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A Molecular Phylogeny of the Pheasants and Partridges Suggests That These Lineages Are Not Monophyletic

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Cytochrome *b* and D-loop nucleotide sequences were used to study patterns of molecular evolution and phylogenetic relationships between the pheasants and the partridges, which are thought to form two closely related monophyletic galliform lineages. Our analyses used 34 complete cytochrome *b* and 22 partial D-loop sequences from the hypervariable domain I of the D-loop, representing 20 pheasant species (15 genera) and 12 partridge species (5 genera). We performed parsimony, maximum likelihood, and distance analyses to resolve these phylogenetic relationships. In this data set, transversion analyses gave results similar to those of global analyses. All of our molecular phylogenetic analyses indicated that the pheasants and partridges arose through a rapid radiation, making it difficult to establish higher level relationships. However, we were able to establish six major lineages containing pheasant and partridge taxa, including one lineage containing both pheasants and partridges (*Gallus, Bambusicola* and *Francolinus*). This result, supported by maximum likelihood tests, indicated that the pheasants and partridges do not form independent monophyletic lineages. © 1999 Academic Press

INTRODUCTION

The pheasants and Old World partridges are thought to represent two closely related taxa within the order Galliformes (tribes Phasianini and Perdicini, respectively; Johnsgard, 1986, 1988). The pheasants are relatively large birds with most species exhibiting extreme sexual dichromatism. Typically, male pheasants are brightly colored and have well developed ornamental traits such as elongated tails, crests, and specialized fleshy structures. Even monochromatic species of pheasants exhibit some degree of ornamentation. Pheasants are confined to Asia, except for the Congo Peafowl (*Afropavo congensis*), which has a restricted distribution in Africa. In contrast, the Old World partridges are smaller and widely distributed in Asia, Africa, and Europe. Most partridge species are monochromatic and primarily dull colored. None exhibits the extreme or highly specialized ornamentation characteristic of the pheasants.

Although the order Galliformes is well defined, taxonomic relationships are less clear within the group (Verheyen, 1956), due to the low variability in anatomical and osteological traits (Blanchard, 1857, cited in Verheyen, 1956; Lowe, 1938; Delacour, 1977). In addition to the study of anatomical traits (e.g., Verheyen, 1956), other traits such as tail molt patterns (Beebe, 1914) or combinations of morphological and behavioral traits (e.g., Delacour, 1977) also have been employed in attempts to ascertain relationships within the order. Johnsgard (1986, 1988) and Sibley and Ahlquist (1990) provide detailed reviews of galliform systematics and the relationships among the pheasants and partridges.

Johnsgard (1986, 1988) concludes that the pheasants and partridges probably form two monophyletic lineages in the subfamily Phasianinae (Fig. 1A). Using DNA hybridization, Sibley and Ahlquist (1990) also indicate that both the pheasants and the partridges are monophyletic. Johnsgard (1986) suggests that the pheasants evolved from a generalized partridge-like ancestor and that the early radiation of the partridge and pheasant lineages probably occurred in southeast Asia. Four major pheasant lineages are recognized by Johnsgard (1986): (1) the gallopheasants and their allies; (2) the peafowl and their allies; (3) the tragopans and their allies; and (4) the junglefowl (Fig. 1B). Johnsgard (1988) also constructed a dendrogram of the partridge genera, but considered it highly speculative.

Akishinonomiya *et al.* (1995) sequenced the hypervariable domain I of the D-loop (mitochondrial control region) to examine relationships both among pheasant taxa and between pheasants and partridges. Although Akishinonomiya *et al.* (1995) examined species from only three of Johnsgard's (1986) four proposed pheasant lineages, his results provide some support for these

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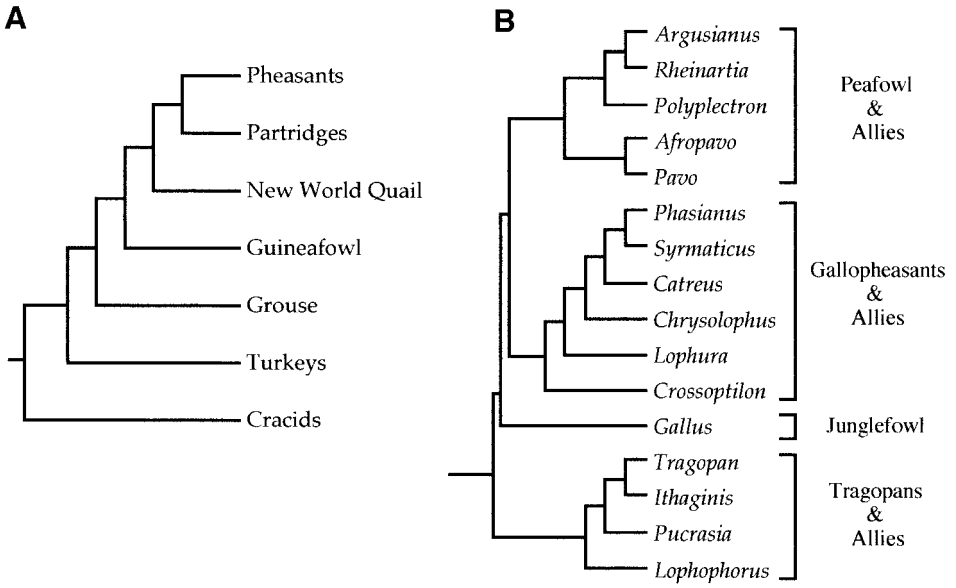

FIG. 1. Johnsgard's (1986) hypothesized relationships among (A) Galliformes and (B) the pheasants.

major lineages. Unfortunately, possibly due to limited taxon sampling, the data presented by Akishinonomiya *et al.* (1995) does not resolve the relationships within the partridges or the relationship between the pheasants and the partridges. Akishinonomiya *et al.* (1995) did note a high degree of uncorrected sequence identity between the bamboo partridge (*Bambusicola*) and members of the junglefowl (*Gallus*) and peafowl (*Pavo*) genera, leading those authors to suggest tentatively that the ancestor of *Bambusicola* may also have been the ancestor of a lineage that evolved into the *Gallus* and *Pavo* clades. However, the reliability of this result was not examined, and no data were provided to indicate whether similar results are found when more sophisticated methods of phylogenetic analysis are employed. Moreover, neither the basal members of the *Pavo* clade (*Argusianus* and *Polyplectron*) nor any other pheasant genus examined show a high degree of similarity to *Bambusicola*.

In this paper, we present phylogenetic analyses based upon complete DNA sequences of the mitochondrial cytochrome *b* gene from all but one monospecific pheasant genus, including representatives of each proposed major lineage, as well as several partridge genera. We used the molecular data to examine hypotheses of the evolution of the pheasants and partridges, focusing on evolutionary relationships: (1) among the pheasants; (2) between the pheasants and the partridges; and (3) with other galliforms. We also reexamined hypervariable domain I D-loop sequences from galliforms (Akishinonomiya *et al.*, 1995; Kimball *et al.*, 1997; Lopez *et al.*, unpublished GenBank submissions) to assess the congruence of estimates of the phylogeny obtained using this region of the mitochondrial genome with those obtained using cytochrome *b*.

MATERIALS AND METHODS

Molecular Biology Techniques

We extracted DNA from blood or tissue (breast muscle) and amplified the cytochrome *b* gene by PCR using standard protocols described elsewhere (Kimball *et al.*, 1997). Sequencing reactions were performed as described previously by Kimball *et al.* (1997) or using the Thermo-Sequenase dye terminator kit (Amersham) according to the manufacturer's recommendations. The primers used for both PCR amplification and sequencing are listed in Table 1.

TABLE 1

Amplification and Sequencing Primers for Cytochrome *b*

Name[a]	Sequence (5′ → 3′)	Source
L14731	ATCGCCTCCCACCT(AG)AT(CG)GA	This study
L14851	TACCTGGGTTCCTTCGCCCT	Kornegay *et al.*, 1993
L14990	ATCCAACATCTCAGCATGATGAAA	Modified, Kornegay *et al.*, 1993
L15164	GCAAACGGCGCCTCATTCTT	This study
H15298	CCTCAGAATGATATTTGTCCTCA	Modified, Kornegay *et al.*, 1993
L15311	CTCCCATGAGGCCAAATATC	Modified, Kornegay *et al.*, 1993
H15400	AGGGTTGGGTTGTCGACTGA	This study
L15662	CTAGGCGACCCAGAAAACTT	This study
H15670	GGGTTACTAGTGGGTTTGC	This study
L15737	CCTATTTGCTTACGCCATCCT	This study
H15826	CGGAAGGTTATGGTTCGTTGTTT	This study
H16065	TTCAGTTTTTGGTTTACAAGAC	Modified, Kornegay *et al.*, 1993

[a] Names indicate light (L) or heavy (H) strand and the position of the 3′ end of the oligonucleotide numbered according to the chicken mitochondrion (Desjardins and Morais, 1990).

Primers designed for this study were based upon galliform sequence data.

