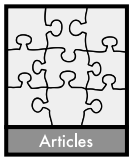

J. Avian Biol. 39: 438–445, 2008
doi: 10.1111/j.2008.0908-8857.04270.x

Received 14 May 2007, accepted 5 November 2007

A multigene phylogeny of Galliformes supports a single origin of erectile ability in non-feathered facial traits

Rebecca T. Kimball and Edward L. Braun

R. T. Kimball (correspondence) and E. L. Braun, Dept. of Zoology, Univ. of Florida, P.O. Box 118525, Gainesville, FL 32611 USA. E-mail: rkimball@ufl.edu

Many species in the avian order Galliformes have bare (or ''fleshy'') regions on their head, ranging from simple featherless regions to specialized structures such as combs or wattles. Sexual selection for these traits has been demonstrated in several species within the largest galliform family, the Phasianidae, though it has also been suggested that such traits are important in heat loss. These fleshy traits exhibit substantial variation in shape, color, location and use in displays, raising the question of whether these traits are homologous. To examine the evolution of fleshy traits, we estimated the phylogeny of galliforms using sequences from four nuclear loci and two mitochondrial regions. The resulting phylogeny suggests multiple gains and/or losses of fleshy traits. However, it also indicated that the ability to erect rapidly the fleshy traits is restricted to a single, well-supported lineage that includes species such as the wild turkey *Meleagris gallopavo* and ring-necked pheasant *Phasianus colchicus*. The most parsimonious interpretation of this result is a single evolution of the physiological mechanisms that underlie trait erection despite the variation in color, location, and structure of fleshy traits that suggest other aspects of the traits may not be homologous.

Species within the Phasianidae (pheasants, partridges, guineafowl, grouse and turkeys) are well known for extreme ornamental traits, including fleshy regions around the head and neck. These fleshy traits are present in only some phasianid species, and (when present) vary from simple featherless regions on the head and/or neck (particularly around the eye) to highly modified and specialized structures such as combs and wattles (Table 1). While most fleshy traits are smooth skin, some are covered in small papillae. The coloration of these traits varies, although red (and to a lesser extent blue) predominate. The exact location of the fleshy region also varies, and may include the top of the head, the side of the head, the area just around the eyes, the neck, or a combination of these locations (Table 1). Even when fleshy traits occur in the same location on different species, the traits often differ in shape and appearance.

The function of these fleshy traits is poorly understood. Among carrion eaters, like vultures, fleshy heads and necks are thought to be an adaptation to avoid microbial infections. However, this is unlikely to explain fleshy traits in the non-carrion eating galliforms. Some grouse and megapodes have featherless inflatable air sacs used for vocalizations (Johnsgard 1973, Jones et al. 1995). However, airsacs with feathers also function in vocalizations, so the absence of feathers on some airsacs is unlikely to be related to their function in vocalizations. The most likely function of galliform fleshy traits is heat loss (e.g. Crowe and Withers 1979, Buchholz 1996) and/or intra- or intersexual selection (e.g., Zuk et al. 1995, Buchholz 1995, 1997, Ligon et al. 1998). Experiments on the red junglefowl *Gallus gallus* and wild turkey *Meleagris gallopavo* have shown that females use fleshy traits in mate choice decisions to the exclusion of ornamental plumage or other aspects of male display (Buchholz 1995, 1997, Zuk et al. 1995, Ligon et al. 1998). There is also evidence for sexual selection on the wattles of ring-necked pheasants *Phasianus colchicus* (reviewed in Mateos 1998), and supraorbital combs of grouse and ptarmigan (e.g. Brodsky 1988, Holder and Montgomerie 1993, Rintamäki et al. 2000). However, the results for ring-necked pheasants and various species of grouse and ptarmigan have been mixed and suggest that fleshy traits may have a more limited role in sexual selection in these species. An important role for fleshy traits in sexual selection is also suggested by studies which have demonstrated that the color and size of these traits is dependent upon testosterone in some species (e.g., Kimball 2006).

