Resolution of the phylogenetic position of the Congo peafowl, *Afropavo congensis*: a biogeographic and evolutionary enigma

REBECCA T. KIMBALL[1*], EDWARD L. BRAUN[1,2] AND J. DAVID LIGON[1]

[1]*Department of Biology, University of New Mexico, Albuquerque, NM 87131, USA*
(rkimball@unm.edu)
[2]*National Center for Genome Resources, 1800 Old Pecos Trail, Santa Fe, NM 87505, USA*

SUMMARY

Afropavo congensis, the Congo peafowl, has long fascinated ornithologists because of its uncertain phylogenetic position and unusual geographic distribution. While some researchers have placed *Afropavo* as a sister taxon to the true peafowl, *Pavo* species, others have suggested relationships with the guineafowl or an Old World partridge, *Francolinus*. These divergent opinions are due, at least in part, to (i) the unique morphological characteristics, lack of elaborate ornamentation, and monogamous mating system in *Afropavo* which differentiates it from *Pavo*; and (ii) the restricted distribution of *Afropavo* in Zaire, which is far removed from the Asian distribution of all other pheasant species. We obtained complete cytochrome *b* and partial D-loop sequences of *Afropavo* and compared them to *Pavo*, guineafowl, *Francolinus* and other galliform taxa. Our results strongly support a close relationship between *Afropavo* and *Pavo*, and we were able to reject alternative phylogenetic hypotheses. Molecular clock estimates of the divergence time place the separation of *Afropavo* and *Pavo* in the late Miocene. We also discuss other relatives of *Afropavo* and *Pavo* and use this information to propose hypotheses regarding the evolution of ornamentation and sexual dimorphism within this group of pheasants.

1. INTRODUCTION

The Congo peafowl, *Afropavo congensis*, may be the most famous African bird (Crowe & Kemp 1986), because its unusual geographic distribution and uncertain taxonomic affinities have perplexed ornithologists since its discovery by Chapin (1936). Chapin (1936) initially placed *Afropavo* in a monospecific genus allied with the true peafowl (*Pavo* species). However, *Afropavo* shows substantial morphological differences to *Pavo*. For example, *Afropavo* exhibits relatively slight sexual dimorphism. In contrast to *Pavo*, male *Afropavo* lack the spectacular train or any specialized feathers containing ocelli ('eye' spots).

Along with differences in the degree of sexual dimorphism, *Afropavo* also differs from *Pavo* in reproductive behaviours (Taibel 1961). *Afropavo* is monogamous, forms long-term pair-bonds, and lays small clutches of about three eggs. In contrast, *Pavo* is promiscuous or polygynous, establishes no pair-bonds, and females may lay clutches of six or more eggs (Taibel 1961; Johnsgard 1986).

The distribution of *Afropavo* presents a biogeographic puzzle. *Afropavo* occupies lowland rainforest in Zaire, several thousand miles from *Pavo* and other pheasants (Phasianini; Johnsgard 1986), all of which are restricted to south-east Asia. The major galliform radiations in Africa are the guineafowl (subfamily Numidinae) and the Old World partridges (tribe Perdicini), particularly the francolins (genus *Francolinus*).

The unique distribution, reproductive behaviour, and external morphology of *Afropavo* have led to several hypotheses regarding its relationships within the galliforms. A majority of studies have concluded that *Afropavo* is sufficiently distinct to warrant placement in a monospecific subfamily, the Afropavoninae (e.g. Ghigi 1949; Verheyen 1956; Gysels & Rabaey 1962; Hulselmans 1962). However, there is disagreement about the placement of the Afropavoninae within the galliforms. Based upon anatomical traits, Verheyen (1956) viewed the Afropavoninae as most closely related to the African guineafowl. Mainardi (1963), using immunological distances, found that *Pavo* is closely related to the guineafowl, and concluded that *Afropavo* is related to the guineafowl as well. Ghigi (1949) describes *Afropavo* as an aberrant francolin, and suggests that the Afropavoninae is related to *Francolinus* and the guineafowl.