Southern hybridization was conducted using standard methods (Ausubel *et al.*, 1994). Briefly, selected DNA samples (see Table 2) were digested using *Eco*RI, separated by agarose gel electrophoresis, transferred to Hybond N+ (Amersham) under alkaline conditions, and hybridized in 50% formamide buffer to a segment of cytochrome *b* corresponding to the region amplified from *Gallus gallus* using primers L15662 and H16065 and labeled with ^{32}P.

Sequence Alignment and Taxon Selection

The species we examined are listed in Table 2. Avian cytochrome *b* sequences are uniform in length (1143 bp), so alignment was straightforward. D-loop domain I sequences were aligned using the default parameters in ClustalW (Thompson *et al.*, 1994), followed with optimization by eye. Regions with many gaps were removed from analyses (see Table 3). The D-loop sequence of *Francolinus* had many unresolved bases (Kimball *et al.*, 1997), and removing these sites left fewer sites for analysis. Therefore, most D-loop analyses excluded *Francolinus*. We deleted all unresolved sites for analyses that included *Francolinus*.

Phylogenetic Analyses

Maximum parsimony analyses (unweighted parsimony and transversion parsimony) were performed using PAUP 3.1.1 (Swofford, 1993). Constraint trees

TABLE 2

Species Examined and Source of Sequence Data

Group	Species	Common name	Cyt. *b*[a]	D-loop[b]
Cracids	*Ortalis vetula*	Plain Chachalaca	L08384	—
	Crax pauxi	Helmeted Currassow	AF068190	—
Turkeys	*Meleagris gallopavo*	Turkey	L08381	—
Grouse	*Tympanchus phasianellus*	Sharp-tailed Grouse	AF068191	—
Guineafowl	*Numida meleagris*	Helmeted Guineafowl	L08383	AF013765
New World Quail	*Callipepla gambelii*	Gambel's Quail	L08382	—
	Cyrtonyx montezumae	Montezuma Quail	AF068192	—
Partridges	*Alectoris barbara*	Barbary Partridge	Z48771	Y08556
	Alectoris chukar	Chukar	L08378	D66890
	Alectoris graeca	Rock Partridge	Z48772	Z80942
	Alectoris magna	Przevalski's Partridge	Z48776	—
	Alectoris melanocephala	Arabian Partridge	Z48773	—
	Alectoris philbyi	Philby's Partridge	Z48774	—
	Alectoris rufa	Red-legged Partridge	Z48775	Y08555
	Bambusicola thoracica	Chinese Bamboo Partridge	AF028790	D66889
	Coturnix coturnix	Japanese Quail	L08377	D82924
	Coturnix sinensis	Blue-breasted Quail	—	D66888
	Francolinus francolinus[c]	Black Francolin	AF013762	AF013766
	Perdix perdix	Grey Partridge	AF028791	D66891
Pheasants	*Afropavo congensis*	Congo Peafowl	AF013760	AF013764
	Argusianus argus	Great Argus Pheasant	AF013761	D66898
	Catreus wallichi[c]	Cheer Pheasant	AF028792	—
	Chrysolophus pictus[c]	Golden Pheasant	AF028793	D66895
	Crossoptilon crossoptilon[c]	White-eared Pheasant	AF028794	—
	Gallus gallus	Red Junglefowl/Chicken	AF028795	X52392
	Gallus lafayettei	Sri Lanka Junglefowl	—	D66893
	Gallus sonnerati	Grey Junglefowl	—	D66892
	Gallus varius	Green Junglefowl	—	D64163
	Ithaginis cruentus	Blood Pheasant	AF068193	—
	Lophophorus impejanus	Himalayan Monal	AF028796	—
	Lophura nycthemera	Silver Pheasant	L08380	D66897
	Pavo cristatus	Indian Peafowl	L08379	D66900
	Pavo muticus	Green Peafowl	AF013763	D64164
	Phasianus colchicus[c]	Ring-neck Pheasant	AF028798	D66894[d]
	Polyplectron bicalcaratum[c]	Gray Peacock–Pheasant	AF028799	D66899
	Pucrasia macrolopha	Koklass Pheasant	AF028800	—
	Syrmaticus humiae	Mrs. Hume's Pheasant	—	D66896
	Syrmaticus reevesi[c]	Reeve's Pheasant	AF028801	—
	Tragopan temminckii[c]	Temminck's Tragopan	AF028802	—

[a] Cytochrome *b* sequences from this study and Kornegay *et al.*, 1993; Randi, 1996; and Kimball *et al.*, 1997.

[b] D-loop sequences from Desjardins and Morais, 1990; Akishinonomiya *et al.*, 1995; Kimball *et al.*, 1997; and Lopez *et al.*, unpublished GenBank submission.

[c] Examined using Southern blot analysis.

[d] Akishinonomiya *et al.*, 1995 lists D66894 as *Phasianus colchicus*, while the database entry lists D66894 as *Phasianus versicolor.*

were constructed using MacClade 3.05 (Maddison and Maddison, 1992) and then the most parsimonious trees given the constraints were identified using PAUP 3.1.1 (Swofford, 1993). Parsimony analyses used at least 100 random addition sequence replicates and the following settings: TBR branch swapping, collapse yes, mulpars yes, steepest descent no. We used MacClade 3.05 (Maddison and Maddison, 1992) to reconstruct character evolution using parsimony.

The reliability of specific groupings in parsimony trees was assessed using the bootstrap (Felsenstein, 1985). We estimated the bootstrap proportion in parsimony analyses using 1000 replicates, with 10 random addition sequence replicates for each bootstrap replicate. A number of studies have suggested that the bootstrap proportion is a conservative estimator of the probability that a clade is correct, as long as the method used to estimate phylogenetic relationships is consistent (Hillis and Bull, 1993; Rodrigo *et al.*, 1994). In fact, several studies have suggested that for maximum parsimony bootstrap values ≥70%, the probability of a clade being correct is at least 95% (Hillis and Bull, 1993), although some authors have questioned whether accepting monophyly of a group whose bootstrap proportion is relatively low (around 70–80%) in the absence of prior expectation might inflate type I error (Rodrigo *et al.*, 1994). We feel that clades showing less than 50% bootstrap in all analyses are unreliable and we have collapsed these in nucleotide analyses. We consider clades to be well supported when the bootstrap proportion is ≥70% and the clade is present in multiple data sets.

Maximum likelihood estimation was performed using DNAml (Felsenstein, 1993) or PUZZLE (Strimmer and von Haeseler, 1997) using either the F84 (described by Kishino and Hasegawa, 1989) or the HKY85 (Hasegawa *et al.*, 1985) models of DNA sequence evolution. We accommodated site-to-site rate heterogeneity using a four-category discrete approximation of a γ distribution (Yang, 1994) with $\alpha = 0.2$ estimated by maximum likelihood using PUZZLE ($\alpha = 0.2$ corresponds to four equiprobable categories with relative rates of 0.0002, 0.0382, 0.4882, and 3.4733). To determine whether the incorporation of site-to-site rate heterogeneity resulted in a significant improvement in the model, we used the likelihood ratio test and compared the test statistic ($\delta = 2\,[ln\ L_1 - ln\ L_2]$) to the χ^2 distribution with one degree of freedom (corresponding to the addition of the shape parameter of the γ distribution). This test appears to be robust as long as the number of parameters represented by the different models is clear, as it is in this case (Huelsenbeck *et al.*, 1996). For maximum likelihood analyses of cytochrome *b* sequences, we conducted six random addition sequence replicates in DNAml using a transition–transversion ratio of 10, rate categories corresponding to a discrete approximation to a γ distribution with $\alpha = 0.2$, and the global rearrangements option (75,802 trees were examined). Justification of these parameters is presented under Results. Comparison of alternative phylogenetic trees was performed using the test proposed by Kishino and Hasegawa (1989) as implemented in DNAml.

Distance analyses were performed using PHYLIP (Felsenstein, 1993) and MOLPHY (Adachi and Hasegawa, 1996). We used the K2P (Kimura 2-parameter) + γ (Jin and Nei, 1990) and F84 (Kishino and Hasegawa, 1989) models of DNA sequence evolution, since these models accommodate site-to-site rate heterogeneity and unequal nucleotide frequencies, respectively. Distance estimates were calculated using transition–transversion ratios of 4 and 10 for cytochrome *b*, and K2P + γ distance estimates were computed using a coefficient of variation of 2.24 (which is equivalent to $\alpha = 0.2$). Protein distances were calculated from translated cytochrome *b* sequences using both ProtML (Adachi and Hasegawa, 1996) with options -D (distance matrix) and -mf (mtREV24 with empirical amino acid frequencies, as described by Adachi and Hasegawa, 1995) and ProtDist (Felsenstein, 1993) with the PAM model of evolution (Dayhoff *et al.*, 1978). Trees were inferred from distance matrices using neighbor joining (Saitou and Nei, 1987).