As might be expected based upon the differences in appearance, there are substantial differences among species in the use of these fleshy traits during displays. Independent of the inflatable air sacs used in vocalizations of some grouse and megapodes, some galliform species have the unique ability to erect rapidly (in seconds to minutes) and contract the fleshy traits. The ''erectile'' fleshy traits include the spectacular lappets and horns of tragopans, the wattles of ring-necked pheasants and related species, the supraorbital combs of grouse and ptarmigan, and the snood of wild turkeys. In contrast, other species lack the ability to erect

Table 1. Species and information about fleshy traits.

Species	Common name	Location	Type of trait	Color
Phasianidae				
Afropavo congensis	Congo peafowl	Neck, around eye	Bare skin	Red
Alectoris chukar	Chukar partridge	–	–	–
Alectoris rufa	Red-legged partridge	–	–	–
Bambusicola thoracica	Chinese bamboo partridge	–	–	–
Catreus wallichii	Cheer pheasant	Side of head	Wattles	Red
Chrysolophus pictus	Golden pheasant	Side of head	Eye ring	Yellow
Coturnix japonica	Japanese quail	–	–	–
Crossoptilon crossoptilon	White-eared pheasant	Side of head	Wattles	Red
Falcipennis canadensis	Spruce grouse	Above eye	Supraorbital comb	Red
Gallus gallus	Red junglefowl	Top, side of head	Comb, wattles, ears	Red
Gallus lafayettei	Ceylon junglefowl	Top, side of head	Comb, wattles, ears	Red, yellow
Gallus sonneratii	Gray junglefowl	Top, side of head	Comb, wattles, ears	Red
Gallus varius	Green junglefowl	Top, side of head	Comb, wattles, ears	Red, blue, yellow
Lophophorus impejanus	Impeyan monal	Around eye	Eye ring	Blue
Lophura inornata	Salvadori's pheasant	Side of head	Wattles	Red
Lophura nycthemera	Silver pheasant	Side of head	Wattles	Red
Lophura swinhoii	Swinhoe's pheasant	Side of head	Wattles	Red
Meleagris gallopavo	Wild turkey	Neck, Head	Snood	Red
Pavo cristatus	Indian peafowl	Side of head	Bare skin	White
Pavo muticus	Green peafowl	Side of head	Bare skin	Blue, yellow
Perdix perdix	Gray partridge	–	–	–
Phasianus colchicus	Ring-necked pheasant	Side of head	Wattles	Red
Polyplectron bicalcaratum	Gray peacock-pheasant	Around eye	Bare skin	Gray
Polyplectron chalcurum	Bronze-tailed peacock-pheasant	–	–	–
Polyplectron emphanum	Palawan peacock-pheasant	Around eye	Bare skin	Red
Polyplectron germaini	Germain's peacock-pheasant	Around eye	Bare skin	Red
Polyplectron inopinatum	Mountain peacock-pheasant	–	–	–
Polyplectron malacense	Malay peacock-pheasant	Around eye	Bare skin	Orange
Pucrasia macrolopha	Koklass pheasant	–	–	–
Syrmaticus ellioti	Elliot's pheasant	Side of head	Wattles	Red
Syrmaticus reevesii	Reeve's pheasant	–	–	–
Tragopan blythii	Blyth's tragopan	Side of head, neck	Lappets, horns	Yellow, blue
Tragopan temminckii	Temminck's tragopan	Side of head, neck	Lappets, horns	Blue, red
Tympanuchus phasianellus	Sharp-tailed grouse	Above eye	Supraorbital comb	Yellow
Odontophoridae				
Colinus virginianus	Northern bobwhite	–	–	–
Cyrtonyx montezumae	Montezuma quail	–	–	–
Oreortyx pictus	Mountain quail	–	–	–
Numididae				
Guttera pucherani	Crested guineafowl	Side of head, neck	Bare skin	Red, blue
Numida meleagris	Helmeted guineafowl	Top, side of head	Casque, wattles	Red, blue
Cracidae				
Crax rubra	Great curassow	Above bill	Casque	Yellow
Ortalis vetula	Plain Chachalaca	Neck	Gular pouch	Red
Megapodidae				
Alectura lathami	Australian brush turkey	Head, neck	Wattle	Red, yellow
Leipoa ocellata	Malleefowl	Around eye	Bare skin	Gray, pale blue
Megapodius layardi	Vanuatu megapode	Around eye	Bare skin	Red