Other studies have supported a relationship between *Afropavo* and *Pavo*. While still separating *Afropavo* into a

*Author for correspondence.

unique subfamily, examination of the soluble eye lens and muscle proteins (Gysels & Rabaey 1962) and the pelvic limb muscles (Hulselmans 1962) supported placement of the Afropavoninae closer to *Pavo* than to the guineafowl. Analysis of karyotype data also supports a close relationship between *Afropavo* and *Pavo* (de Boer & van Bocxstaele 1981). Lowe (1938) performed a detailed morphological study and he too reported several similarities that united *Afropavo* and *Pavo*.

Molecular sequence evidence has been used to resolve a number of longstanding phylogenetic questions, such as the one addressed in this paper. Here we present complete DNA sequences of the mitochondrial cytochrome *b* gene and a portion of the D-loop (mitochondrial control region), which we used to resolve relationships among *Afropavo* and its hypothesized relatives.

2. MATERIALS AND METHODS

(*a*) *DNA extraction, amplification, and sequencing*

We extracted DNA from blood or liver using the Puregene DNA isolation kit (Gentra Systems, Inc.). The mitochondrial cytochrome *b* gene and D-loop were PCR amplified using a set of ten primers (available upon request from the authors). Amplifications used PCR Supermix (GibcoBRL) with 35 cycles (1 min at 94 °C, 1 min at 48–55 °C, 1 min at 72 °C), followed by a 5 min extension. Samples were examined by gel electrophoresis, cleaned (QIAquickPCR clean-up, Qiagen, Inc.), quantitated, and diluted for sequencing.

Sequencing reactions were performed using the Applied Biosystems (ABI) PRISM dye terminator kit (Perkin Elmer) and analysed using the ABI Model 377 DNA sequencer as described (Nelson *et al*. 1997). Despite repeated sequencing attempts, some nucleotides in the D-loop of *Francolinus francolinus* were unresolved. These sites were deleted from phylogenetic analyses.

(*b*) *Sequence alignment and taxon selection*

We sequenced cytochrome *b* from *Afropavo congensis*, *Argusianus argus*, *Francolinus francolinus*, and *Pavo muticus* and a portion of the D-loop from *Afropavo congensis*, *Francolinus francolinus* and *Numida meleagris* (accession numbers AF013760–AF013766). These were aligned with published cytochrome *b* sequences from Kornegay *et al*. (1993), which included *Alectoris chukar*, *Coturnix coturnix*, *Lophura nycthemera*, *Numida meleagris*, *Ortalis vetula*, *Pavo cristatus*, (L08377–L08380, L08383–L08384), and *Gallus gallus* (Desjardins & Morais 1990; X52392); and with published D-loop sequences from Desjardins & Morais (1990) and Akishinonomiya *et al*. (1995), which included *Argusianus argus*, *Chrysolophus pictus*, *Gallus gallus*, *G. lafayettei*, *G. sonneratii*, *G. varius*, *Lophura nycthemera*, *Pavo cristatus*, *Pavo muticus*, *Polyplectron bicalcaratum*, *Syrmaticus humiae* (X52392, D64163–D64164, D66892–D66893, D66895–D66900). Avian cytochrome *b* sequences are uniform in length (1143 bp), so alignment was straightforward. D-loop sequences were aligned using ClustalW (Thompson *et al*. 1994), and regions with many gaps were removed from analyses. Both alignments are available upon request from the authors. We performed preliminary analyses and excluded distantly related taxa present in Kornegay *et al*. (1993) and Akishinonomiya *et al*. (1995) which were not hypothesized relatives of *Afropavo*, following the suggestions of Kim (1996).

