fresh eggs ($n \geq 20$ for each species; Table 6.2). Penguin eggs are roughly ellipsoid so

we calculated egg volume = length × breadth^2 × π × 6^{-1} and assumed that A-egg and B-egg volumes provide independent estimates of egg mass. Visual inspection revealed a pair of points, A- and B-eggs of *Pygoscelis antarctica*, that deviated strongly from an otherwise exceptionally tight linear relationship (data not shown); both eggs were ~10 g larger than expected from their linear dimensions (A-egg residual = 4.1, Bonferroni p = 0.0140; B-egg residual = 6.4, Bonferroni p = 0.0001). After first confirming that the dimensions reported by Lishman (1985) were likely correct (Belliure et al. 1999), we excluded *Pygoscelis antarctica* from the regression analysis and estimated A- and B-egg mass for this species also.

The resulting ordinary least-squares regression equation was adequate for prediction (fresh egg mass = -1.28 ± 1.32 + 1.08 ± 0.01 × (calculated volume), n = 20, $F_{1,18}$ = 7080, p < 0.0001, adj. r^2 = 0.997). Egg dimensions for the 5 species requiring egg-mass estimation were within the range of the 10 species included in the regression. We validated our egg mass estimation to assess bias. First, we retained the species with the largest, *Eudyptes schlegeli*, and smallest, *Eudyptula minor*, eggs and iteratively excluded each of the 8 remaining species. Second, we used the ordinary least-squares regression equation from each set of 9 species to estimate A- and B-egg mass for the 1 excluded species. Finally, we compared estimated and fresh egg mass for the 1 excluded species. The mean difference between estimated and fresh mass was +0.26% for A-eggs and -0.30% for B-eggs. So, we are confident that egg mass estimation did not strongly influence the results presented here.

We used phylogenetic generalized least squares (PGLS; Pagel 1999, Freckleton et al. 2002) regression models to test for a life history slowdown, evolutionary mismatch and their interaction. PGLS is analogous to ordinary least-squares regression in that the error distribution is Gaussian; however, in PGLS the independence assumption is relaxed (Freckleton et al. 2002). The lack of independence among taxa is accounted for in PGLS by incorporating phylogenetic covariance directly; this sets the expected residual correlation structure (Freckleton et al. 2002). We use a well-supported, dated molecular phylogeny to specify phylogenetic covariance among penguins (Figure 6.1; Baker et al. 2006). We modeled character evolution with Pagel's (1999) correlation structure, which assumes constant variance through time. Pagel's (1999) methodology provides a maximum likelihood estimate of phylogenetic autocorrelation, lambda (λ),

which specifies the optimal branch length transformation for correlated characters (Freckleton et al. 2002). Character evolution is independent of phylogeny when $\lambda = 0$ and conforms exactly to Brownian motion when $\lambda = 1$. When $0 < \lambda < 1$ the influence of phylogeny is weaker than strict Brownian motion (Freckleton et al. 2002). When closely related species have inversely related characters $\lambda < 0$.

We use adult female mass as a size-covariate and transform variables logarithmically (base *e*) where appropriate. We characterize ESD as the mass difference between B- and A-eggs. We use "dummy" variables, coded as 0 or 1, to distinguish *Eudyptes* and non-*Eudyptes* penguins and to assess variation in ESD within *Eudyptes*. Tests of predictions are reported with one-tailed *p*-values and test statistics associated with covariates are reported with two-tailed *p*-values. Alpha is set at 0.05 and parameter estimates are reported with standard errors. All analyses were conducted in APE (Paradis et al. 2004; R Development Core Team 2011).

Results

Consistent with life history theory, annual fecundity was inversely correlated with age of first reproduction (Figure 6.2, $\lambda = -0.20$, $\beta_{\ln(\text{age of first breeding})} = -0.91 \pm 0.13$, n = 14, $t_{11} = -6.8$, $p < 0.0001$), even while accounting simultaneously for adult female body mass ($t_{11} = 1.14$, $p > 0.2$). This inverse relationship was homogeneous across *Eudyptes* and non-*Eudyptes* taxa (interaction: $\lambda = -0.22$, n = 14, $t_9 = 0.26$, $p > 0.8$; dummy variable: $\lambda = -0.22$, $t_{10} = -1.29$, $p > 0.2$). Consistent with a life-history slowdown in *Eudyptes*, age of first reproduction was 52% later ($\lambda = 0.02$, n = 14, *Eudyptes* vs. non-*Eudyptes*: 6.4 ± 0.8 vs. 4.2 ± 0.6 yrs, $t_{12} = 2.41$, one-tailed $p = 0.016$) and annual fecundity 48% lower ($\lambda = -0.47$, n = 14, 0.50 ± 0.09 vs. 0.96 ± 0.02 chicks fledged $\times$ yr^{-1}, $t_{12} = 4.57$, one-tailed $p = 0.0003$) than those of other 2-egg clutch penguin spp. Consistent with an evolutionary mismatch between clutch size and realised fecundity, *Eudyptes* also fledged 43% fewer chicks per 2-egg clutch ($\lambda = 0.52$, n = 14, 0.52 ± 0.17 vs. 0.91 ± 0.11 chicks fledged $\times$ 2-egg clutch^{-1}, $t_{12} = 2.27$, one-tailed $p = 0.0211$).

It is unclear whether extreme intraclutch ESD in *Eudyptes* resulted from a decrease in relative A-egg size, an increase in relative B-egg size or a combination of