their fleshy traits rapidly, although in some species these more static fleshy traits can exhibit small changes in size and color over longer time periods. These static traits include the combs and wattles in the red junglefowl, the wattles of the helmeted guineafowl *Numida meleagris*, the bare flesh on the heads of peafowl (*Pavo* spp.), and the fleshy, colored regions around the eyes of peacock-pheasants (*Polyplectron* spp.).

Only a limited amount of information is known about the underlying physiology of fleshy traits, though what is known suggests variability within the phasianids. As indicated above, coloration and size appear to be affected by testosterone in many species (Kimball 2006). In the red junglefowl, the red color of the comb (as well as the wattle, and in some individuals, the earlobe), as well as the red coloration of the snood and caruncles in the wild turkey, is due to the presence of blood in the underlying capillary sinuses (Lucas and Stettenheim 1972, Stettenheim 2000). Carotenoids in the epidermis may also contribute to the red coloration (e.g., Stettenheim 2000), such as has been demonstrated for the supraorbital combs of grouse (e.g., Mougeot et al. 2007). While epidermal carotenoids may be important for coloration in some species, it is not universal as the red caruncles of wild turkey do not have carotenoids in the epidermis (McGraw et al. 2005). The red coloration of ring-necked pheasant wattles increases with carotenoid supplementation (e.g., Smith et al. 2007), and the carotenoids are thought to be deposited in the small papillae that cover the wattles (Lucas and Stettenheim 1972).

Overall, other than lacking feathers, the high degree of variability among these traits combined with the

observation that they are present in only a subset of species suggests they may have multiple independent origins. If this inference is correct, they should not be considered homologous. However, analyses of developmental pathways have revealed surprising connections between characters that were not thought to be homologous, such as the involvement of the *pax*-6 gene in the regulation of development of non-homologous visual systems across the animal kingdom (Gehring and Ikeo 1999). Thus, apparently homologous genes can be expressed in apparently non-homologous structures suggesting traits can exhibit partial homology (Mindell and Meyer 2001).

An earlier study using the mitochondrial cytochrome *b* suggested that fleshy traits are evolutionarily labile, but it also suggested the ability to erect rapidly or enlarge fleshy traits may have had a single evolutionary origin (Kimball et al. 2006). However, these conclusions, particularly regarding the single origin of erectile ability, were only supported when a codon-based model of sequence evolution was used in Bayesian analyses; other analyses of cytochrome *b* in galliforms provided conflicting conclusions (e.g., Kimball et al. 1999)

To better address the evolution of fleshy traits and the erectile ability, we wanted to obtain a well-resolved and well-supported phylogeny of the galliforms, particularly among the phasiands. To do this, we combined sequence data from two mitochondrial protein coding regions (cytochrome *b* [CYB] and NADH dehydrogenase subunit 2 [ND2]) with the sequences of four nuclear introns (intron G of ovomucoid [OvoG], intron 7 of β-fibrinogen [BFib7], intron 3 of the dimerization cofactor of hepatocyte nuclear factor 1 [DCoH3], and intron 1 of Rhodopsin [Rhod1]), and used these data to estimate a galliform phylogeny that would allow us to examine the evolution of erectile ability and fleshy traits in the Phasianidae.

Methods

Molecular methods

Species were selected to represent a range of galliform lineages. Taxon sampling is biased towards the most speciose galliform family, the Phasianidae, many of which are highly ornate and exhibit a wide range of fleshy traits. DNA samples used for PCR amplification have been described previously (Kimball et al. 1999, 2001, Randi et al. 2000, 2001, Armstrong et al. 2001, Cox et al. 2007). We used published and novel sequences collected for this study. Primers and gene information for novel sequences can be obtained from Cox et al. (2007).