(*c*) *Phylogenetic analyses*

Nucleotide distance analyses were performed using DNAdist in PHYLIP (Felsenstein 1993) and MEGA (Kumar *et al*. 1993). The models of DNA substitution we used were maximum likelihood (Kishino & Hasegawa 1989) and Tamura–Nei (Tamura & Nei 1993). Both models allow unequal nucleotide frequencies, which occur in cytochrome *b* (e.g. Kornegay *et al*. 1993) and the D-loop (Marshall & Baker 1997), and a variable transition–transversion ratio. We used a transition–transversion ratio of 10 for cytochrome *b*, a value that has been used in other studies (e.g. Nunn & Cracraft 1996), and is similar to other estimates for mitochondrial genes (Wakeley 1996). We used a transition–transversion ratio of 2 for the D-loop, since this region does not show a strong transition–transversion bias (R.T.K. and E.L.B., unpublished observation). For Tamura–Nei distance estimates, we used a gamma distribution to accommodate rate heterogeneity among sites. We estimated $\alpha = 0.45$ for cytochrome *b* and $\alpha = 0.37$ for the D-loop using the method of moments (Tamura & Nei 1993). Protein distances were calculated from translated cytochrome *b* sequences using ProtDist (Felsenstein 1993) and the PAM model of evolution (Dayhoff *et al*. 1978).

Trees were inferred from distance matrices using the neighbour-joining (Saitou & Nei 1987) and Fitch–Margoliash (Fitch & Margoliash 1967) methods. Fitch–Margoliash trees were generated using global rearrangements and ten random addition sequence replicates in the Fitch program (Felsenstein 1993).

Maximum parsimony analyses were performed using PAUP 3.1.1 (Swofford 1993). Bootstrap replicates involved a heuristic search with ten random addition sequence replicates. Both distance and parsimony analyses used 1000 bootstrap replicates.

Maximum likelihood estimation was performed using DNAml (Felsenstein 1993). We used empirical base frequencies and a transition–transversion ratio of 10 for cytochrome *b*, and 2 for the D-loop. To accommodate rate heterogeneity, we used four substitution categories corresponding to a discrete approximation of a gamma distribution with $\alpha = 0.5$ (four equally probable categories with rates of 0.0291, 0.2807, 0.9248, and 2.7654; see Yang 1994). This model estimated more likely trees than a model assuming homogeneous substitution rates (*ln* likelihood cytochrome *b*: -5413.4 versus -5789.1; D-loop: -2031.2 versus -2198.0). Maximum likelihood analyses were performed using global rearrangements and ten random addition sequence replicates. For the statistical comparison of alternative phylogenetic trees, we used the test proposed by Kishino & Hasegawa (1989), varying the position of *Afropavo* (and *Pavo* when necessary) to reflect proposed hypotheses.

(*d*) *Molecular clock analyses*

Branch lengths are variable in many phylogenetic trees, confounding estimates of divergence times (Berbee & Taylor 1993). Therefore, we estimated divergence times using branch lengths calculated by DNAmlk (Felsenstein 1993), a maximum likelihood program that assumes a molecular clock, using the most likely tree generated by DNAml and the parameters discussed above.

We estimated that the cracid–phasianid split, represented by the root of the cytochrome *b* tree (figure 1*a*), occurred *ca*. 50 million years ago (MA) using the earliest known fossil cracid, *Gallinuloides wyomingensis* (Olson 1985) from the Lower Eocene. The D-loop molecular clock was calibrated using the earliest member of the genus *Gallus* from the Upper