RESULTS

Molecular Evolution of Cytochrome b and D-loop Sequences

All cytochrome *b* sequences contained an open reading frame that encoded a protein with significant identity to other cytochrome *b* proteins. The hemeligating histidines and other conserved residues (Howell, 1989) could be identified, suggesting that our sequences were functional cytochrome *b* genes, rather than nuclear pseudogenes (e.g., Kornegay *et al.*, 1993; Arctander, 1995). An analysis of nuclear pseudogenes and their functional counterparts by Sorenson and Quinn (1998) indicated that nuclear pseudogenes often accumulate mutations that would result in amino acid changes in highly conserved regions even in the absence of indels (e.g., Arctander, 1995), making an examination of such regions a suitable method to detect nuclear pseudogenes.

We examined several other lines of evidence to determine whether the sequences we analyzed represented nuclear pseudogenes (see Sorenson and Quinn, 1998). Sequences for two species that we analyzed (*Phasianus colchicus* and *Polyplectron bicalcaratum*) were confirmed using mitochondrially enriched tissues. Southern blot analysis of eight species (see Table 2) demonstrated that only one restriction fragment hybridized with a cytochrome *b* probe. Branch lengths of nuclear pseudogenes tend to be shorter than their functional counterparts (Sorenson and Fleischer, 1996; Sorenson and Quinn, 1998). An examination of cytochrome *b* phylograms (e.g., Fig. 2) suggests that se-

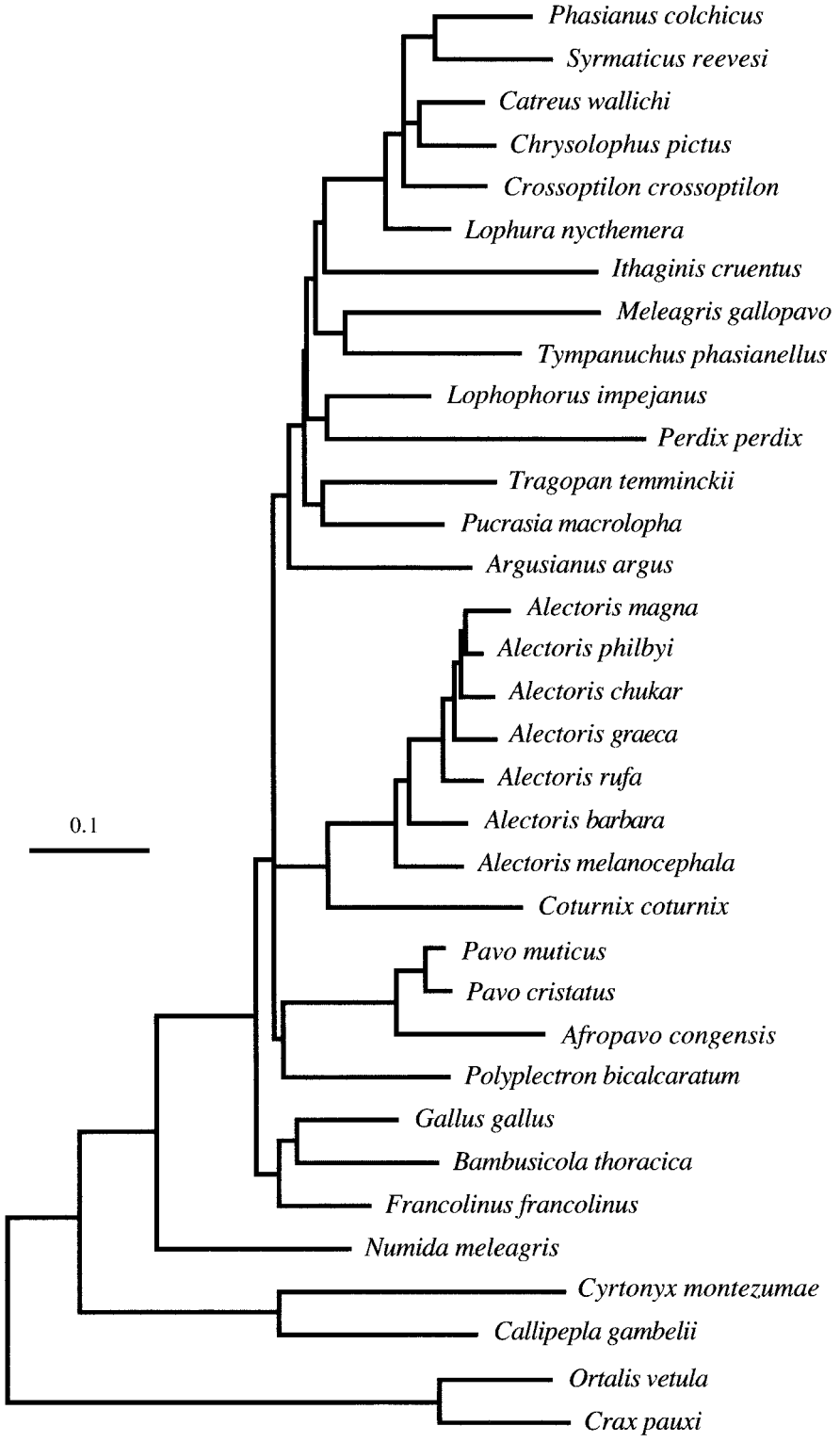

FIG. 2. Most likely tree identified using cytochrome *b* nucleotide data. *In* likelihood = −11698.3.

quences obtained from blood samples were not associated with short branch lengths.

As previously observed (e.g., Kornegay *et al.*, 1993), the base composition of the cytochrome *b* sequences was highly biased, with the strongest bias in third-codon positions. The 1143 bp cytochrome *b* alignment contained 527 variable sites, of which 422 were informative (parsimony) sites. Most of the variable sites were in the third position of codons, with 359 variable and 321 informative third-codon positions. In the translated

data set, 113 amino acids were variable, and 62 amino acid positions were informative.

We identified 16 equally parsimonious trees by unweighted parsimony analysis of the cytochrome *b* nucleotide alignment (tree length = 2427; CI excluding uninformative sites = 0.307). Previous analyses of mitochondrial genes, including cytochrome *b*, have indicated that analysis of transversions may improve phologenetic reconstruction, particularly at deeper branches (e.g., Mindell and Thacker, 1996). Therefore, we performed transversion parsimony and identified a total of 66 equally parsimonious trees (tree length = 675; CI excluding uninformative sites = 0.381). These analyses indicate that there is less homoplasy in the transversion data, suggesting that transversion analyses may be superior for the estimation of deeper branches. However, trees estimated using either method are largely congruent (Fig. 3), suggesting that the differences between the transition and the transversion data partitions are fairly modest. Parsimony analyses were largely congruent with distance analyses (unpublished data); thus we did not present the results of distance analyses.

Previous analyses have suggested that mitochondrial protein coding sequences exhibit substantial site-to-site rate heterogeneity (Kumar, 1996). Our previous analysis of cytochrome *b* sequences from a more limited set of galliforms indicates that these sequences do exhibit substantial site-to-site rate heterogeneity (Kimball *et al.*, 1997). We found that incorporating site-to-