the two. So now, we characterize A- and B-egg allometry for the 2-egg clutch Spheniscidae. A-egg allometry differs markedly between *Eudyptes* and non-*Eudyptes* taxa (Figure 6.3A, $\lambda = 0.93$, n = 16, interaction: $t_{12} = 3.92$, $p < 0.0020$, dummy variable: $t_{12} = 3.77$, $p < 0.0027$). A-egg allometry is positive in non-*Eudyptes* taxa ($\beta_{\ln(\text{female mass})} = 0.57 \pm 0.06$, $t_{12} = 8.81$, $p < 0.0001$) but not in *Eudyptes* ($\beta_{\ln(\text{female mass})} = 0.01 \pm 0.13$, $t_{12} = 0.09$, $p > 0.9$). B-egg allometry is positive and uniform across 2-egg clutch Spheniscidae (Figure 6.3B, $\lambda = 0.83$; n = 16, interaction: $t_{12} = -0.65$, $p > 0.5$, $\beta_{\ln(\text{female mass})} = 0.57 \pm 0.04$, $t_{12} = 13.83$, $p < 0.0001$); however, relative B-egg size is uniformly larger in *Eudyptes* ($\lambda = 0.83$; n = 16; $\beta_{\ln(\text{dummy variable})} = 0.27 \pm 0.05$, $t_{13} = 4.92$, $p < 0.0004$). The striking difference between A- and B-egg allometry suggests that variation in ESD within *Eudyptes* may be attributable, in part, to the A-egg interaction. To characterize the contribution of the A-egg to variation in ESD we divided the 6 *Eudyptes* spp. into 2 groups based on deviation from A-egg allometry of non-*Eudyptes* taxa (Figure 6.3A): the three larger species (*E. sclateri*, *E. chrysolophus* and *E. schlegeli*) have large negative deviations while the three smaller species (*E. moseleyi*, *E. pachyrhynchus* and *E. robustus*) have small positive deviations (Figure 6.3A). Compared to non-*Eudyptes* taxa, relative A-egg size is smaller in the three larger ($\lambda = 0.96$, n = 16, dummy variable: $t_{12} = -3.79$, $p < 0.0026$) but not in the three smaller (dummy variable: $t_{12} = 0.24$, $p > 0.8$) *Eudyptes* species. Variation in ESD within *Eudyptes* is attributable to a 5.4% increase in relative B-egg size across the genus (Figure 6.3B) and to a 5.6% decrease in relative A-egg size in the three larger species (Figure 6.3A).

Finally, we test whether the evolution of intraclutch ESD in 2-egg clutch Spheniscidae can be explained by an interaction between a life-history slowdown and evolutionary mismatch. Intraclutch ESD was independent of female mass ($\lambda = 2.29$, n = 14, $t_{12} = -0.45$, $p > 0.7$) and mean egg mass ($\lambda = 2.24$, n = 14, $t_{12} = -0.39$, $p > 0.7$), so these potential size covariates were excluded from models explaining ESD. As expected, intraclutch ESD was positively correlated with age of first reproduction in *Eudyptes* but not in other 2-egg clutch penguin spp. (Figure 6.4, $\lambda = -0.47$, n = 14; interaction: $\beta_{(\text{age of first reproduction:dummy variable})} = 9.62 \pm 2.46$, $t_{10} = 3.91$, 1-tailed $p = 0.0015$; main effects: $\beta_{(\text{age of first reproduction})} = -0.44 \pm 1.08$, $t_{10} = -0.41$, $p > 0.6$; $\beta_{(\text{dummy variable})} = -23.08 \pm 13.85$, $t_{10} = -1.67$, $p > 0.1$).

Discussion

Lack (1968) suggested that extreme intraclutch ESD in *Eudyptes* penguins might represent a rare, transitional stage in the evolution of a 1-egg clutch, and we provide life-history context for his prescient observation. Consistent with the evolution of a slower life history in *Eudyptes*, age of first reproduction is 52% later and annual fecundity 48% lower compared to non-*Eudyptes* taxa. Despite this marked life-history slowdown, clutch size has not decreased in *Eudyptes* as expected: all 6 spp. retain a 2-egg clutch. The apparent inconsistency between a slowdown in some life-history traits (age of reproduction and annual fecundity) but not in others (clutch size) is reinforced by comparison with the emperor penguin (*A. forsteri*), the only penguin species with a 1-egg clutch and an annual reproductive cycle (Williams 1995). Although *A. forsteri* is 7.5-times larger on average than *Eudyptes* taxa (Table 6.1) relative egg size (calculated as ($\log_e$(egg mass) × $\log_e$(female mass)$^{-1}$; *Eudyptes* mean ± standard error vs. *A. forsteri*; B-egg: 0.611 ± 0.002 vs. 0.610) and annual fecundity (0.59 ± 0.06 vs. 0.63 chicks × yr^{-1}) are remarkably similar. However, age of first reproduction is actually 18.5% *later* on average in *Eudyptes* (6.4 ± 0.6 vs. 5.4 yrs). It is not possible to infer whether the *Aptenodytes* lineage evolved from a 1- or 2-egg clutch ancestor because the sister group to Spheniscidae, Ciconiidae (storks), is too distantly related to be informative (diverged ~67 myr before present; Pacheco et al. 2011). However, the current breeding distribution and unique life-history characteristics of the much larger *Aptenodytes* penguins (winter breeding, foot incubation, extremely prolonged chick rearing, Williams 1995) suggest that they evolved under a different selective regime than *Eudyptes*.

The similarities in life history between *A. forsteri* and *Eudyptes* taxa raise an important question, why have all 6 spp. in *Eudyptes* failed to evolve a 1-egg clutch? The extant radiation of *Eudyptes* penguins diversified ~7 myr before present (Baker et al. 2006) and contains > 26 myr of evolutionary history (sum of branch lengths within *Eudyptes*; Figure 6.1). Retention of a 2-egg clutch across a marked life history slowdown, 5 speciation events and > 26 myr of evolutionary history suggests that a 2-egg clutch became canalized prior to the diversification of the extant *Eudyptes* radiation (see below). This raises a related question, how should egg size evolve when clutch size is canalized and life history slows down? Comparison with *A. forsteri* indicates that B-

egg size is exactly what one would predict if *Eudyptes* penguins had a 1-egg clutch, and this corroborates Williams (1990) suggestion that B-egg size might be optimized to enhance survival in a 1-chick brood. A-egg size has decreased in the 3 *Eudyptes* spp. with the most extreme ESD and this may provide a means of reducing costs of A-egg production in these species. Variation in intraclutch ESD is positively correlated with age of first reproduction in *Eudyptes* but not in other 2-egg clutch penguin species; this suggests that extreme intraclutch ESD arose in *Eudyptes* as a consequence of an interaction between selection favouring a slower life history and clutch size canalisation.