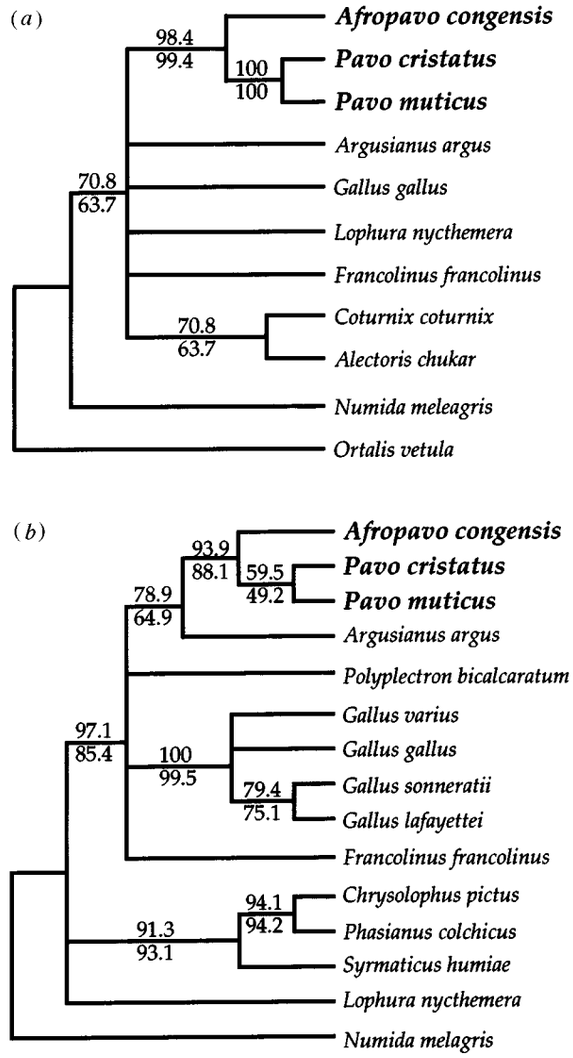

Figure 1. Phylogenetic trees for the relationship between *Afropavo* and other galliforms. Numbers represent per cent of 1000 bootstrap replicates from neighbour-joining trees inferred using Tamura–Nei distances (above lines), and from trees inferred using maximum parsimony (below lines). Branches with less than 50% bootstrap support were collapsed. (*a*) Phylogenetic tree inferred using cytochrome *b* nucleotide data and rooted with the cracid, *Ortalis vetula*; (*b*) phylogenetic tree inferred using D-loop nucleotide data and rooted with *Numida meleagris*.

Miocene (8 MA; see Helm-Bychowski & Wilson 1986). Other fossil data was used to check the results of the molecular clock.

3. RESULTS

(*a*) *Cytochrome* b *and D-loop sequence comparisons*

All cytochrome *b* sequences contained an open reading frame that encoded a 380 amino acid protein with significant identity to other cytochrome *b* proteins. The haem-ligating histidines and other conserved residues (Howell 1989) could be identified, suggesting our sequences were functional cytochrome *b* genes, rather than nuclear pseudogenes (e.g. Kornegay *et al*. 1993; Arctander 1995). The base composition of the novel cytochrome *b* sequences was highly biased, as previously observed (e.g. Kornegay *et al*. 1993). The cytochrome *b* alignment contained 397 variable sites, of which 233 are informative (parsimony) sites. Most variable sites were third codon positions; 62 amino acids were variable, with 23 informative amino acid positions.

Alignment of the D-loop required the introduction of a number of gaps to maximize overall identity. None of the D-loop sequences used in this analysis were unusually divergent, suggesting that they represent mitochondrial sequences. The base composition of our 358 bp D-loop alignment is somewhat biased (26.6% A, 29.2% T, 29.2% C, 15% G), as observed in other avian D-loop alignments (Marshall & Baker 1997). Our alignment contains 121 variable sites and 94 informative sites.

(*b*) *The phylogenetic position of* Afropavo

Distance and parsimony analyses of cytochrome *b* and D-loop sequence alignments indicated that *Afropavo* is most closely related to the two *Pavo* species (figure 1). We obtained high bootstrap support for an *Afropavo–Pavo* clade in all analyses performed with both data sets (range of bootstrap support for cytochrome *b*: 98–100%; for D-loop: 88–97%). In fact, all analyses placed *Afropavo* as a sister taxon of *Pavo*.

We did not exclude third codon positions, as did Kornegay *et al*. (1993), because these positions contain significant phylogenetic information. Some of our analyses incorporate site-to-site variation in substitution rates, therefore including rapidly evolving third position sites should not affect phylogenetic inference. To confirm this, we performed an analysis using cytochrome *b* protein sequences and also observed an *Afropavo–Pavo* clade.

The maximum likelihood trees estimated using the cytochrome *b* and D-loop alignments contain all well supported clades, including the *Afropavo–Pavo* clade (figure 2). We compared the most likely tree obtained, which contained an *Afropavo–Pavo* clade, with trees corresponding to four alternative hypotheses suggested for *Afropavo* (figure 3). With both data sets, the most likely tree had a significantly higher likelihood than three of the four alternative hypotheses (figure 3). All alternative topologies were rejected with at least one of the two data sets, strongly supporting the placement of *Afropavo* and *Pavo* together in a clade distinct from the guineafowl and *Francolinus*.

