

S20-2 Using molecular phylogenetics to interpret evolutionary changes in morphology and behavior in the Phasianidae

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Abstract We used molecular phylogenies derived from mitochondrial and nuclear DNA sequence data to interpret changes in morphological and behavioral characters among several genera of phasianids. This approach has been difficult to develop because relationships among the Phasianidae are poorly resolved. Bayesian analyses of cytochrome *b* with more complex, codon-based models improved resolution relative to previous maximum parsimony analyses. We mapped several characters (presence of fleshy traits, ability to rapidly enlarge fleshy traits, and frontal displays) on to our molecular phylogeny to explore trait evolution. We also reconstructed traits incorporating uncertainty (unresolved nodes) in the phylogeny by reconstructing gains and losses of traits using a set of trees obtained through Markov chain Monte Carlo sampling. Assuming equal weights for gains and losses, we conclude that fleshy traits have been gained and lost multiple times in the course of trait evolution, with wide 95% confidence intervals. In contrast, the ability to rapidly enlarge those fleshy traits is restricted to a single, well-supported clade in which it appears to have been gained only once. Like the presence of fleshy traits, the presence of a frontally-oriented display appears to have evolved many times, perhaps in correlation with the evolution of specific ornamental traits.

Key words Galliformes, Cytochrome *b*, Homology, Ornaments, Sexual selection

1 Introduction

In the past decade, molecular sequencing technologies have led to a renaissance in the field of systematics. One of its most exciting aspects is its potential to construct molecular phylogenies for examining other evolutionary questions. This approach can establish whether specific morphological and behavioral traits of living species have been gained or lost during evolution, provide information on trends and directions in evolutionary changes, and test hypotheses regarding speciation. Examining these questions effectively, nevertheless, requires that the molecular phylogeny is well supported.

Many members of the Phasianidae (pheasants, partridges, grouse and turkeys) exhibit extreme ornamental traits and elaborate displays. For example, many species have plumage that is brightly colored, exhibits striking patterns or is highly modified. Many phasianids also have bare patches of colored flesh in a variety of shapes and locations on head and neck. These fleshy traits vary in color, though red is the most common. In some species, their color and shape may remain relatively constant over time while varying in others. The patches are often involved in displays and appear to be important in female mate choice in

some species (Ligon, 1999).

We have now examined the evolution of specific ornamental traits in two phasianid genera using well-supported molecular phylogenies. The patterns of speciation and trait evolution in *Tragopan*, a genus of five Indo-Himalayan species, were examined using sequences of the mitochondrial cytochrome *b* gene (CYB) and its control region (CR) (Randi et al., 2000). Evolutionary relationships within *Polyplectron*, a genus of six southeast Asian and Sunda species, were also examined using CYB, CR and intron G of the nuclear ovomucoid gene (OVOG; Kimball et al., 2001).

Males of the strikingly sexually dimorphic species of *Tragopan* wear fleshy throat lappets and horns that can be enlarged rapidly. The horns project and vibrate during display, while the lappet expands to cover the breast; lappets are brightly colored in blue, red, yellow and/or orange. The mtDNA phylogeny for tragopans is better supported than alternative topologies inferred from morphological and behavioral traits, and is consistent with the geographic distribution of *Tragopan* species (Randi et al., 2000). Evolutionary diversification in tragopans is compatible with allopatric speciation in two different episodes about 4 and 2 million years ago, possibly driven by uplift of the Himalayas. Lappets of similar coloration or patterns occur in different

clades, suggesting that lappet coloration and pattern has evolved independently more than once. Thus, differences in ornamental traits in different species probably reflect differences in selection on an ancestral lappet template. Randi et al. (2000) concluded that as each population became isolated by vicariant events, female preference could have selected for unique color patterns that ultimately resulted in independent evolutionary trajectories for the lappets in each species.

Peacock-pheasants of the genus *Polyplectron* are characterized by ocelli (ornamental eyespots) in the plumage of all but one species (*P. chalcurom*). The ocelli appear in many parts of the plumage (tail, tail coverts, wing, wing coverts and mantle), their size and distribution varying among species. All species perform lateral displays, but those with ocelli are more frontal in orientation towards females. The two least ornamented species have long been considered primitive (e.g., Johnsgard, 1999), and were originally placed in another genus. This evolutionary scenario implies that ocelli in *Polyplectron* are derived. Our phylogenetic analyses indicated the opposite, that the ocellated species are basal and the two least ornamented derived (Kimball et al., 2001). Thus, the absence and reduction of ocelli and related display behaviors reflect relatively recent losses of a trait thought to be under sexual selection. Examination of other morphological and behavioral traits suggested that the less ornamented species have not shifted to a different target in sexual selection. Analyses of CYB also provided weak evidence that the closest relatives of *Polyplectron* are the true peafowl (*Pavo*), the Congo peafowl (*Afropavo*), and the argus pheasants (*Argusianus* and *Rheinartia*), although alternative hypotheses could not be eliminated. Thus, it remains unclear whether the ocelli of *Polyplectron*, *Pavo*, and *Argusianus* have a common evolutionary origin.