PCR products were cleaned for sequencing by precipitation using an equal volume of PEG:NaCl (20%: 2.5M). Sequencing of PCR products was done using either ABI BigDye Terminator v. 1.0, BigDye Terminator v. 3.1, or Beckman DTCS Quickstart chemistries. Manufacturers' recommendations were followed, except reaction volumes were cut to 1/2-1/6 of the recommended volume. Sequences were analyzed on an ABI Prism 3100-Avant genetic analyzer (PE Applied Biosystems), or a CEQ 8000 (Beckman-Coulter) genetic analysis system. Double-stranded contigs were assembled using Sequencher 4.1 (Gene Codes Corp.).

Many of the nuclear loci were heterozygous, and some of the alleles differed in size within an individual. These PCR products were cloned using the pGEM-T Easy vector (Promega Corp.) to obtain clean sequences in each direction. Plasmid preparations were done using Eppendorf Perfectprep Plasmid Mini kit, and the resulting plasmids were sequenced in each direction. In these cases, original sequences from the PCR products, as well as those from the plasmids, were used in assembly of the final contig.

Alignment and phylogenetic analysis

Sequences of the mitochondrial coding regions were equal in length and did not have any insertions or deletions, so alignment was straightforward. Nuclear intron sequences were initially aligned using ClustalX (Thompson et al. 1997) and optimized by eye. Sequences collected for this study have been deposited in GenBank (Accession numbers DQ306959-DQ307021, EF569434-EF569485).

Maximum parsimony (MP) analyses were performed using PAUP* 4.0b10 (Swofford 2003) and maximum likelihood (ML) analyses were performed using both PAUP* and RAxML 2.2.3 (Stamatakis 2006). The appropriate model for ML analyses of the combined matrix and each independent nuclear locus or mitochondrial region was determined using the Akiake Information Criterion from the model set examined by Modeltest 3.6 (Posada and Crandall 1998). In PAUP*, ML trees of the independent loci and the combined matrix were identified using a heuristic search with 10 random sequence additions. RAxML was used to conduct partitioned ML analysis, with each independent nuclear locus or mitochondrial region defined as a partition as well as to analyze individual partitions. Support for groups was assessed using the bootstrap, using 1000 bootstrap replicates and a heuristic search with 10 random sequence additions in PAUP* for MP and using 500 bootstrap replicates in RAxML for ML. A partitioned Bayesian analysis was performed using MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003), using the same partitions as in the RAxML analysis. For each partition, we either used the Modeltest results or we substituted the best fitting model implemented in MrBayes. We ran four chains (three of which were heated) for 5×10^6 generations and discarded the first 2×10^6 generations, a strategy that achieved convergence based upon a second analysis using the same model and settings (but different random seeds).

We defined fleshy traits as any region bare of feathers that was found on the head or neck. Information about fleshy traits and displays were taken from the literature (Johnsgard 1973, 1988, 1999, Roles 1976, Delacour and Amadon 1973, Delacour 1977, Jones et al. 1995, Madge and McGowan 2002, and references therein). We performed a parsimony reconstruction of fleshy traits using MacClade 4.0 (Maddison and Maddison 2000) using the fully-resolved ML tree generated by RAxML (Fig. 1). We weighted gains and losses equally and used the parsimony analysis to reconstruct the ancestral condition, estimate the number of transitions, and establish the minimum and