Penguins have the slow life histories characteristic of pelagic seabirds (Weimerskirch 2002) and intraspecific clutch-size invariance is ubiquitous among pelagic seabirds (Hamer et al. 2002; *Procellariiformes* (125 spp.; 1 egg); *Sphenisciformes* (18 spp.; 1 or 2 eggs); *Charadriiformes* (Alcidae, 22 spp., 1 or 2 eggs; Laridae, 55 spp., 1, 2 or 3 eggs). So, why is extreme intraclutch ESD restricted to Spheniscidae and specifically to *Eudyptes*? We suggest that the answer to this question involves a combination of limited opportunities for the evolution of extreme intraclutch ESD in pelagic seabirds and particularly high costs of migration in penguins. Extreme intraclutch ESD requires clutch size > 1. Clutches of 2 and 3 eggs are relatively rare in pelagic seabirds (Hamer et al. 2002) and this suggests that there have been limited opportunities for extreme intraclutch ESD to evolve. Penguins are flightless and flipper-propelled swimming is an expensive means of transport. Field metabolic rate of penguins is intermediate among seabirds (double-labelled water estimates; Shaffer 2011); however, mean swimming speed (2.1 $m \times s^{-1}$, n = 7; Croxall and Davis 1990) of penguins is 82% slower than mean ground speed (11.8 $m \times s^{-1}$, n = 25; Spear and Ainley 1997) of other pelagic seabirds. Intermediate energy expenditure coupled with slow travel speed suggest that the energetic and temporal costs of migration are exceptionally high in the Spheniscidae and this has been confirmed empirically for *Eudyptes* (Bost et al. 2009, Green et al. 2009). We would expect high temporal and energetic costs of migration in *Eudyptes* to exert strong, persistent stabilizing selection on the timing of life history events.

Eudyptes penguins are characterized by high primary reproductive investment (2-egg clutch), but low realised fecundity (0.59 ± 0.06 chicks $\times$ yr^{-1}). This aberrant trait combination provides a rare example of evolutionary mismatch and offers novel support

to the interpretation that *Eudyptes*' 2-egg clutch is "maladaptive" (*sensu* Crespi 2000). Failure to evolve a 1-egg clutch in *Eudyptes* would be less problematic if production costs of the 'extra' egg were negligible, and it has been argued that this is the case given that A-egg mass only represents 2-5% of adult female mass (Table 6.1; Williams 1995). However, this previous assessment of low production costs only considers the direct energetic costs of the A-egg. Inferring that *Eudyptes*' 2-egg clutch constitutes a genus-wide evolutionary maladaptation is contingent on high production costs for the A-egg. So, now we quantify the direct and indirect energetic costs of A-egg production for *E. chrysolophus* and, using incubation metabolic rate (1242.9 kJ × d^{-1}; Brown 1984), represent these costs as incubation-day equivalents. In *E. chrysolophus* the A-egg is composed of 14.1 g yolk and 5.7 g albumen (dry-matter; Crossin et al. 2010). Assuming the yolk is composed of 58% lipid and 42% protein (*E. pachyrhynchus*; Grau 1982), that lipid and protein contain 38.91 kJ × g^{-1} and 17.99 kJ × g^{-1}, respectively (Whittow 1986), and 75% conversion efficiency, the direct cost of A-egg production is 703 kJ and constitutes 0.6 incubation-day equivalents. Indirect costs accrue across males and females because all *Eudyptes* taxa have shared incubation (Williams 1995). During the 4.2-day laying interval female *E. chrysolophus* metabolize 342.5 g of reserves, which are composed of 35.3% water, 55.5% lipid and 9.2% protein (Croxall 1982). The 7998.5 kJ of metabolized reserves constitute 6.4 incubation-day equivalents. There are no comparable mass-loss data for males; however, assuming an average daily metabolic rate (1319.6 kJ × d^{-1}; Brown 1984), which is ~6% higher than incubation metabolic rate, males are expected to lose 4.5 incubation-day equivalents during the laying interval (potentially decreasing the probability of successfully retuning to relieve their partner). So, a pair of *E. chrysolophus* loses a minimum of 11.5 incubation-day equivalents (14,000 kJ) of body reserves to A-egg production, and this represents 33% of the 35-day incubation period. This estimate represents the minimum production cost for the A-egg because energetic, temporal and survival costs associated with foraging to attain the lost reserves are not included, nor is the temporal cost of the laying interval.

We acknowledge that constraint-based arguments for evolutionary mismatch are open to criticism; however, it is interesting, albeit anecdotal, that when *E. chrysolophus* is maintained in captivity (abundant food resources, low energetic demands and no migration) and allowed to incubate eggs and raise chicks systematic, early loss of the A-

egg persists (n = 7) and fledging success remains low (0.43 ± 0.20 chicks per 2-egg clutch; n = 7; RWS, unpublished data). If a 2-egg clutch coupled with extreme intraclutch ESD is part of an adaptive strategy evolved to exploit rare times of abundant resources then we would expect a highly flexible response to exploit those opportunities, but this has not been observed in captives. Taken together, these novel lines of evidence provide support for the interpretation that the persistence of a 2-egg clutch might not be part of an adaptive strategy.

We suggest that *Eudyptes*' extreme intraclutch ESD evolved in the context of clutch-size invariance (a canalized 2-egg clutch) stemming from a unique interaction between the physiology of follicle (yolk) development and selection favouring a slower life history that resulted in temporal overlap between migration and reproduction (Crossin et al. 2010). In birds, including penguins, the number of recruited follicles exceeds clutch size, and pre-ovulatory follicles are resorbed after clutch completion (Haywood 1993; Crossin et al. 2011). Clutch size is typically determined by arresting follicle development at the end of the follicle hierarchy (Haywood 1993), not by selective abortion of earlier developing follicles within the hierarchy (Goerlich et al. 2010). In penguins, development of the A-follicle precedes that of the B-follicle, by ~4 days (Astheimer and Grau 1985; Grau 1982, Crossin et al. 2010); this suggests that development of the A-follicle will be disproportionately affected by migration overlap, with migration overlap potentially contributing to the extent of ESD (Crossin et al. 2010). Consistent with this, *E. pachyrhynchus* exhibits the lowest ESD (17.5%) within *Eudyptes* and little or no overlap between follicle development and migration (Grau 1982; Williams 1995), while *E. chrysolophus* exhibits among the highest ESD (46.8%) and extensive overlap between follicle development and migration (Williams 1990; Crossin et al. 2010). There is a widespread fitness advantage associated with early onset of egg laying in birds (Williams 2012). If this fitness advantage was large when *Eudyptes* made the transition to a pelagic over-wintering ecology and their life history slowed down, then selection could have favoured migration-reproduction overlap with the energetic cost of the A-egg being off set by the increased survival probability of the B-chick. Under such a selective regime, the physiology of clutch-size determination could favour the B-egg and preclude the subsequent elimination of the A-follicle.