The difficulty in establishing relationships among phasianid genera that have ocelli underlines the difficulties of developing analyses applicable at a larger evolutionary scale, despite the clear potential of this approach to provide testable hypotheses for behavioral evolution. Analyses of CYB, CR, and OVOG sequences indicate that phasianids have undergone a relatively rapid radiation, making relationships among phasianid lineages extremely difficult to establish (Kimball et al., 1999; Armstrong et al., 2001). Such lack of resolution has limited the unravelling of evolutionary pathways within the family. Here we focus on three questions concerning phylogenetic relationships among the phasianids. First, can more realistic models of molecular evolution improve resolution of phasianid relationships? Secondly, can new insights into trait evolution be gained with improved phylogenetic resolution? Thirdly, can phylogenetic uncertainty be incorporated in the examining of patterns of evolutionary change?

2 Materials and methods

We used CYB sequences from a variety of sources (e.

g., Kimball et al., 1999; Randi et al., 2000, 2001). Maximum parsimony (MP) bootstrap analyses were conducted using PAUP* 4.0b10 (Swofford et al., 2002), and Bayesian analyses were conducted in MrBayes 2.01 (Huelsenbeck and Ronquist, 2001), both following the methods of Braun and Kimball (2002). Model selection used Akaike weights calculated as described in Burnham and Anderson (1998), with likelihood values corresponding to maximum values from the Bayesian analyses. Information about traits and behaviors was taken from Johnsgard (1999) and references therein. Numbers of gains and losses for specific morphological and behavioral characters were reconstructed using MacClade 4.0 (Maddison and Maddison, 2000) from 5 000 trees sampled by MrBayes. The average number of changes under multiple equally parsimonious reconstructions is reported when gains and losses are weighted equally and the 95% confidence interval (95% CI) is given.

3 Results and discussion

3.1 Resolution of phasianid radiation

Additional MP analyses of CYB using a larger number of taxa still failed to produce many well-supported clades at the base of the phasianids (Fig. 1). No better results were obtained from larger CR and OVOG alignments (unpubl. obs.). However, analyses of CYB in which parameters were added to describe differences in rates at different codon positions resulted in substantial improvements in model fit. Some of the well-supported clades that appear in these new analyses are also supported by analyses of other mitochondrial and nuclear markers (e.g., Dimcheff et al., 2002; unpubl.

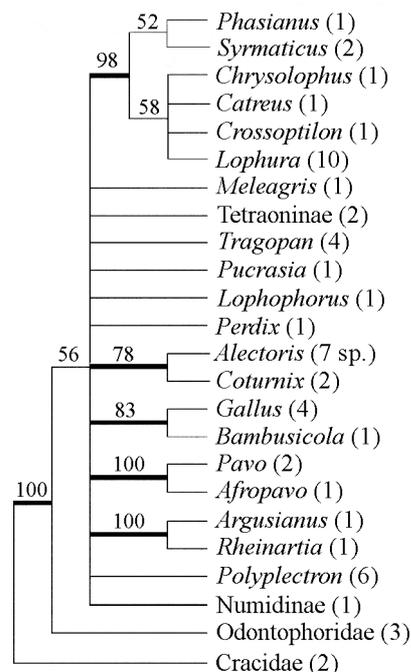


Fig. 1 MP bootstrap analysis

Numbers in parentheses indicate the number of species included in that branch. Branches were collapsed when bootstrap support was <50%, and are presented as thick lines when bootstrap support was >70%.

obs.). Although the model used in these analyses is relatively parameter-rich, we feel there is still substantial room for improvement and additional resolution may be obtained using novel models.