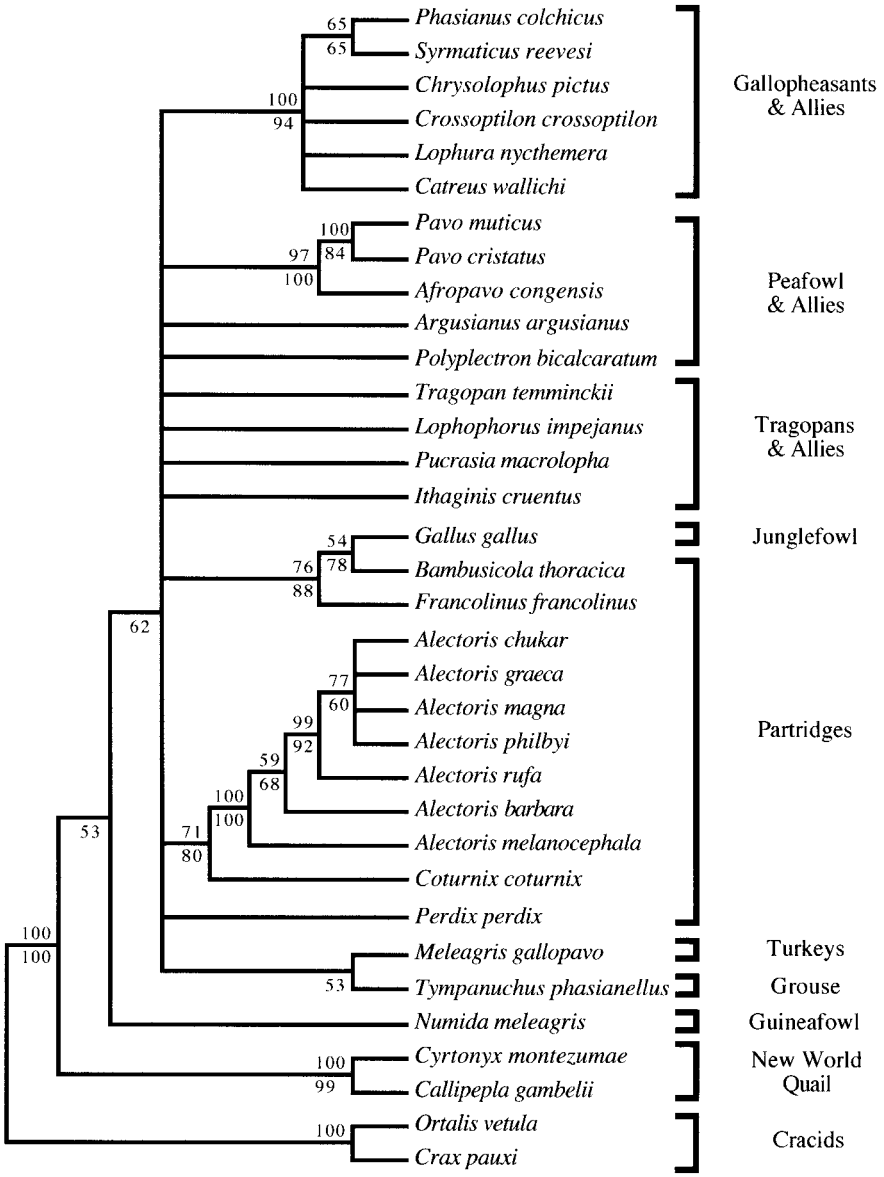

FIG. 3. Bootstrap consensus tree of cytochrome *b* nucleotide sequences. Numbers are percentage bootstrap support for unweighted parsimony (above branch) and transversion parsimony (below). No data are given if bootstrap values are <50%.

site rate heterogeneity resulted in a significant improvement in the estimates of likelihood (ln L = −11698.25 [$\alpha = 0.2$]; ln L = −13625.14 [no rate heterogeneity]; $\delta = 1926.88$, significant at $P < 0.001$). Based upon these results, we used a discrete approximation of a γ distribution with $\alpha = 0.2$ for estimation of phylogenies by maximum likelihood.

Previous analyses have also indicated that mitochondrial sequences show an extremely high transition–transversion ratio (Wakeley, 1996). However, estimation of the transition–transversion ratio is not completely straightforward, and it has been reported that maximum likelihood methods underestimate the transition–transversion ratio of mitochondrial sequences (Purvis and Bromham, 1997). For this reason, the maximum likelihood estimate of the transition–transversion ratio for the galliform cytochrome *b* data analyzed in this study, which corresponds to 3.8, probably represents a minimum value for the actual transition–transversion ratio. In fact, pairwise estimates of the transition–transversion ratio, calculated using a K2P + γ correction for multiple substitutions (Jin and Nei, 1990), range from 1.2 to 18.4, with comparisons between more closely related taxa consistently corresponding to the higher estimates of the transition–transversion ratio. For this reason, we have used a transition–transversion ratio of 10, as have several previous phylogenetic analyses of avian cytochrome *b* sequences (e.g., Nunn and Cracraft, 1996; Nunn *et al.*, 1996; Kimball *et al.*, 1997).

Phylogenetic analyses of inferred cytochrome *b* amino acid sequences were largely congruent with estimates of phylogeny based upon cytochrome *b* nucleotide sequences (Fig. 4). However, as previous studies of galliform cytochrome *b* sequences have indicated (Kornegay *et al.*, 1993; Randi, 1996; Kimball *et al.*, 1997), the bootstrap support for most specific groupings was extremely low. Previous studies have shown that the use of amino acid sequences rather than nucleotide sequences for phylogenetic analyses of mitochondrial protein coding genes may discard more information than noise (Milinkovitch *et al.*, 1996), which may reflect the functional constraints upon the proteins encoded by the mitochondrial genome (see Naylor *et al.*, 1995).

We conducted phylogenetic analyses of the hypervariable domain I of the D-loop using galliform sequence data available from the National Center for Biotechnology Information (Table 2). After alignment of these sequences and elimination of regions where homology of nucleotides was ambiguous (see Table 3), we were left with a 350-bp alignment of D-loop sequences that exhibited a somewhat biased nucleotide composition (see Kimball *et al.*, 1997). This alignment contained 115 variable sites and 79 informative sites. Inclusion of *Francolinus* and deletion of sites that are ambiguous in the *Francolinus* sequence resulted in an alignment of 306 bases of which 96 were variable and 60 were informative.

Estimates of phylogeny obtained using domain I of the D-loop (Fig. 5) are largely congruent with those obtained using cytochrome *b* (compare Figs. 3 and 5), although there are differences in the taxon composition of these two data sets. The congruence between these data sets may seem surprising since the hypervariable domain I of the D-loop is generally thought to be inadequate for the analysis of mid- to deep-level branches in avian phylogeny due to problems with saturation. Furthermore, the fact that substantially fewer sites were available for phylogenetic analyses of the hypervariable domain I of the D-loop (350 bp) than there were for phylogenetic analyses of the complete cytochrome *b* gene (1143 bp) suggests that the D-loop analyses would present additional problems. However, the similarity between the estimates of phylogeny obtained using domain I of the D-loop and cytochrome *b* suggests that the D-loop alignment contained substantial phylogenetic information.

These results are consistent with recent simulation studies that suggest that rapidly evolving sequences may actually have extremely desirable properties for phylogenetic reconstruction, even for divergent taxa (Hillis, 1998; Yang, 1998). It is possible to find empirical support for these simulations, such as the study of Lewis *et al.* (1997), which showed that accurate phylogenies of liverworts that diverged over 400 million years ago could be inferred using only third-codon positions from *rbc*L sequences, despite the high degree of divergence at these sites. Unweighted parsimony analysis of the galliform taxa for which both cytochrome *b* and D-loop sequences were available (see Table 2) indicate that there are similar levels of homoplasy in the alignments of cytochrome *b* and domain I of the D-loop. For these taxa, we identified a single most parsimonious tree using the cytochrome *b* alignment (tree length = 1169; CI excluding uninformative sites = 0.440) and two most parsimonious trees using the domain I D-loop alignment (tree length = 268; CI excluding uninformative sites = 0.480). Based upon these results, we feel that analyses of D-loop sequences show potential for resolution of avian phylogenies at multiple levels.

Relationships within the Pheasants

The four major lineages of pheasants (Fig. 1B) proposed by Johnsgard (1986) are largely supported in at least some of our analyses. However, within the lineages, we inferred different branching orders from those proposed by Johnsgard (1986). In the junglefowl lineage, our analyses suggested the inclusion of additional genera not previously suggested to be pheasants.

Monophyly of the gallopheasant lineage is well supported by cytochrome *b* bootstrap analyses (Fig. 3), and

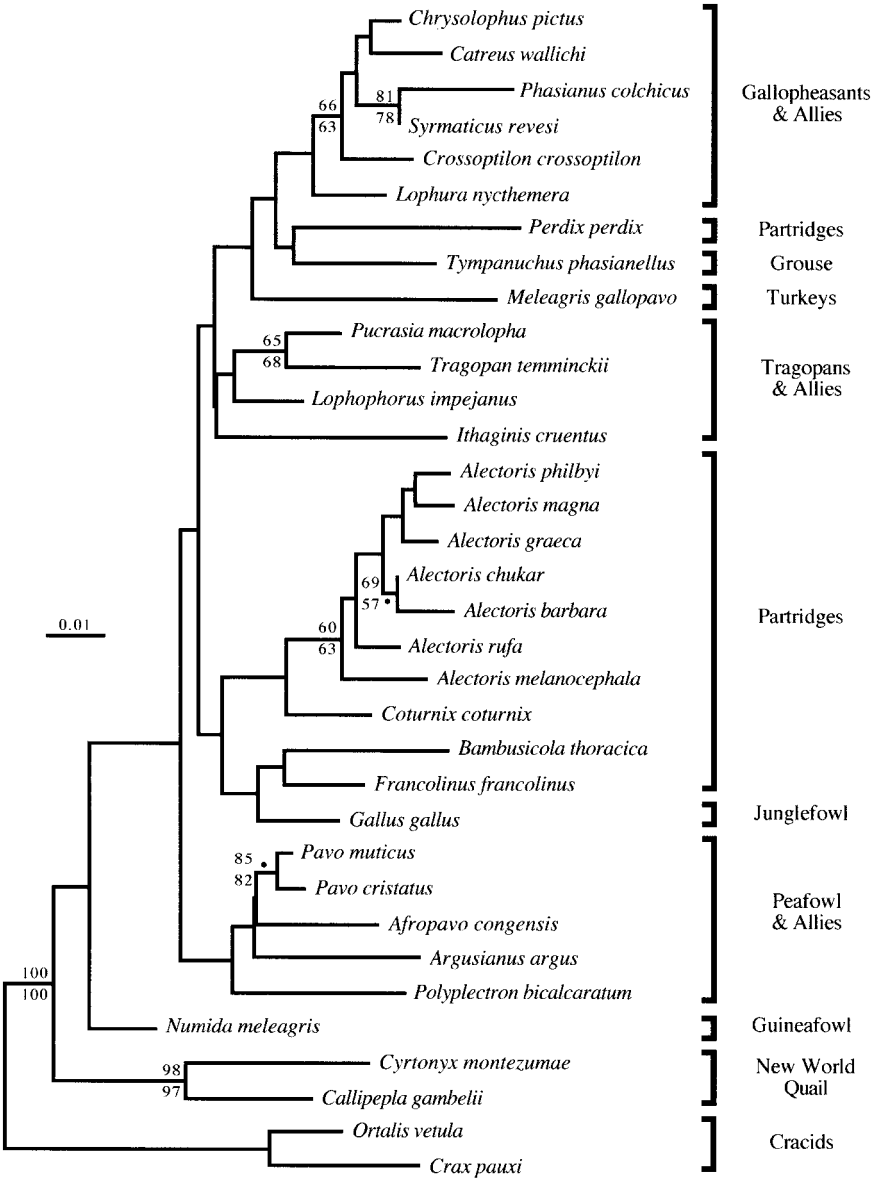

FIG. 4. Analysis of cytochrome *b* amino acid sequences. Numbers are percentage bootstrap support for the mtREV24 model (above branch) and the PAM model (below) of evolution. No data are given if bootstrap values are <50%.