(*c*) *Which taxa are most closely related to* Pavo *and* Afropavo?

The placement of *Pavo* within the galliforms has been under some debate (see Johnsgard 1986). Many authors have placed *Pavo* in a clade with *Argusianus* and *Polyplectron*. The position of *Argusianus* could not be established using cytochrome *b*, but the placement of *Argusianus* in the *Afropavo–Pavo* clade was marginally supported by analysis of the D-loop (figure 1). This clade is also present in trees inferred from cytochrome *b* protein

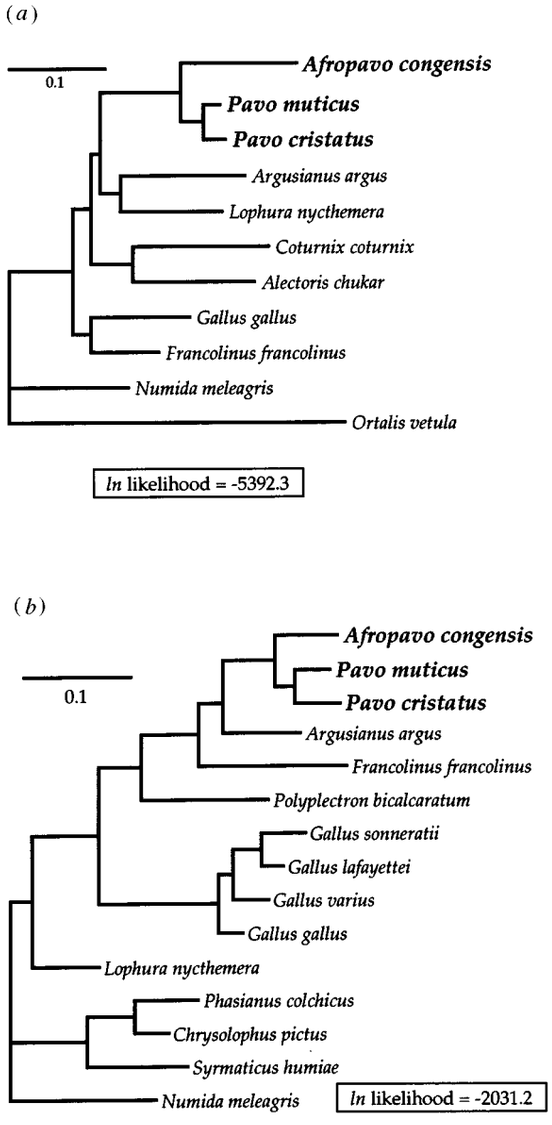

Figure 2. Maximum likelihood trees inferred from (*a*) cytochrome *b*, and (*b*) D-loop nucleotide data.

sequence data (55% bootstrap support). Maximum likelihood analyses of cytochrome *b* could not exclude the presence of a clade containing *Argusianus*, *Afropavo*, and *Pavo* (*ln* likelihood -5397.0, δ *ln* likelihood $= -4.7 \pm 5.9$), but analysis of the D-loop excluded the presence of an *Argusianus–Lophura* clade, present in the most likely cytochrome *b* tree (*ln* likelihood -2062.2, δ *ln* likelihood $= -31.0 \pm 13.8$). These analyses provide support for the inclusion of *Argusianus* in the *Pavo–Afropavo* clade.