In conclusion, the 6 spp. of *Eudyptes* penguins have an invariant 2-egg clutch but only attempt to raise one chick (Williams 1995); this bizarre combination of reproductive traits constitutes a genus-wide evolutionary mismatch between clutch size and realized fecundity. This mismatch is associated with reversed hatching asynchrony (St. Clair et al. 1996) and maternal egg ejection (St. Clair et al. 1995), which appear to have coevolved with extreme intraclutch ESD in *Eudyptes*. Reversed hatching asynchrony ensures systematic loss of the A-chick when the smaller A-egg is retained until hatching (St. Clair 1992; St. Clair 1996). Maternal egg ejection typically occurs at or before the time the B-egg is laid (St. Clair et al. 1995). While acknowledging that our constraint-based explanation is controversial, we argue that an invariant 2-egg clutch coupled with extreme intraclutch ESD, high production costs of the A-egg, systematic loss of the A-egg or A-chick (in the wild) and an apparent inability of abundant food resources to rescue the A-egg or A-chick (in captivity) are more consistent with maladaptation than they are with an adaptive strategy. We suggest that the persistent failure to evolve a 1-egg clutch constitutes a genus-wide evolutionary maladaptation unique to *Eudyptes* and that extreme intraclutch ESD arose as a correlated response to selection favouring a slower life history.

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Tables and Figures

Table 6.1. Life history characteristics of penguin species used in comparative analyses.

Common name *Scientific name*	Female mass (g)	A-egg mass (g)	B-egg mass (g)	Age of first reproduction (yr)	Annual fecundity (chicks × yr^{-1})	Chicks fledged per clutch
Emperor *Aptenodytes forsteri*	24000 [44]	469.4 [44]	--	5.3 [19]	0.63 [44]	0.63 [44]
Adélie *Pygoscelis adeliae*	3890 [44]	122.8 [44]	115.3 [44]	5.8 [1]	0.99 [1,21,32,34,36,46]	0.99 [1,21,32,34,36,46]
Gentoo *Pygoscelis papua*	5860 [44]	128.2 [44]	130.0 [44]	3.5 [19]	0.83 [6,27,30,41,43,44]	0.83 [6,27,30,41,43,44]
Chinstrap *Pygoscelis antarctica*	3893 [44]	102.2 [21]	102.5 [21]	4.8 [36]	0.81 [21,36]	0.81 [21,36]
Magellanic *Spheniscus magellanicus*	3708 [2]	124.9 [5]	124.7 [5]	7.5 [3]	0.52 [4,17,27,44,47]	0.52 [4,17,27,44,47]
Black-footed *Spheniscus demersus*	2880 [24]	106.8 [44]	104.8 [44]	5.2 [40]	0.62 [11]	0.62 [11]
Peruvian *Spheniscus humboldti*	3820 [35]	121.2 [10,44]	125.1 [10,44]	2.5 [48]	1.53 [26]	0.92 [26]
Galapagos *Spheniscus mendiculus*	1768 [44]	79.6 [44]	80.9 [44]	--	--	--
Little *Eudyptula minor*	1048 [44]	53.7 [20]	53.5 [20]	2.6 [13]	1.22 [25,29]	0.76 [25,29]
White-flippered *Eudyptula albosignata*	1148 [8]	60.0 [8]	59.7 [8]	2.6 [8]	1.16 [8]	1.16 [8]
Yellow-eyed *Megadyptes antipodes*	4900 [44]	139.4 [22]	136.9 [22]	3.2 [31]	1.27 [14,22,28]	1.27 [14,22,28]
Erect-crested *Eudyptes sclateri*	3617 [44]	81.6 [15]	150.9 [15]	--	--	--
Royal *Eudyptes schlegeli*	4100 [39]	100.3 [44]	159.3 [44]	8.1 [7]	0.49 [44]	0.49 [44]
Macaroni *Eudyptes chrysolophus*	3950 [44]	92.7 [44]	149.4 [44]	7.5 [12]	0.49 [33,42,45]	0.49 [33,42,45]
Rockhopper *Eudyptes moseleyi*	2290 [9]	88.4 [16]	118.4 [16]	4.7 [18]	0.72 [18]	0.72 [18]
Fiordland *Eudyptes pachyrhynchus*	2645 [37]	99.4 [44]	118.5 [44]	5.5 [35]	0.50 [37]	0.50 [37]

Common name *Scientific name*	Female mass (g)	A-egg mass (g)	B-egg mass (g)	Age of first repro-duction (yr)	Annual fecundity (chicks × yr^{-1})	Chicks fledged per clutch
Snares *Eudyptes robustus*	2700 44	103.3 23	132.5 23	6.3 38	0.77 44	0.77 44

Sources. [1]Ainley and Demaster 1980, [2]Bertellotti et al. 2002, [3]Boersma 2011, [4]Boersma et al. 1990, [5]Boersma et al. 2013, [6]Bost and Jouventin 1991, [7]Carrick and Ingham 1970, [8]Challies 2011, [9]Cherel et al. 1999, [10]Corado and Hall 2009, [11]Crawford et al. 2006, [12]Croxall and Davis 1999, [13]Daan and Cullen 1990, [14]Darby and Seddon 1990, [15]Davis and Renner 2003, [16]Demongin et al. 2010, [17]Frere et al. 1998, [18]Guinard et al. 1998, [19]Jouventin and Weimerskirch 1981, [20]Kemp and Dann 2001, [21]Lishman 1985, [22]Massaro 2010, [23]Massaro and Davis 2005, [24]Nagy et al. 1984, [25]Nisbet and Dann 2009, [26]Paredes et al. 2002, [27]Putz et al. 2001, [28]Ratz et al. 2004, [29]Reilly and Balmford 1975, [30]Reilly and Kerle 1981, [31]Richdale 1957, [32]Spurr 1975, [33]Stahl 1985, [34]Taylor 1962, [35]Taylor et al. 2002, [36]Trivelpiece et al. 1990, [37]Warham 1974a, [38]Warham 1974b, [39]Warham 1971, [40]Whittington 2005, [41]Williams 1980a, [42]Williams 1980b, [43]Williams 1991, [44]Williams 1995, [45]Williams and Croxall 1991, [46]Yeates 1968, [47]Yorio 2001, [48]Zavalaga and Paredes 2009.
Note. Female mass: all single-study data points taken from the chick-rearing period except a) *Eudyptes schlegeli* mid-point of range 3200 – 5000g; b) *Eudyptula minor*; c) *Pygoscelis antarctica* mean of 3 studies. A- and B-egg mass: all single study data points except a) *Eudyptes pachyrhynchus* mean of 2 studies; b) *Eudyptes chrysolophus* mean of 3 years, South Georgia Island; c) *Pygoscelis adeliae* mean of 2 studies; d) *Spheniscus humboldti* unpublished data; e) A- and B-egg mass for *Pygoscelis antarctica*, *Spheniscus humboldti*, *Spheniscus mendiculus*, *Eudyptula albosignata* and *Eudyptes robustus* was estimated using a ordinary least-squares regression equation derived from fresh egg mass of 10 2-egg clutch penguins species: fresh egg mass = $-1.28 + 1.08 \times \text{length} \times \text{breadth}^2 \times \pi \times 6^{-1}$, $F_{1,18} = 7080$, $p < 0.0001$, adjusted $r^2 = 0.997$. Age of first reproduction: average of male and female mean ages of first reproduction uncorrected for within-cohort mortality. Annual fecundity: values for *Spheniscus humboldti* and *Eudyptula minor* account for successful second clutches.