3.2 Reconstruction of ancestral states in phasianids

Examining the evolution of many traits is difficult, because traits may be diverse and data on character states hard to obtain. Therefore, we only examined several traits of particular interest. Fleishy wattles are not only prevalent in many lineages, but have also been shown to be the targets of sexual selection in some species (Ligon, 1999). Mapping their presence and absence on to our phylogeny reveals that they have probably been gained and lost multiple times (gains: mean = 5.5, 95% CI = 1.5–7.7; losses: mean = 3.9, 95% CI = 1.5–8.0). In the species of *Tragopan*, the fleshy parts can be enlarged rapidly during display. It is an ability that appears in only one well-supported clade (Fig. 2), an ability that thus appears to have evolved only once (95% CI = 1.0–1.0).

Although lateral displays are common in most phasianids, those in several species (e.g., *Polyplectron* spp.) are more frontal in orientation. The evolution of frontally oriented display appears labile, with multiple gains (mean = 4.5, 95% CI = 2.0–6.2) and losses (mean = 2.9, 95% CI = 1.0–6.0), suggesting that such displays are not homologous among all species. Evolution of frontal displays may be

driven by changes in ornamentation and sexual selection, as in *Polyplectron*. While additional sequence data may allow us to further refine our estimates of gains and losses, it is clear that insights about trait evolution can be obtained even without complete resolution of all lineages.

3.3 Using phylogeny to examine homology

The types and differences in position of fleshy traits among phasianids might suggest that such traits are not homologous. However, the ability to rapidly enlarge fleshy traits in one clade of phasianids (Fig. 2) suggests the existence of physiological mechanisms likely to be homologous in the fleshy traits in this particular lineage. Thus, it is possible that superficially different fleshy traits develop in response to common regulatory gene(s) that arose once (Mindell and Meyer, 2001), and therefore that the fleshy traits in this lineage have a homologous genetic base.

4 Conclusions

We have demonstrated that combining more complex models with Bayesian methods can improve phylogenetic reconstruction, providing improved resolution in existing data sets. The addition of further sequence data may ultimately produce a well-resolved phasianid phylogeny. Even available CYB data, nevertheless, can track character evolution, particularly for single traits appearing in single lineages. Incorporating phylogenetic uncertainty into an-

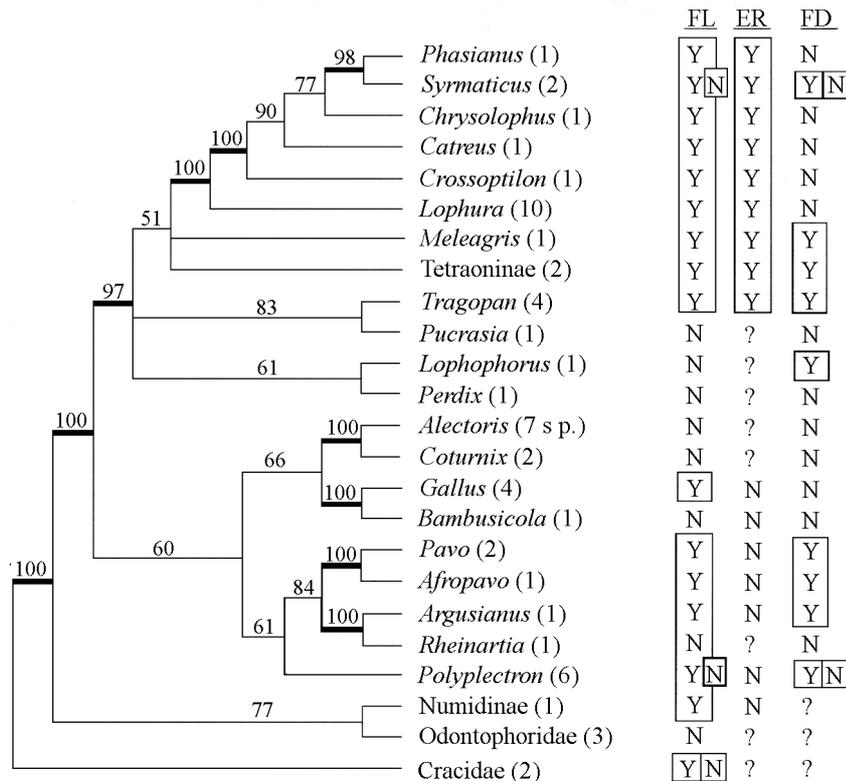


Fig. 2 Bayesian analysis and character state distribution

Numbers in parentheses indicate the number of species included in that branch. Branches were collapsed when the posterior probability of the clade was <50%, and presented as thick lines when posterior probability was >95%. FL = fleshy traits, ER = rapidly enlarging wattles, FD = frontal display, Y = present, N = absent, ? = unknown.

central state reconstruction can also provide useful information when some nodes are poorly supported.

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