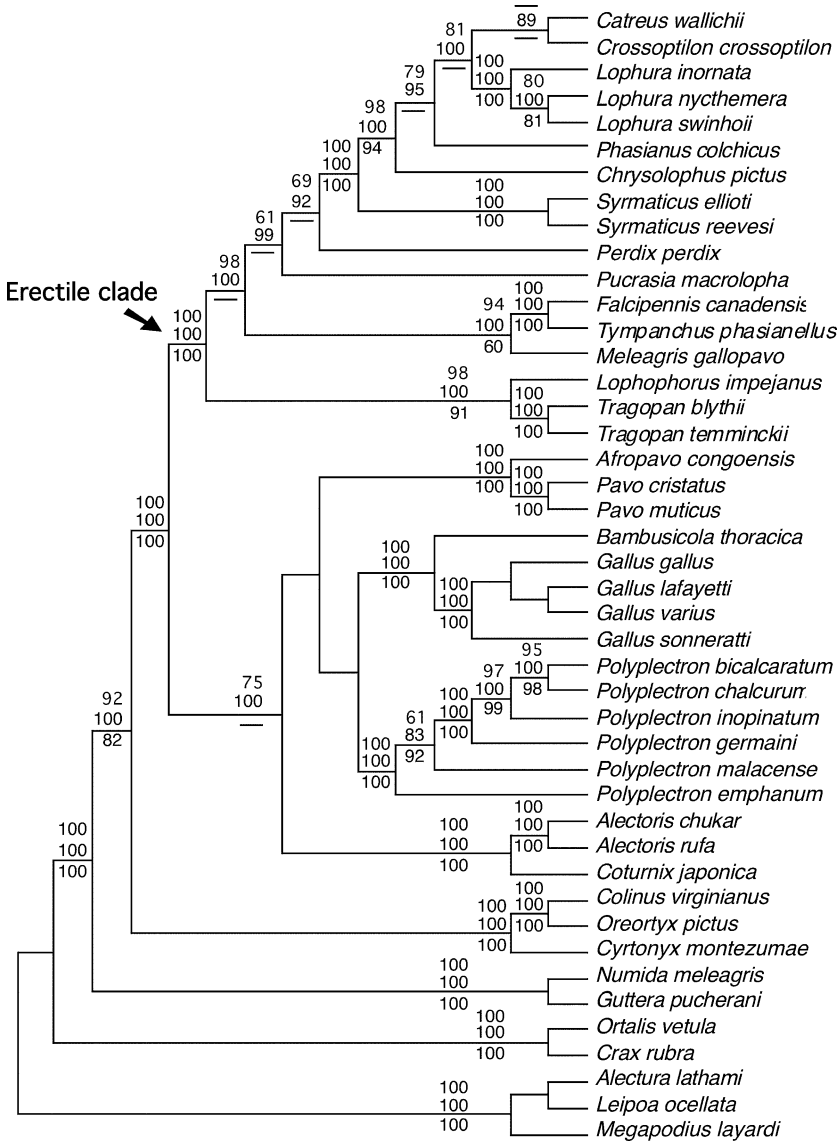

Figure 1. Phylogeny based upon four nuclear introns (BFib7, DCoH3, OvoG, and Rhod1) and two mitochondrial coding regions (CYB and ND2). Shown is the ML cladogram from a partitioned search in RAxML. At each node is the percentage of ML bootstrap support from RAxML (top), Bayesian posterior probability expressed as a percentage (middle), and MP bootstrap support (bottom) for nodes that received at least 50% support in at least one analyses. Nodes receiving less than 50% support values or absent in one type of analysis are indicated by a dash.

maximum numbers of gains and losses. We also performed a maximum likelihood reconstruction using Mesquite (Maddison and Maddison 2003) on the same fully-resolved ML tree, but this time incorporating branch length information (using ML branch length estimates). Using likelihood, we tested both a one rate (gains = losses) model and a two rate model (allowing a different gain and loss rate).