Table 6.2. Species and sample sizes of A- and B-eggs used to estimate fresh egg mass from linear dimensions.

Common name	Scientific name	A-egg (n)	Length (mm)	Width (mm)	Volume (cc)	Mass (g)	B-egg (n)	Length (mm)	Width (mm)	Volume (cc)	Mass (g)
Adélie	*Pygoscelis adeliae*	73 [2]	69.2	55.3	110.8	120.8	73 [2]	68.4	55.2	109.1	113.4
Gentoo	*Pygoscelis papua*	20 [3]	68.3	58.2	121.1	128.7	20 [3]	67.4	57.4	116.3	124.1
Chinstrap	*Pygoscelis antarctica*	51 [2]	67.1	52.3	96.1	113.6	56 [2]	67.0	52.4	96.3	112.2
Black-footed	*Spheniscus demersus*	70 [3]	69.6	52.1	98.9	106.8	70 [3]	67.6	52.0	95.7	104.8
Little	*Eudyptula minor*	94 [1]	55.8	42.0	51.5	53.7	94 [1]	54.6	42.2	50.9	53.5
Erect-crested	*Eudyptes sclateri*	50 [3]	68.1	47.1	79.1	83.7	50 [3]	82.5	57.3	141.8	150.7
Royal	*Eudyptes schlegeli*	31 [3]	69.7	50.8	94.2	100.3	28 [3]	80.7	59.2	148.1	159.3
Macaroni	*Eudyptes chrysolophus*	52 [3]	68.1	49.6	87.7	92.6	70 [3]	78.2	57.8	136.8	144.6
N. Rockhopper	*Eudyptes moseleyi*	122 [3]	62.3	46.8	71.4	76.0	119 [3]	70.2	52.9	102.9	109.1
E. Rockhopper	*Eudyptes filholi*	37 [3]	63.5	48.0	76.6	79.6	37 [3]	70.5	53.7	106.4	112.0
Fiordland	*Eudyptes pachyrhynchus*	54 [3]	67.3	51.1	92.0	98.9	54 [3]	70.9	54.2	109.1	116.6

Sources. [1]Kemp and Dann 2001, [2]Lishman 1985, [3]Williams 1995.
Notes. Published fresh mass for the A- and B-eggs of *Pygoscelis antarctica* was almost exactly 10 g larger than expected from linear dimensions. So, *Pygoscelis antarctica* was excluded from the predictive analysis and fresh egg mass was also estimated for this species.

Figure 6.1. Time-calibrated molecular phylogeny of extant penguin species with Bayesian posterior support probabilities (from Baker et al. 2006). Extreme intraclutch egg-size dimorphism in *Eudyptes* is associated with evolutionary mismatch between clutch-size and number of chicks fledged per clutch. *Aptenodytes patagonicus*, *Spheniscus mendiculus* and *Eudyptes sclateri* are not included in genera means for chicks fledged per clutch.