Polyplectron has been suggested to be a member of the *Afropavo–Pavo–Argusianus* clade (Johnsgard 1986). Our D-loop analyses could not resolve the position of

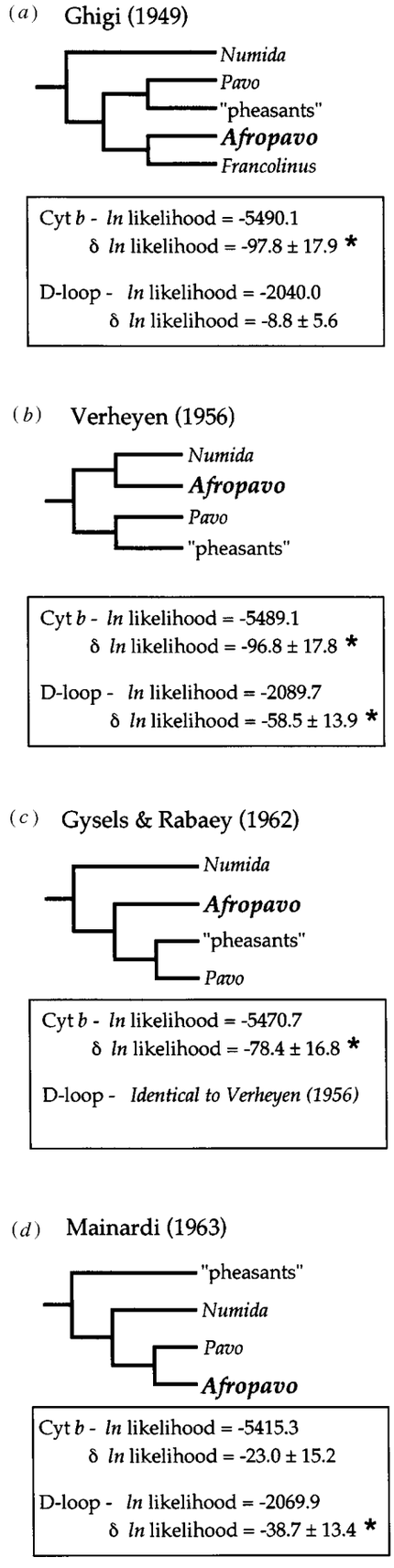

Figure 3. Alternative relationships hypothesized for *Afropavo congensis*. *Ln* likelihood values indicated by the asterisk are significantly worse than the most likely tree (figure 2), according to the Kishino & Hasegawa (1989) test. Topology (*b*) and (*c*) are indistinguishable with the taxon composition of the D-loop data set, since the tests used unrooted trees. All alternative topologies could be rejected by at least one of the two data sets.

Table 1. *Molecular clock estimates of divergence times*
(Values are million years ago.)

	Cytochrome *b*	D-loop
Pavo–Pavo split	4.2	6.2
Pavo–Afropavo split	14.3	9.2
Argusianus–Afropavo split	n.a.[1]	15.3
pheasant–partridge radiation	32.8	n.a.[2]
Numida–pheasant split	37.2	n.a.[2]

[1]Value not calculated since *Argusianus* was not supported as a member of the *PavoAfropavo* clade using cytochrome *b*.
[2]These values were not calculated due to rate differences between *Numida* and the pheasants.

Polyplectron. Akishinonomiya *et al*. (1995) placed *Polyplectron* as a basal member of the *Afropavo–Pavo–Argusianus* clade, though support was low (53.4% bootstrap support).

(*d*) *Rate variation in molecular evolution*

Overall, molecular evolution of galliform mitochondrial DNA sequences is relatively slow, with approximately 0.5% and 0.7% changes per million years in cytochrome *b* and the analysed portion of the D-loop, respectively. It is well established that the rate of molecular evolution is variable (Berbee & Taylor 1993; Hillis *et al*. 1996), and deviations from rate constancy are apparent in our data (figure 2). Rate differences are also evident when only transversions are used to calculate distances, sometimes exceeding those calculated using both transitions and transversions (R. T. K. and E. L. B., unpublished observation). The cytochrome *b* sequence of *Afropavo* has evolved more rapidly than that of the *Pavo* species (figure 2*a*). This increase reflects synonymous and non-synonymous substitutions, since *Afropavo* shows a similar acceleration in protein distance trees (R. T. K. and E. L. B., unpublished observation). Substantial rate differences between *Afropavo* and *Pavo* are not apparent in the D-loop, although rate differences occur between other taxa (figure 2*b*). Both cytochrome *b* and D-loop analyses suggest that *Numida* evolved more slowly than other galliforms, a result consistent with allozyme markers (Randi *et al*. 1991), suggesting that the relatively slow rate of molecular evolution in *Numida* is a general phenomenon.