is also present in the most likely tree (Fig. 2). Analysis of inferred cytochrome *b* amino acid sequences strongly supported a clade containing most members of this group, but support for inclusion of the *Lophura* species examined is weak (Fig. 4). While a previous analysis of D-loop nucleotide sequences supported *Lophura* as a member of the gallopheasant clade (Akishinonomiya *et al.*, 1995), our current reanalysis did not support the inclusion of *Lophura* within the gallopheasant clade (Fig. 5).

Our results supported the peafowl clade proposed by Johnsgard (1986), although the results are not completely straightforward. All of our analyses strongly supported an *Afropavo–Pavo* clade (also see Kimball *et al.*, 1997), but the positions of *Argusianus* and *Polyplectron* are problematic. Some analyses of cytochrome *b* placed *Polyplectron* in the peafowl clade, but could not resolve the position of *Argusianus* (e.g., Fig. 2 and distance analyses). Previous analyses of the D-loop provided some support for the inclusion of *Argusianus* and *Polyplectron* within the peafowl clade (Akishinonomiya *et al.*, 1995; Kimball *et al.*, 1997). Analysis of cytochrome *b* protein sequences placed both taxa within the peafowl clade, though bootstrap support was weak (Fig. 4).

The tragopan clade proposed by Johnsgard (1986) is the least well supported by our data. Analysis of cytochrome *b* protein sequences weakly supports the inclusion of *Tragopan*, *Pucrasia*, *Lophophorus*, and *Ithaginis* in a clade (Fig. 4), though branching order

TABLE 3

Alignment of Sequences of the Hypervariable Domain I of the D-loop (Mitochondrial Control Region) from Members of the Galliformes

Gallus gallus	TTTTATTTTT	TAACCTAACT	CCCCTACTAA	GTGTACCCCC	CCTTTCCCC-	--AGGGGGGG
Gallus lafayettei	.....	.....	.....	.....	C	CC.....
Gallus sonnerati	.....	.....	.....	.....	C	CC.....
Gallus varius	.....	.....C....	.....	.....	C	CC.....
Lophura nycthemera	CAACT.....	.....	......T.G.	.....	C	CC.....
Chrysolophus pictus	CAAAC.....	....TC....	......T.G.	A.....	..C.....C	CC.....
Phasianus colchicus	CA.AC.....	....TC....	......T.G.	A..C.....	..C.....C	CC.....
Syrmaticus humiae	CAAAC.....	....TC...C	......T.G.	A.....	C	CC.....
Pavo cristatus	ACA.T.....	C	......T.G.	.....	C	CC.....
Pavo muticus	ACACT.....	C	.....G.	.....	C	CC.....
Afropavo congensis	ACA.T.....	.....	...-..T.G.	.....	.-.....C	CC.....
Argusianus argus	CAACT.....	C	.....G.	.....	C	CC.....
Polyplectron bicalcaratum	.AACT.....	.....	.....	.....	C	CC.....
Bambusicola thoracica	...CT.....	C	......T.G.	.....	C	CC.....A..
Perdix perdix	.AA.C.....	....TG....	.....AT.	.....	C	CC....A...
Coturnix coturnix	ACACT.....	.....	.....T.	.....	C	CC.....
Coturnix sinensis	ACACT.....	.....	.....T.	.....	C	CC.....
Alectoris chukar	ACACT.....	.....A....	.....CT.	.....	C	CC.....
Alectoris graeca	ACACT.....	.....A....	.....CT.	.....	C	CC.....
Alectoris rufa	ACACT.....	.....A....	.....CT.	.....	C	CC.....
Alectoris barbara	ACACT.....	.....	.....G.CT.	.....	C	CC.....
Numida meleagris	AC.C.....	.....	.....G.	.....	C	CC.....

Gallus gallus	TATACTATGC	ATAATCGTGC	ATACATTTAT	ATACCACATA	TA--TTATGG	TACCGGTAAT
Gallus lafayettei	.....	.....	.....	.....	..--.....	.....
Gallus sonnerati	.....T	.....	.....	.....	..--.....	.....
Gallus varius	.....	.....	.....	.....	..--.....	.....
Lophura nycthemera	.....T	.....	.....	.....	..--C.....	.....C.
Chrysolophus pictus	.....	.....	.....	.....	..--C.....	.....C.
Phasianus colchicus	.....T	.....	.....	.....	..TAC.....	.....C.
Syrmaticus humiae	.....	.....	.....	.....	..--C.....	.....C.
Pavo cristatus	.....	.....	.....	.....	..--.....	.CA.....
Pavo muticus	.....	.....	.....	.....	C.--.....	.CA.A.....
Afropavo congensis	.....T	.....	.....	.....	C.--.....	.CA.A.....
Argusianus argus	.....T	.....T..A.	.....	.....	C.--.....	.CA.A.....
Polyplectron bicalcaratum	.....T	.....	.....	.....	..--.....	.....
Bambusicola thoracica	.....T	.....	.....	.....	..--.....	.....
Perdix perdix	...G.....T	.C.G-.....	...TC...G.	G.T..C....	C.--.....	..A.A...C.
Coturnix coturnix	.....	.....	.....	..T.....	..--C.....	.....
Coturnix sinensis	.....T	.....	.....	..T.....	..--.....	.....
Alectoris chukar	..C.....	.....	...T.....	..G..C....	..---....A	..G.....
Alectoris graeca	..C.....	.....	...T.....	..G..C....	..---....A	..G.....
Alectoris rufa	..C.....	.....	...T.....	..G..C....	..---....A	..G.....
Alectoris barbara	.....	.....	...T.....	..G..C....	..---.....	..G.....
Numida meleagris	.....T	.....T....	.....	.....	C.--.....	.....C.

differs from that proposed by Johnsgard (1986; see Fig. 1B). Maximum likelihood analysis suggests that *Tragopan* and *Pucrasia* form a clade, to the exclusion of *Lophophorus* and *Ithaginis* (Fig. 2). Nucleotide analyses cannot resolve the phylogenetic position of any of the members of this hypothesized clade (Fig. 3). The low support for the existence of this clade suggests that if these genera actually do form a clade, their divergence took place relatively early in the evolution of the pheasants.

The biggest difference between our results and the lineages proposed by Johnsgard (1986) are in the

TABLE 3—*Continued*

Gallus gallus	ATATACTATA	TAT-GTACTA	AACCCAT-TA	TATGTATACG	GGCATTAACC	TATATTCCAC
Gallus lafayettei	.....	...-.....	.....-..	.....	.....T.	..C.....C.
Gallus sonnerati	.....	...-.....	.....-..	.....	.....	.....C.
Gallus varius	.....	...-.....	.....-..	.....	.A.....	..C.....C.
Lophura nycthemera	.....T....	..C-.....	.....-..	.....G...	.A...A.CA.	C..-A...C.
Chrysolophus pictus	.....T....	ATC-.....	.....-..	.....	.A.....CA.	C.-CAG....
Phasianus colchicus	.....T....	ATC-.....	.....-..	.....G...	.A.....CA.	CT-TGA..C.
Syrmaticus humiae	.....T....	ATC-.....	.....-..	.....G...	.A.....A.	..ATAC..T.
Pavo cristatus	.C.....	..C-.....	.....-..	.....G...	.A.....CA.	..CT....C.
Pavo muticus	.C.....	..C-.....	.....-..	.....G...	.A.....CA.	...C....C.
Afropavo congensis	.C..T.....	..C-.....	.....-..	.....G...	.A.....CA.	C..-.A..C.
Argusianus argus	.G...-.G..	..C-.....	.....-..	.....G...	.A.....CA.	CTA-GC....
Polyplectron bicalcaratum	..C..T.G..	..C-.....	.....-..	.....G...	.A...A.CA.	CT.-A...C.
Bambusicola thoracica	.....	..C-.....	.....-..	..C.....A	.A.....CT.	.....C....
Perdix perdix	.....T....	..CC.....	GG.....-..	.....A...	.A.....CA.	...CAG..C.
Coturnix coturnix	.....T....	..C-.....	.....-..	.....	.....C-A	...TG...C.
Coturnix sinensis	.G...T....	..C-.....	.....-..	.....	.A.....-A	G..T.G..C.
Alectoris chukar	.....-.G..	..C-.....	.....-..	.....C...	.A...A.CAA	C..TAG..C.
Alectoris graeca	.....-.G..	..C-.....	.....-..	.....C...	.A...A.CAA	C..TAG..C.
Alectoris rufa	.....-.G..	..C-.....	.....-..	.....G...	.A...A.CAA	C..TAG..C.
Alectoris barbara	...C.-.G..	..C-.....	.....-..	.....C...	.A...A.CAA	C..-A...C.
Numida meleagris	.....T..C.	...-.....	.....A..	.....A...	.A...A..TA	CC.CCA..C.