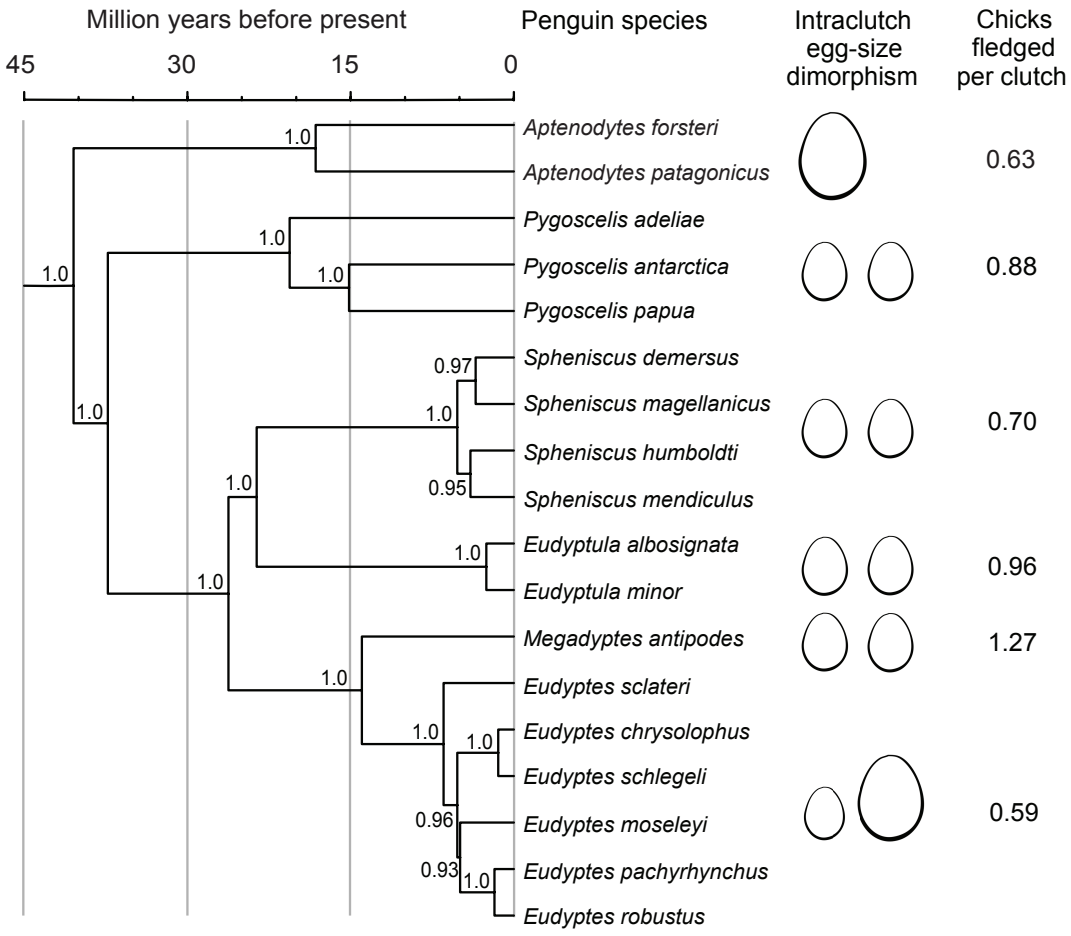


Figure 6.2. Phylogenetic generalized least-squares regression model demonstrating a uniform, inverse relationship between annual fecundity and age of first reproduction for 2-egg clutch penguin species. Gray-filled open circles represent *Eudyptes* and solid black circles represent *Pygoscelis*, *Spheniscus*, *Eudyptula* and *Megadyptes*. The white-filled open circle represents *Aptenodytes forsteri*, the only extant 1-egg clutch penguin species with a annual reproductive cycle. *A. forsteri* was not included in the analysis and is displayed only for comparison.

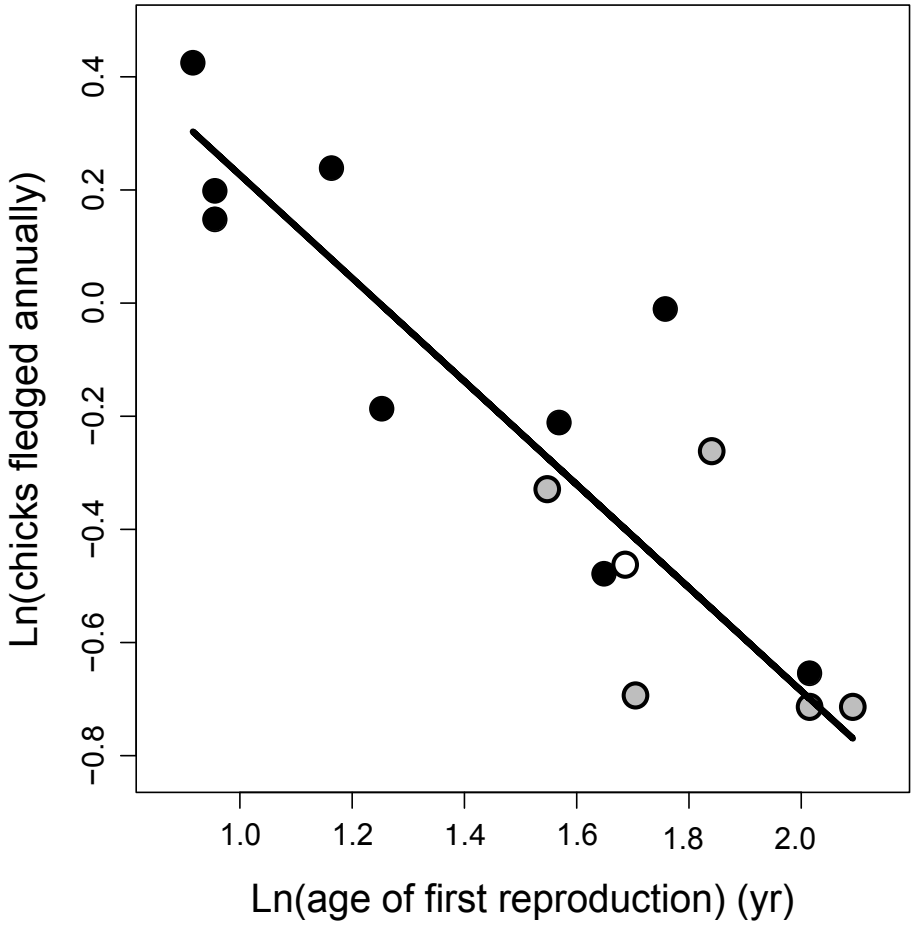


Figure 6.3. Phylogenetic generalized least-squares regression models depicting A-egg (A) and B-egg (B) allometry for 2-egg clutch penguin species. Gray-filled open circles represent *Eudyptes* and solid black circles represent *Pygoscelis*, *Spheniscus*, *Eudyptula* and *Megadyptes*. For consistency, the full regression model (interaction and main effects) is plotted in each panel.