Results

Characteristics of the data sets

The combined alignment was 6153 bp in length, of which 2184 bp were mitochondrial and 3969 bp were nuclear intron data. A single, large insertion (579 bp) in BFib7 of the Mountain quail *Oreortyx pictus* was excluded from analyses. There were two micro-inversions of 20 and 21 bp that were also excluded in the taxa showing the derived inversion state. The resulting data set included 5533 sites, of which 3349 were nuclear. Of these sites, 53% were variable and 41% were parsimony informative. The mitochondrial and nuclear data had similar proportions of variable sites (52% and 53%, respectively), though the mitochondrial data had a higher proportion of informative sites (45% as compared to 38%). The consistency index (CI), excluding uninformative sites, was 0.399 for the entire data set, with the mitochondrial partition having a much lower CI (0.286) than the nuclear intron partition (0.648). This suggests that the higher proportion of parsimony informative sites in the mitochondrial dataset reflects a higher degree of homoplasy rather than the existence of more phylogenetic signal.

Relationships among Phasianidae

Analysis of the combined data set resulted in a well-resolved phylogeny (Fig. 1) with many highly supported nodes. The differences among the phylogenies estimated by ML (the topologies from PAUP* and the partitioned ML tree from RAxML were identical), Bayesian, and MP were minor and largely reflected the lower resolution of the MP analyses. MP analyses generally gave lower levels of bootstrap support than were found in ML bootstrap analyses (Fig. 1). No well-supported nodes differed between analyses.

Based on these results, the Phasianidae is comprised of two major clades. One of these includes the grouse, wild turkey, ring-necked pheasants and other typical pheasants, as well as the gray partridge *Perdix perdix*. This phasianid clade (''erectile clade'', Fig. 1) was present in all individual partitions, often with strong support (bootstrap values were 90% or greater in all but CYB and OVOG). Finally, there is a large ($\sim$300 base pair) deletion in BFib7 that unites this derived clade, providing a distinct molecular synapomorphy defining this clade.

The second clade within the Phasianidae has lower support and less resolution (Fig. 1). This grouping includes: 1) junglefowl and relatives, 2) peafowl and relatives, 3) peacock-pheasants, and 4) the Japanese quail *Coturnix japonica* and relatives. There was little support for relationships among these four lineages, all of which are separated by short internodes (data not shown).

Evolution of fleshy traits and erectile ability in the Phasianidae

The most parsimonious reconstruction of the ancestral condition is the presence of fleshy traits. Similarly, likelihood reconstruction using the partitioned ML tree indicated an 80.25% probability that presence of fleshy traits is ancestral (a similar value, 86.5%, was obtained using the unpartitioned ML tree). If we assume that fleshy traits are homologous (e.g., they have similar developmental controls) and use parsimony to map the presence and absence of fleshy traits on our estimated phylogeny, there are a minimum of eight transitions (gains and losses). A single transition rate model (i.e., an instantaneous rate of gains equals that of losses) is not significantly more likely than a two-transition rate model, based on a likelihood ratio test (for the partitioned likelihood tree ln likelihood $-$ 24.444 vs. $-$24.253, respectively; $P > 0.05$). Parsimony reconstruction suggests there have been four to eight losses and zero to four gains, though this difference cannot be examined statistically.

In contrast to the multiple transitions reconstructed for the presence or absence of fleshy traits, the ability to erect rapidly fleshy traits is found in one well-supported suprageneric clade (erectile clade, Fig. 1) suggesting a single origin of this ability. The possibility of a single underlying physiological mechanism for the rapid erection of fleshy traits suggests that the morphologically distinct fleshy traits within this clade may have some underlying homologous features. Interestingly, inflatable air sacs occur in two distantly related clades, represented in our phylogeny by the Australian brush turkey *Alectura lathami* (Jones et al. 1995) and the sharp-tailed grouse *Tympanchus phasianellus* (Drovetski 2002), suggesting independent evolutionary origins of this trait.

Discussion

Relationships among Phasianidae

Earlier molecular phylogenies of various phasianid taxa have provided support for monophyly of many genera and for relationships within genera (e.g., Kimball et al. 1997, 2001, Randi et al. 2000, 2001). However, relationships among many genera have been more problematic, exhibiting low levels of support or differences among studies (e.g., Kimball et al. 1999, Armstrong et al. 2001, Crowe et al. 2006a,b). The inclusion of multiple nuclear and mitochondrial markers in this study resulted in phylogeny that was largely resolved with well-supported nodes.