Gallus gallus	ATTTCTCCCA	ATGTCCA-TT	CTATGC---A	TGATCTAGGA	CA-TACTCAT	TTACCCTCCC
Gallus lafayettei	.....	.....-.C	TC...A---.	..G..C...T	..A.C..ATC	.AT..TA...
Gallus sonnerati	C	.....-.C	TC...A---.	..G..C.A.T	..-.C..AT.	CAC.T.A...
Gallus varius	C	....A..-..	.C...A---.	.....C.A.T	..T...GTCG	.CCATAC..T
Lophura nycthemera	...CA.....	....A.T-A.	.ACC.T--A.	..C....AC.	..TA.GA.C.	CACA...A..
Chrysolophus pictus	C	....A..TA.	.C....--A.	..C..CCAA.	.TA.C.AAGC	.CC.T.--A.
Phasianus colchicus	.....	....A..-AC	.....--A.	C-TC.CCAAG	ACA.TAATGC	.CTTA.--..
Syrmaticus humiae	.....	....A..-G.	.....--A.	..C..CTA..	.TTAC.CTCC	AAC.T.--..
Pavo cristatus	....A....C	.C..T..-A.	.A.....---.	..T..CTA..	..TA..--.C	.C.TA.CTA.
Pavo muticus	....A....C	.C..T..-AC	.A.....---.	.AC..CTA..	..TA..--.C	C..TA..TA.
Afropavo congensis	....A....C	.C..A..-A.	TC.....---.	..C..CTA..	..TA..--GC	...TAT.T..
Argusianus argus	....A.....	.CCC.-.-.C	.C.....---.	..C..CTA..	..T..A--G.	CC.....AA.
Polyplectron bicalcaratum	.....	....A..--.	.C.....---.	..CCAC.A.G	ACTA.....C	.ACAA.....
Bambusicola thoracica	C	....A..-A.	.....---.	..C......G	..-..AAGCC	.C.T...A.A
Perdix perdix	..C.....TC	.C..G..-.A	T..AC.---.	.TC....A..	.TA....TGC	.AC.T.--A.
Coturnix coturnix	C	....A..--.	TAG...---.	..C..C.A..	..TA.AC...	ACGTT.--A.
Coturnix sinensis	.....	....A..--.	TA....---.	..C..CTA..	..T..AA.TC	.C-T.T--A.
Alectoris chukar	.....	.CCC.-.-.A	TC...T---.	.ATCTACA.G	TCA...A...	GGCTT.CAA.
Alectoris graeca	.....	.CCC.-.-.A	TC...T---.	.ACCTACA.G	TCA...A...	GGCTT.CAA.
Alectoris rufa	.....	.CCC...-.A	TC...T---C	AACC.ACAAG	ACA..GC...	GATT...AA.
Alectoris barbara	.....	.CCC.-.-.A	T....T---.	CAGCTACA.G	ACA..GC.CC	GACT..CAA.
Numida meleagris	...C.....	.ATGTAC-.A	GA.C.TGTA.	..C..CC...	..TA.A.T..	AA-T..--A.

junglefowl clade. Johnsgard (1986) suggested that the junglefowl clade contains only members of the genus *Gallus* (Fig. 1B). However, cytochrome *b* nucleotide analyses supported a relationship between *Gallus* and partridges of the genera *Bambusicola* and *Francolinus* (Fig. 3). Maximum likelihood and protein sequence analyses also suggested the presence of this clade (Figs. 2 and 4). D-loop analyses strongly supported the presence of a *Gallus–Bambusicola* clade (Fig. 5), whether or not *Francolinus* was included in the alignment. However, the position of *Francolinus* could not be resolved using the reduced D-loop nucleotide data. Removal of *Francolinus* from the cytochrome *b* data set resulted in high support for a *Gallus–Bambusicola* clade, indicating that our results are not dependent upon inclusion of *Francolinus* (unpublished observation).

TABLE 3—*Continued*

Gallus gallus	CATAGACAGT	TCCAA-----	----------	----------	----------	----------
Gallus lafayettei	-...C.T.A.	CTTT.-CCAC	TAACAAGTCA	CCTAACTATG	AATGGTTACA	GGACATACAT
Gallus sonnerati	-...C.TG.C	.TAT.TCCAC	TACCAGGCCA	CCTAACTATG	AATGGTTGCA	GGACATACAC
Gallus varius	-.C.TC..AC	..T-.T----	----------	----------	----------	----------
Lophura nycthemera	-CC.C.----	----------	----------	----------	----------	----------
Chrysolophus pictus	-CA.AC----	----------	----------	----------	----------	----------
Phasianus colchicus	-CAGT.----	----------	----------	----------	----------	----------
Syrmaticus humiae	-CA..T----	----------	----------	----------	----------	----------
Pavo cristatus	-C..TC----	----------	----------	----------	----------	----------
Pavo muticus	-CC.TT----	----------	----------	----------	----------	----------
Afropavo congensis	-CC.TC----	----------	----------	----------	----------	----------
Argusianus argus	-C..CC----	----------	----------	----------	----------	----------
Polyplectron bicalcaratum	-C.GCC----	----------	----------	----------	----------	----------
Bambusicola thoracica	-CCG..----	----------	----------	----------	----------	----------
Perdix perdix	-CA.C-----	----------	----------	----------	----------	----------
Coturnix coturnix	-C...-----	----------	----------	----------	----------	----------
Coturnix sinensis	-CAGC-----	----------	----------	----------	----------	----------
Alectoris chukar	-C..T-----	----------	----------	----------	----------	----------
Alectoris graeca	-C..TT----	----------	----------	----------	----------	----------
Alectoris rufa	-C..T-----	----------	----------	----------	----------	----------
Alectoris barbara	-C..A-----	----------	----------	----------	----------	----------
Numida meleagris	-CATC-----	----------	----------	----------	----------	----------

Gallus gallus	----------	------ACCA	CTATCAAGCC	ACCTAACTAT	GAATGGTTAC	AGGACATAAA
Gallus lafayettei	CTAACCTTAA	TGCTCTT..T	...A.....	....	....	........C.
Gallus sonnerati	TTAACTTTAA	TGCTCTT..T	.CCA..G.T.	......TC..	.....A.C.T	...T....CG
Gallus varius	----------	------....	...A..G.T.	..T.....	....	CC
Lophura nycthemera	----------	----------	----------	----------	----------	----------
Chrysolophus pictus	----------	----------	----------	----------	----------	----------
Phasianus colchicus	----------	----------	----------	----------	----------	----------
Syrmaticus humiae	----------	----------	----------	----------	----------	----------
Pavo cristatus	----------	----------	----------	----------	----------	----------
Pavo muticus	----------	----------	----------	----------	----------	----------
Afropavo congensis	----------	----------	----------	----------	----------	----------
Argusianus argus	----------	----------	----------	----------	----------	----------
Polyplectron bicalcaratum	----------	----------	----------	----------	----------	----------
Bambusicola thoracica	----------	----------	----------	----------	----------	----------
Perdix perdix	----------	----------	----------	----------	----------	----------
Coturnix coturnix	----------	----------	----------	----------	----------	----------
Coturnix sinensis	----------	----------	----------	----------	----------	----------
Alectoris chukar	----------	----------	----------	----------	----------	----------
Alectoris graeca	----------	----------	----------	----------	----------	----------
Alectoris rufa	----------	----------	----------	----------	----------	----------
Alectoris barbara	----------	----------	----------	----------	----------	----------
Numida meleagris	----------	----------	----------	----------	----------	----------

Relationships between the Pheasants and the Partridges

We analyzed relatively few partridge genera. Like Randi (1996), we supported monophyly of the *Alectoris* partridges (Figs. 2–5) and the presence of a *Coturnix–Alectoris* clade (Figs. 2–4). However, the relationship between *Coturnix* and *Alectoris* could not be resolved in analyses of the hypervariable domain I of the D-loop (Fig. 5). We did support monophyly of the genus *Coturnix*, unlike Akishinonomiya *et al.* (1995). Increased taxon sampling or the analysis of the pheasant and partridge taxa together may explain the differences between our results and those of Akishinonomiya *et al.* (1995). None of the analyses could resolve the position of *Perdix*, which appears to be distantly related to all other taxa sampled (Figs. 2–5).