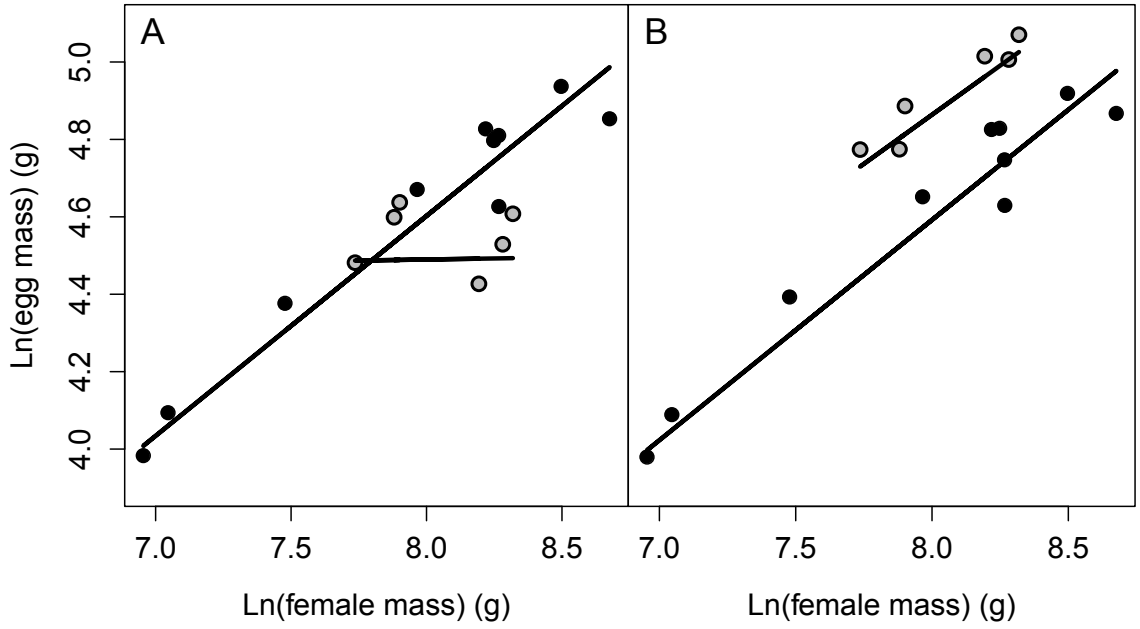
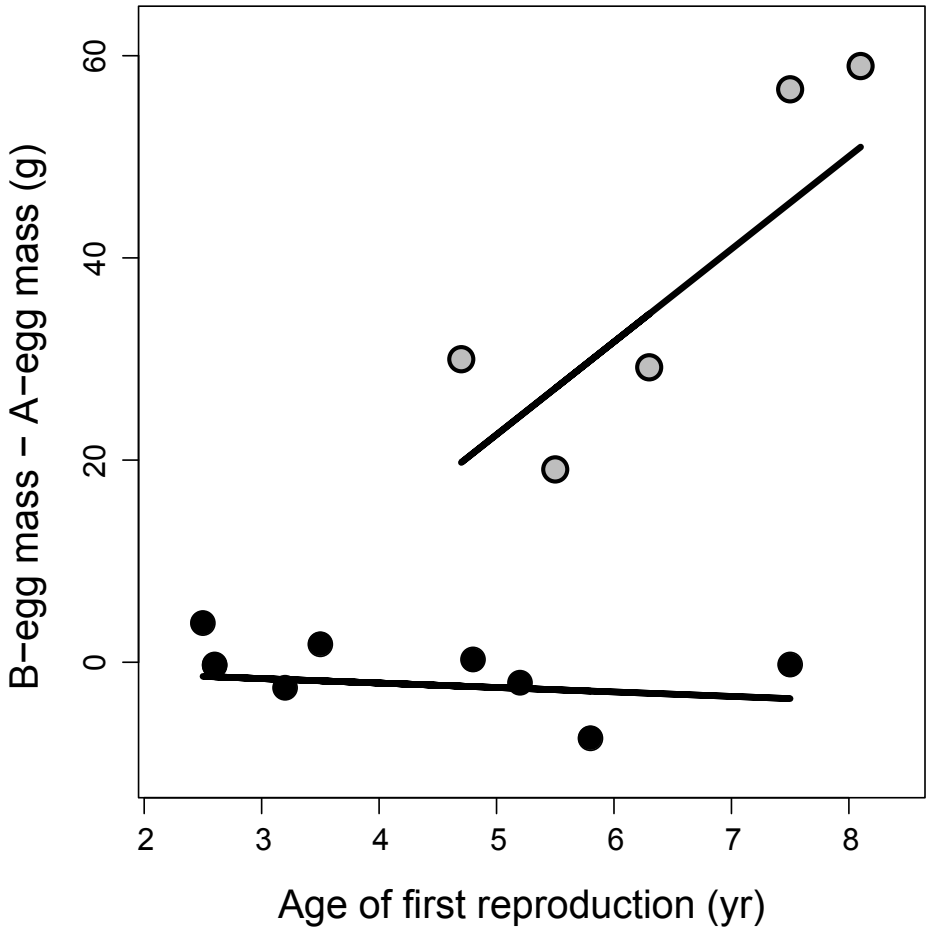


Figure 6.4. Phylogenetic generalized least-squares regression model explaining egg-size dimorphism (B-egg mass – A-egg mass) for 2-egg clutch penguin species. Egg-size dimorphism is positively correlated with age of first reproduction in *Eudyptes*, but not in other 2-egg clutch penguin species. Gray-filled open circles represent *Eudyptes* and solid black circles represent *Pygoscelis*, *Spheniscus*, *Eudyptula* and *Megadyptes*.



Chapter 7.

General synthesis and future directions

This thesis explores process-based explanations for the evolution of a small but diverse set of polymorphisms in birds. The polymorphisms addressed here involve a disparate set of taxonomic groups sampled from across the hierarchy (families within an order, genera within a family, and within a single species) of avian phylogeny (Jetz et al. 2012). Exploring polymorphisms at multiple taxonomic levels is important because different process (e.g. trait-dependent diversification vs. intra- or intersexual competition) are operating at different taxonomic levels and consideration of processes at one level can offer insights into process operating at another level. For example, Székely et al. (2004) were able to explain SSD for body mass and wing cord in a comparative phylogenetic analysis across the Charadriiformes using proxies for sexual selection (acrobatic display flights and social mating system), but these proxies for sexual selection were not able to account for bill-length SSD. Székely et al. (2004) suggested that the processes driving the evolution of disproportionate bill-length SSD might not be operating on the breeding grounds and that they might be operating at another time, and potentially in another place, in the annual cycle. Disproportionate bill-length SSD is common in sandpipers that exhibit differential migration and this prompted the intraspecific work presented here assessing whether intersexual competition on the wintering might be promoting / maintaining disproportionate bill-length dimorphism in the western sandpiper, a small shorebird that exhibits female-biased SSD, disproportionate bill-length dimorphism and differential migration. Comparison to a null model of bill-length dimorphism specific to the western sandpiper suggested that the extent of dimorphism was larger at some wintering sites and this is consistent with niche differentiation, but it is not conclusive. Similar data from additional sites could help to demonstrate the generality of the findings consistent with niche differentiation presented here.

This work raised an important question: is intersexual competition sufficient to promote and maintain disproportionate bill-length SSD in differential migrants, such as the western sandpiper? This question cannot be addressed with the data presented in this thesis, but it is an important question because migratory birds have the ability to redistribute themselves to other wintering sites if conditions are poor or deteriorate. Long-term climate cycles, such as the Pacific decadal oscillation (PDO; associated with the El Niño–Southern Oscillation) could be an additional source of disruptive selection on bill length in shorebirds that overwinter along the Pacific coast of the Americas and rely on food resources derived from the marine environment. O'Hara et al. (2007) reported a trend for a reduction in pre-migratory body condition (size-corrected body mass) for three species of *Calidris* sandpiper wintering at coastal sites on the Pacific coast of Ecuador following a strong PDO event. To determine the potential impacts of PDO events on the evolution of disproportionate bill-length dimorphism, one would first need to determine whether PDO events have an impact on the abundance and quality of the invertebrate prey of migratory shorebirds and then whether wintering populations exposed to PDO are associated with increased bill-length dimorphism. To test the hypothesis that PDO events exert disruptive selection on bill length, one could sample sites on the Pacific and Atlantic coasts of southern North America, Central America and northern South America to test whether bill-length dimorphism is larger at sites on the Pacific coast.