Our results are in agreement with many aspects of recently published studies, though we also find well-supported differences from those studies as well. The erectile clade, which is one of the two major phasianid lineages, has been evident in previous studies. However, taxon sampling was too limited in some studies to determine the limits of this group (e.g., Dimcheff et al. 2002, Kaiser et al. 2007) or the clade has limited support (Crowe et al. 2006a,b, Kimball et al. 2006, Kolm et al. 2007).

The relationships within the erectile clade revealed by this study differ from recently published studies. For example, our results support a clade containing the wild turkey and the grouse, a relationship initially suggested by the DNA-DNA hybridization (Sibley and Ahlquist 1990). In contrast to our strong support, this relationship has not been found in all studies (e.g., Kimball et al. 1999, Armstrong et al. 2001, Crowe et al. 2006a,b), or has been weakly supported (e.g., Gutiérrez et al. 2000 – if their phylogeny is rooted to *Gallus*, Dimcheff et al. 2002, Kaiser et al. 2007, Kolm et al. 2007). Our results also support the placement of the gray partridge at the base of a large clade containing typical pheasants, rather than the weakly supported with wild turkey (Crowe et al. 2006a,b). The grouping of the Impeyan monal *Lophophorus impejanus* with the tragopans occurs in many other published studies (e.g., Crowe et al. 2006a,b, Kolm et al. 2007), though in other studies these taxa are often united with the koklass pheasant *Pucrasia macrolopha*, in strong contrast to the results presented here.

The second phasianid clade has been resolved differently across studies. Several studies have also found these taxa to form a clade (e.g., Crowe et al. 2006a, Kimball et al. 2006) though support for this clade is weak in these studies. Other studies have found these lineages to form a grade at the base of the erectile clade rather than a second clade (e.g., Crowe et al. 2006b, Kaiser et al. 2007, Kolm et al. 2007). Whether a grade or a clade, relationships among the lineages differ among data sets and are never well-supported (e.g., Crowe et al. 2006a,b, Kimball et al. 2006, Kaiser et al. 2007, Kolm et al. 2007). While our data clearly suggest that a clade is more likely than a grade, relationships among the lineages within this clade will require more data to resolve fully.

Evolution of fleshy traits and erectile ability in the Galliformes

Our analyses suggest that the ancestral galliform had fleshy traits and that the number of losses probably exceeds the number of gains. Buchholz (1994) examined all galliform species and concluded that fleshy traits were primitive in the order. However, this does not clarify whether the distribution in the derived phasianids reflects a combination of maintenance and elaboration in some lineages and loss in others (homologous traits) or if instead it reflects multiple origins of fundamentally distinct characters (non-homologous traits). Testosterone appears to be important for trait appearance or use in display in a number of phasianid species (Kimball 2006). However, the traits differ in other ways such as color, location, and shape (e.g., Table 1). Even where the color of two fleshy traits is the same, the factors underlying the coloration may be distinct (e.g., the red comb of red junglefowl reflects blood flow through the underlying capillaries while the red wattles of ring-necked pheasants reflects carotenoids deposited in the papillae covering the wattles). These differences suggest that the fleshy traits in phasianids reflect multiple independent gains of fundamentally unique traits.