TABLE 3—*Continued*

Gallus gallus	-TCTCACTCT	CATGTTCTCC	CCCCAACAAG	TCACCTAA-C	TATGAATGGT	TACAGGACAT	ACATTTAACT	ACC-ATGTTC	TAACCCATTT
Gallus lafayettei	-...A..CT.	A...C...T.	...TG...G.	.....-.	.....	.....	..CC.CC.A.	C.TT.....	.....
Gallus sonnerati	G.T.A.T..C	A...C..GT.	...TT.....	.....-.	.....	C.....	GA.CC...A.	C.TT.....	.....
Gallus varius	-...A.TA..	AG..C...A.	...T....G.	.....-.	.....	.....	....C.....	...-...A..	.....
Lophura nycthemera	-----TAA.A	GG.--C.GGG	-A..C.....	C....AT.-A	C.....	.....	..TA....A.	...ATGT.CT	ATC.A.....
Chrysolophus pictus	-----.A.--	GC.--CTA.A	-A.TTC..G.	..C..AT.TT	C.....	.....	..-A.GT.A.	..TAGG-.AT	.TC.....
Phasianus colchicus	-----.A.--	G..--CTA.A	-A.TTC..G.	.....AT.-.	C.....	.....	..-A.GC.A.	..TAGG-.AT	..C.....
Syrmaticus humiae	-----.ACTA	GC.--C.AGA	-G..CT....	.....AT.A.	.....	.....	..TA.CT.A.	..AATA-...	.TC.T.....
Pavo cristatus	-----C..T.	.TC--....A	GA..C.....	.....-.	.....	.....	.ATC..-CA.	.TTACA.C..	.TC.....
Pavo muticus	-----C.CTG	.TC--C.A.A	-A..C.....	C.....-.	.....	C.....	.ACG..-CA.	.TTACA.C..	.CC.....
Afropavo congensis	-----C.CA.	..---..A.A	-A..CC..G.	.....-.	.....	C.....	.ATA.G--..	.TTACA.C..	.CC.....C.
Argusianus argus	-----..G..	GC.--C.AGA	-A..CT....	.....A..-.	.....	C.....	.ATA..--.A	.TTAC..C.A	C.C.....
Polyplectron bicalcaratum	-----..GG.	T..--CTA.A	-A..CT....	..C....A.	.....	.....	.AC..C-.A.	..TGTA--..	..C.....
Bambusicola thoracica	-----.....	GC.--CAATT	.A.T.....	.....-.	.....	...G.....	.A.C....TA	TA.-...C..	..C.T.....
Perdix perdix	------ACTG	.C.--A.AAA	-G..C.....	G....AT.A.	...T.....	.....	.ATAC.T.A.	.TTCTATCA.	CTC.A.....
Coturnix coturnix	-----TAATA	G.C--.T...	-A.T....G.	A....AT.A.	.....	.G.....	.AGC...CTA	.ATAC.-.AG	CTC.....
Coturnix sinensis	-----C.A.G	AT.--CTAA.	-..A.....	G....AT.GA	C.....	.....	..C.C...TA	CATA....CA	.TC.A.....
Alectoris chukar	-----CACT.	TC.--.....	-...CC....	G.....T.	.....	C.....	..TCC.--AC	.TTT...G.A	CTC.A...C.
Alectoris graeca	-----CACT.	TC.--.....	-...CC....	G.....T.	.....	.....	..TCC.--AC	.TTT...G.A	CTT.A...C.
Alectoris rufa	------TCT.	.C.--C....	-...CC....	.....T.	.....	.....	..T.C.--AC	.TTT...G.G	ATC.A.....
Alectoris barbara	-----CAA..	.C.--.....	-...CC....	.....TA	.....	.....	..CC..--A.	TTTT...G.T	CTC.T...C.
Numida meleagris	-----.TAT.	TG.--C.C.A	-A.TTC..G.	.....ATGAT	C.....	.....	..CC....A.	TATATG-...	.TC.T.....

The available data cannot resolve the branching order of major pheasant and partridge lineages, or determine whether many or all of the typical pheasant lineages evolved from a partridge-like ancestor as proposed by Johnsgard (1986). Instead, our data suggest that the pheasants and partridges we sampled form at least six lineages: peafowl, gallopheasants, tragopans, junglefowl with *Bambusicola* and *Francolinus*, *Alectoris* and *Coturnix*, and a final lineage containing *Perdix*.

Pheasant Monophyly Can Be Excluded Based upon Cytochrome b Sequences

There are several alternative explanations for the surprising relationship between *Gallus* and two partridge genera, *Francolinus* and *Bambusicola*. First,

TABLE 3—*Continued*

Gallus gallus	TGATCGTCCA	CCTCACGAGA	GATCAGCAAC	CCCTGCCTGT	AATGTA-CTT	CATGACCAGT
Gallus lafayettei	.......T..	....	....	....	......-...	....
Gallus sonnerati	.......T..	....	....	....	......-...	....
Gallus varius	.......-..	....	....	.......-..	......-...	-.........
Lophura nycthemera	.......A..	....	....	.......C..	......-...	......T..G
Chrysolophus pictus	.......A..	....	....	....	......-..C	T.....T..G
Phasianus colchicus	.......A..	....	.....C....	........A.	......-..C	......T..C
Syrmaticus humiae	.A.....A..	....	.....C....	.....T....	.......-T.C	.G.......A
Pavo cristatus	.......A..	....	....	.......C..	......-..C	......T..G
Pavo muticus	.......A..	....	....	....	......-...	......T..C
Afropavo congensis	.......A..	....	....	.......C..	......-..C	......T..C
Argusianus argus	.......A..	....	....	.......-..	......-...	.........G
Polyplectron bicalcaratum	......GA..	....	....	.......-..	.......-.GG	T.....T..G
Bambusicola thoracica	.......A..	....	..C.......	....	......-...	......T..G
Perdix perdix	......GA..	....	....	.......C..	......-...	......T..C
Coturnix coturnix	.......A..	....	.....C....	.....T....	....CTAT.C	.G....T..C
Coturnix sinensis	......GA..	....	....	......TC..	.....T-TA.	......T...
Alectoris chukar	.......A..	....	.....C....	....	......-..C	......T..G
Alectoris graeca	.......A..	....	....	....	......-..C	......T..C
Alectoris rufa	.......A..	....	....	....	......-..C	......T..C
Alectoris barbara	.......A..	....	....	....	......-..C	......T..C
Numida meleagris	.......A..	....	....	....	.....C-..A	T.....T..C

Gallus gallus	CTCAGGCCCA	TTCTTTCCCC	CT
Gallus lafayettei	....	....	..
Gallus sonnerati	....	....	..
Gallus varius	.........-.	....	-.
Lophura nycthemera	T.........	....	..
Chrysolophus pictus	T.....G...	....	..
Phasianus colchicus	T.........	....	..
Syrmaticus humiae	....	......T...	..
Pavo cristatus	T.........	....	..
Pavo muticus	T.........	....	..
Afropavo congensis	T.........	....	..
Argusianus argus	T.........	....	T.
Polyplectron bicalcaratum	T.........	....	..
Bambusicola thoracica	....	....	..
Perdix perdix	T.........	....	..
Coturnix coturnix	T.........	....	..
Coturnix sinensis	T.........	....	..
Alectoris chukar	T.........	....	..
Alectoris graeca	T.........	....	..
Alectoris rufa	T.........	....	..
Alectoris barbara	T.........	....	..
Numida meleagris	T.........	....	..

Note. The sequences correspond to positions 3 to 487 of the chicken mitochondrial genome (Desjardins and Morais, 1990). Dots indicate identity to the *Gallus gallus* sequence, dashes indicate gaps, and gray areas indicate regions deleted from phylogenetic analysis.

monophyly of the pheasants and partridges may exist, but the resolution of our data is insufficient to discriminate between monophyly and polyphyly. Alternatively, the lineages are monophyletic, but placement of either *Gallus* or both *Bambusicola* and *Francolinus* is incorrect. To test these hypotheses, we found the most parsimonious trees in which composition of the pheasant and partridge lineages was constrained. Trees identified by maximum parsimony were not significantly less likely than the most likely tree (unpublished observations). However, the most parsimonious trees compatible with monophyly of either the pheasants alone or the pheasants and partridges were significantly less likely than the most likely tree (Table 4).

Including *Gallus* within the partridges or placing both *Bambusicola* and *Francolinus* within the pheasants also produced trees that were significantly less likely than the most likely tree (Table 3). Our rejection of these alternative hypotheses suggests that the lineages are clearly not monophyletic.