The *Eudyptes* penguins exhibit a unique reproductive polymorphism, an extreme form of intraclutch egg-size dimorphism where the second-laid B-egg is substantially larger than the first-laid A-egg. A-eggs almost never produce a fledged chick and the substantial time and energy invested in making the A-egg is wasted; this is maladaptive. Using life history theory, I argue that extreme intraclutch egg-size dimorphism in *Eudyptes* penguins evolved as a correlated response to a life history slowdown and clutch-size canalization. One unresolved question remains in this evolutionary conundrum, why is there such marked variation in the relative size of the A-egg within *Eudyptes*? In the three larger *Eudyptes* species there has been a reduction in the relative size of the A-egg but in the three smaller species the relative size of the A-egg is exactly what one would expect from non-*Eudyptes* taxa with a two-egg clutch. The phylogenetic comparative approach is unlikely to be effective in this instance because

the sample sizes are prohibitively small (n = 3 vs. n = 3). It is possible, however, that captive breeding colonies could offer insights. The extent of migration-reproduction overlap, stemming from the transition to a pelagic lifestyle in *Eudyptes*, has been implicated in the extent of intraclutch egg-size dimorphism within *Eudyptes chrysolophus*, which is primarily associated with variation in the size of the smaller A-egg (Crossin et al. 2010). Captive populations do not have a pre-breeding migration, and this means that the relative size of the A-egg in captive populations is unaffected by migration-reproduction overlap. If the extent of overlap between follicle formation and pre-breeding migration is a critical factor determining the relative size of the A-egg, this would lead one to predict that there should be no difference in the relative size of the A-egg in captive populations of the large and small *Eudyptes* species. If there is no difference in the relative size of the A-egg in captive populations of large and small *Eudyptes* taxa, then this suggests that migration is a critical factor determining the relative size of the A-egg and that there is a difference in the extent of migration-reproduction overlap in large and small *Eudyptes* taxa. If the difference in relative A-egg size is still evident in captive populations of large and small *Eudyptes* taxa, then this suggests that evolutionary, and not ecological factors are involved.

In light of recently characterized fossils, the diversification chronology of the extant radiation of penguins (Sphenisciformes) presented by Baker et al. (2006) has been questioned (Ksepka and Ando 2011). This does not present a problem for the results presented in this thesis, because the results presented here are not explicitly time dependant. Baker et al. (2006) inferred that the extant penguin radiation diversified ~40 M years ago. This estimate was inferred via a two-stage process: first, a molecular phylogeny for the Spenisciformes was inferred and second, this topology was constrained, hard constraints were placed on nodes distant to the Sphenisciformes and then branch lengths were inferred across the phylogeny. Based on a large series of recently characterized fossils, Ksepka and Ando (2011) suggested that ~25 M years was a more realistic estimate for the diversification of the extant penguin taxa. Given the discrepancy in these estimates (25 vs. 40 M years) and recent advances in the simultaneous estimation of phylogenetic topology and chronology available in BEAST (Drummond and Rambaut 2007), the diversification chronology of the Sphenisciformes warrants re-evaluation. The sister-group relationships of the Sphenisciformes, the

Ciconiiformes (Pacheco et al. 2011), are better characterized, more sequence data is available, and the recently characterized fossils would facilitate better temporal calibration (Ksepka and Ando 2011). All of these factors would contribute to a more robust estimate of the phylochronology of Sphenisciformes. If Ksepka and Ando (2011) were correct in estimating that the extant radiation of penguins diversified ~25 M years ago, then this shifts the diversification chronology from the Paleogene to the Neogene and suggests “long fuse,” rather than “short fuse,” diversification dynamics (van Tuinen et al. 2009).

The first data chapter in this thesis presents a phylogenetic comparative analysis examining the indirect effects of sexual selection (acting on males) on two female-specific reproductive characters, egg size and clutch size, in the Galliformes. This comparative study was conducted at the genus-level, using a supertree as the phylogenetic hypothesis. There are limitations on what can be done analytically with a supertree, and this eventually lead me to reconstruct the phylochronology of Galliformes using BEAST (Drummond and Rambaut 2007). BEAST is the only phylogenetic inference platform that presently allows for the simultaneous inference of topology and chronology. Diversification analyses conducted on a set of 10000 taxon-complete phylogenies of the Galliformes confirmed Cretaceous origins and revealed diversification in the Paleogene and Neogene corresponding to the two-pulse model proposed by van Tuinen et al. (2006). Among-family variation in diversification rate was substantial and MEDUSA (Alfaro et al. 2008) analyses provided support for 1-3 increases in diversification rate. The most frequently inferred rate shift corresponds to a sustained increase in diversification rate post-K-Pg, in the clade including Numididae, Odontophoridae and Phasianidae. A second, less common rate shift was associated with an increase in diversification rate in the Phasianidae. Tarsal spurs are common in the Phasianidae and this suggests that tarsal spurs might be acting as a key innovation promoting diversification in this family. This is something that I plan to test using the multi-state speciation and extinction models in Diversitree (Fitzjohn 2010).

In a subsequent analysis of SSD in the Galliformes I used tarsal spurs and mode of parental care as proxies for strength of sexual selection and divergence in the reproductive roles of males and females, respectively. I then used combinations of these trait-based proxies to explain SSD. Tarsal spurs are a complex character; they are

composed of a bony core that is covered by highly cornified integument (Stettenheim 2000). In the Galliformes the integument covering the leg is composed of a combination of scutellate and reticulate scales. Scutellate scales are composed of beta-keratin and reticulate scales are composed of alpha-keratin (Stettenheim 2000). The bony core projecting off the tarsometatarsus interacts with the leg scales in such a way that spurs involving scutellate scales can make a much sharper and more formidable weapon than those involving reticulate scales. Tarsal spurs occur in the Numididae, which have reticulate leg scales, and in the Phasianidae, which have scutellate leg scales. Tarsal spurs in the Numididae are functionally similar, but not identical, to tarsal spurs in the Phasianidae, and this complicates ancestral character state reconstruction for tarsal spurs. However, none of the taxa from the Numididae are included in the phylogenetic comparative analyses presented in this thesis. The reason for this is that in the Numididae tarsal spurs are present in males and females and sample sizes were not sufficient to include taxa where tarsal spurs were present in both sexes. Nevertheless, the ancestral character state reconstructions, and the comparative analyses informed by them, must be considered preliminary until ancestral character state for tarsal spurs can be explored further.

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