Within the Phasianidae, the ability to erect rapidly fleshy traits (excluding air sac inflation) was limited to a single well-supported clade. Thus, while fleshy traits may not be homologous, the most parsimonious interpretation of the results is a single origin of erectile ability (suggesting this aspect of the traits may be homologous). Once the ability to erect fleshy traits had evolved, several factors may have favored the retention of this ability. First, if fleshy traits are important in thermoregulation (e.g., Crowe and Withers 1979, Buchholz 1994, 1996), the ability to manipulate the size of these traits rapidly may allow better regulation of body temperature. In at least some species (see introduction), fleshy traits function as social signals. The ability to retract such traits can allow these status signals to be largely hidden in many social environments (Holder and Montgomerie 1993, but see Hannon and Eason 1995), but displayed in others. It has also been suggested that the ability to enlarge such traits rapidly may serve as a stimulus to females (Ligon 1999), thus males able to make the most rapid changes or capable of the greatest change in size might be favored by females. Finally, fleshy traits are susceptible to damage through male-male competition (e.g., Holder and Montgomerie 1993, Rintamäki et al. 2000), frostbite or predation. Being able to retract such traits may help prevent injury (Holder and Montgomerie 1993). Females may assess the degree of damage to these traits as well as (or instead of) trait size and color (e.g., Brodsky 1988, Holder and Montgomerie 1993), so damaged fleshy traits may reduce a male's success at obtaining mates.

Thus, once erectile ability had evolved, there are multiple advantages for the maintenance of this ability in species that retained fleshy traits. If fleshy traits could be retracted, and therefore largely hidden, why might such traits have been lost in some species within the erectile clade? Shifts in sexual selection to alternative traits (e.g., plumage rather than fleshy traits) or a reduction in the strength of selection (e.g., if the mating system shifted form polygyny to monogamy) could remove selection to retain the fleshy trait. Fleshy traits, even if they can be retracted, may be costly to develop or maintain, and thus may be lost in the absence of positive selection. Thus, even retractable traits may be lost in some situations.

Given that the ability to enlarge fleshy traits rapidly appeared to have evolved a single time (Fig. 1), rather than through multiple independent events, it is interesting that this ability is shared across apparently different fleshy traits. This suggests the existence of some homologous physiological mechanisms underlying erectile ability, even if other aspects of fleshy traits are not homologous (though convergence in erectile ability cannot be excluded at this time). At present, the mechanism behind the erection of fleshy traits is poorly understood though a better understanding of this may shed light on whether or not erectile ability is likely to be homologous. In the wattled pheasant *Lophura bulweri* (not sampled in this study, but united with sampled *Lophura*, Randi et al. 2001), the erection of the large, blue wattles is due to an influx of blood (Delacour 1977). Injection of gelatin into the blood vessels of wild turkey snood causes the snood to ''erect'' (Lucas and Stettenheim 1972), suggesting blood may be involved in snood erection as well. Movement of blood has also been suggested in two other taxa: rock ptarmigan *Lagopus muta* (not sampled in this study, but united with grouse, Dimcheff et al. 2002, MacDonald 1970), and in tragopans, where the head-bobbing display during lappet erection has been suggested to pump blood into the lappet (Islam and Crawford 1998). Although the traits that undergo erection are varied in shape and location (Table 1), the first step to testing the hypothesis of a shared physiology is to determine whether blood flow causes erection in all members of the erectile clade.

The results of this study are consistent with the idea that superficially different traits might exhibit partial homology. The *pax*-6 gene represents a well-studied example of partial homology where a homologous gene plays a role in the development of the non-homologous eyes of vertebrates and invertebrates (e.g., Gehring and Ikeo 1999). The most important outcome of this comparative study is the information uniting galliforms with erectile traits as an excellent group for further studies. If further experiments support the hypothesis that blood flow is involved in erection in all species, it would clearly be of interest to determine whether there are other common physiological mechanisms underlying erectile ability and whether common regulatory gene(s) control the development of fleshy traits in the erectile clade. Such data could provide insights into whether there is some level of homology among fleshy traits in the erectile clade. This study illustrates the ability of comparative studies to focus attention on specific groups in which identification of homology is complex (Mindell and Meyer 2001), but might be rewarding to study at a proximate level.

Acknowledgements – This manuscript was greatly improved by helpful suggestions from Colette St. Mary, Ben Burkley, and anonymous reviewers. Amanda Hudson and Andrew Cox assisted with data collection. This research was supported by the National

Science Foundation (DEB-0228682) to RTK, ELB, and D. W. Steadman.

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