Relationships with Other Galliforms

Johnsgard (1986) and others place the turkeys and grouse into two separate lineages, allied with the pheasants and partridges. Sibley and Ahlquist (1990) suggest that turkeys are closely related to grouse, and include the turkey–grouse lineage in the pheasant and partridge family. Support for a turkey–grouse lineage is found in the most likely tree (Fig. 2) and is weakly supported in bootstrap analyses of nucleotide data (Fig. 3). However, the clade was not present in analysis of protein sequence data. While our data cannot resolve whether or not turkeys and grouse form a clade, these species are not placed outside the pheasant–partridge clade, suggesting that turkey and grouse evolved during the radiation of the pheasants and partridges and not prior to them as has been previously suggested (e.g., Johnsgard, 1986).

Our analyses of cytochrome *b* sequences suggest that guineafowl and New World quail diverged prior to the radiation of the pheasants and partridges (Figs. 2–4). This conclusion is congruent with some molecular analyses of galliform evolution (Sibley and Ahlquist, 1990; Kornegay *et al.*, 1993; Kimball *et al.*, 1997), but differs from morphological (e.g., Verheyen, 1956; Johnsgard, 1986) and allozyme (Randi *et al.*, 1991) analyses which place the New World quail within the pheasant–partridge radiation.

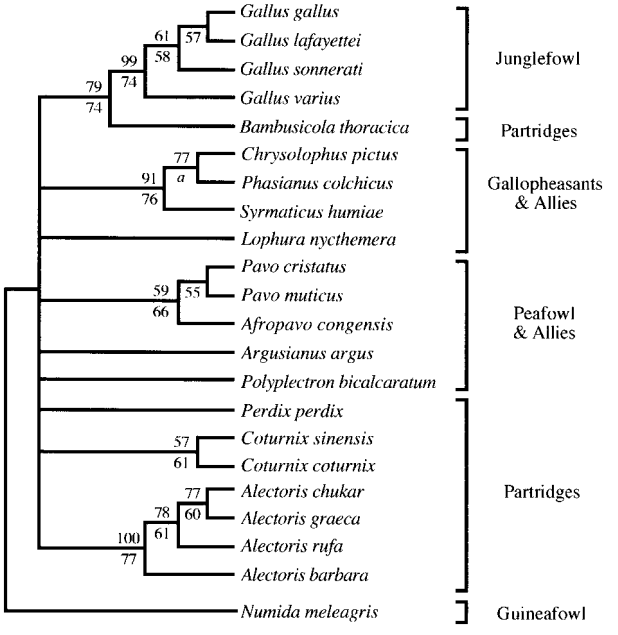

FIG. 5. Bootstrap consensus tree of D-loop nucleotide sequences. Numbers are percentage bootstrap support for unweighted parsimony (above branch) and transversion parsimony (below). No data are given if bootstrap values are <50%. The branch labeled *a* was not supported by transversion parsimony. Instead, transversion parsimony supported a *Phasianus–Syrmaticus* clade at 51%.

TABLE 4

Analysis of Cytochrome *b* Phylogenies in Which the Monophyly of Certain Taxa Was Constrained, Compared with the Most Likely Tree (Fig. 2)

Constrained lineages	δ *ln* likelihood (SD)
Most likely tree	0.0[a]
Pheasants and partridges	−108.5 (27.8)*
Pheasants only	−100.3 (25.9)*
Gallus in Partridges	−69.5 (22.2)*
Bambusicola and *Francolinus* in Pheasants	−56.3 (19.0)*

Note. Except as noted, both pheasant and partridge monophyly constrained to follow Johnsgard (1986).

[a] *ln* likelihood = −11698.3.

* Significantly different from most likely tree.

DISCUSSION

Our results showed that two genera of partridges are present in a clade with *Gallus*, indicating that the pheasant and partridge lineages proposed by Johnsgard (1986) cannot be monophyletic. Using DNA hybridization, Sibley and Ahlquist (1990) reported monophyly of pheasants and partridges. Although they sampled fewer taxa, their study did include *Gallus* and two species of *Francolinus.* However, they did not examine *F. francolinus* and the genus *Francolinus* is probably not monophyletic (e.g., Crowe and Crowe, 1985; Bloomer and Crowe, 1998; Laskowski and Fitch, 1989).

This surprising result cannot be due to contamination. The cytochrome *b* data for *Gallus*, *Bambusicola*, and *Francolinus* were collected in our lab, while the D-loop samples for *Gallus* were from the chicken mitochondrion (Desjardins and Morais, 1990), and *Bambusicola* was from a study by Akishinonomiya *et al.* (1995). Therefore, data from different labs, sequencing different regions of mitochondrial DNA from different individuals, led to the same conclusion.

In addition, other analyses have suggested a relationship between these taxa, though these studies did not assess the reliability of this clade. Analysis of partial ovomucoid sequences resulted in a clade containing *Gallus, Bambusicola,* and two *Francolinus* species (*F. francolinus* and *F. pondicerianus;* Laskowski and Fitch, 1989), though other *Francolinus* species in that study were not placed within the *Gallus* clade. Furthermore, a phenetic analysis of morphological data from 22 species of *Francolinus* and a number of other partridge genera revealed that the nearest neighbor of *F. lathami* and *F. sephaena* is *Bambusicola,* rather than the other species of *Francolinus* examined (Crowe and Crowe, 1985).

The inclusion of *Bambusicola* and *Francolinus* with *Gallus*, as well as the unresolved relationship between the pheasants and partridges, suggests that the terms pheasant and partridge are not phylogenetically useful. Delacour (1977: 25) had noted this as well, stating "In a strictly scientific sense, the term 'pheasant' applies to a group of game birds which do not differ from others by very well-defined or important characteristics . . . many birds of these groups [partridges and quail] differ from one another just as much as they do from some of the so-called 'pheasants', among which, in turn, fairly distantly related genera have usually been placed." It appears that the terms pheasant and partridge should only be used to include suites of related behavioral and morphological characteristics, rather than implying anything about the evolutionary history of galliform birds.

Our results suggest that traits generally associated with pheasants, such as a high degree of dichromatism and exclusive female parental care, evolved multiple times within the galliforms. Members of *Francolinus* and *Bambusicola* are generally monochromatic, with no highly dimorphic or ornamented species. This contrasts with the four species in the genus *Gallus*, all of which exhibit a high degree of ornamentation in males. Behaviorally, *Francolinus* and *Bambusicola* are also like typical partridges, primarily exhibiting monogamy, while in *Gallus*, males often are polygynous and generally do not participate in parental care.

Support for the lability of traits typically associated with pheasants can be found in the gallopheasant clade as well. *Crossoptilon* and *Catreus* are both monochromatic and monogamous, and they exhibit biparental care. Their derived position in the clade suggests that they evolved from a highly dichromatic, "pheasant"-like ancestor and subsequently lost dichromatism. Interestingly, the loss of dichromatism has differed in each lineage. In *Crossoptilon*, the sexes are alike, both exhibiting ornamentation such as ear tufts and elaborated tails; *Catreus* exhibits sexual dimorphism in which the sexes are dull in coloration, but males have elongated tails. These different patterns suggest that *Crossoptilon* and *Catreus* independently evolved monochromatism.

The difficulty of resolving the branching order among the major galliform lineages suggests that these birds underwent a relatively rapid radiation (also see Kornegay *et al.*, 1993; Kimball *et al.*, 1997). Consistent with rapid speciation is the low bootstrap support (Figs. 3 and 5) and short branch lengths separating the major lineages (Figs. 2 and 4). Resolving clades and branching orders during a radiation such as this may be difficult, as there is insufficient time for genetic or morphological changes to accumulate before additional branching occurs. However, the addition of more sequence data for all taxa and the addition of data from more species thought to belong to these lineages may improve evolutionary reconstruction (Hendy and Penny, 1989; Hillis, 1996). It is likely that such measures will at least resolve the position of some taxa that are weakly or inconsistently placed in a clade, such as *Polyplectron* and *Argusianus*.

Some traits may not reflect phylogenetic history when taxa undergo a rapid radiation. Several mechanisms have been proposed to explain why this phenomenon may occur. Introgression of genes may occur prior to the evolution of effective isolating mechanisms, or polymorphisms present in the last common ancestor of these lineages may sort randomly into multiple lineages yielding a pattern inconsistent with the phylogenetic history (reviewed in Maddison, 1996). In addition, traits may "flicker" on and off during a radiation event, since genetic information may not degrade irretrievably for up to 6 million years (Marshall *et al.*, 1994). For example, genes affecting sexual selection or male ornamentation may have turned on and off multiple times in the early evolution of the pheasant and partridge lineages and may therefore not accurately reflect phylogenetic history. We speculate that these effects may have made it difficult to establish relationships among the galliforms (e.g., Delacour, 1977).

Our results indicate that the evolution of the galliforms is complex and suggest that it may be difficult to understand the evolution of the interesting morphological, behavioral, and ecological characteristics of this group. However, better resolution of the deeper branches, by use of additional taxa and sequence data, may allow the reconstruction of at least some of the evolutionary pathways. Use of labile traits to assist in classification of these taxa appears to have led to misleading results, such as placing *Gallus* in a clade with the other "pheasant-like" taxa, and the phylogenetic utility of such traits should be questioned.

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