(*e*) *Divergence time estimates using a molecular clock*

Maximum likelihood estimates of divergence times suggest that *Afropavo* and *Pavo* diverged during the late Miocene, 9–14 MA (table 1). Given the approximately twofold increase in the rate of cytochrome *b* evolution in *Afropavo*, the more recent date is probably more accurate. Estimates using sequence data from both regions of mitochondrial DNA and calibrated using different fossils generally agree with each other (table 1) and with the fossil record of other galliform groups. These fossils include the Pliocene *Pavo* (Mourer-Chauviré 1989), a number of Miocene pheasants and partridges, suggesting a pheasant–partridge radiation in the early Miocene or late Oligocene (Olson 1985; Mourer-Chauviré 1992), and the late Eocene guineafowl *Telecrex* (Olson 1974).

4. DISCUSSION

Our analyses strongly support the placement of *Afropavo* and *Pavo* in a clade that is part of the pheasant and partridge lineage. This supports Chapin's (1936) initial conclusions and those based on morphological (Lowe 1938) and karyotype data (de Boer & van Bocxstaele 1981). This close relationship is surprising, given (i) the differences in morphology and behaviour between *Afropavo* and *Pavo*, and (ii) that the divergence of *Afropavo* and *Pavo* is only two to three times older than the divergence between the two very similar *Pavo* species.

The geographic distribution of *Afropavo* is unique among living pheasants, but is not inexplicable. A land bridge has existed between Asia and Africa since the Miocene (*ca*. 17 MA; Morain 1984), allowing dispersal between these continents. This possibly led to the similarities between Indian and Ethiopian avifaunas (Moreau 1966). Fossil data suggest that peafowl once were far more widely distributed, with Pliocene fossils from France and Moldavia identified as being of the genus *Pavo*, and distinct from *Afropavo* (Mourer-Chauviré 1989). These fossils suggest that the modern distribution of *Pavo* is due to a Pliocene or Pleistocene range restriction, and that the presence of *Afropavo* in Africa may reflect an earlier distribution of the ancestor to *Pavo* and *Afropavo* that included Asia, Africa and Europe.

Our divergence times are supported by multiple lines of fossil evidence. These include the *Pavo* fossils from the Pliocene, 4 MA, (Mourer-Chauviré 1989), the beginning of the pheasant–partridge radiation approximately 32 MA (Mourer-Chauviré 1992), and the divergence between the guineafowl and the pheasants between 35 and 40 MA (Olson 1974). The pre-Pliocene divergence between *Afropavo* and *Pavo* is also consistent with the fossil evidence (see Mourer-Chauviré 1989).

Afropavo lacks the bright iridescent plumage present in males of both *Pavo* species. Most striking in *Afropavo* is the complete absence of the ornamental ocellated train, for which the *Pavo* species are so famous. *Pavo* and *Argusianus* both possess ocellated feathers, suggesting either that their absence in *Afropavo* represents loss of this trait, or that ocelli have independently arisen in *Pavo* and *Argusianus*. The distribution of ocelli and structural specializations of ocellated feathers are unique to each taxon, suggesting that the elaborations arose after the divergence of each lineage, even if ocelli are primitive. *Pavo* species have ocelli only on the specialized, elongated tail coverts of the train. In contrast, the ocelli of *Argusianus* are concentrated on highly elongated secondaries of the wing, while the tail and tail coverts lack ocelli.

In addition to being highly ornamented, *Pavo* and *Argusianus* also exhibit polygynous or promiscuous mating systems with no male parental care, in contrast

to the monogamous mating system with biparental care that occurs in *Afropavo*. Although parental care is evolutionarily labile, to date, a transition from exclusive female parental care to biparental care has not been observed in birds (Székely & Reynolds 1995). This suggests that biparental care and monogamy, as exemplified by *Afropavo*, may be primitive in this group. The mating system in *Pavo* and *Argusianus* is therefore likely to be independently derived, and may have evolved in conjunction with the independent elaboration of male ornamentation in each taxon. These results suggest that many features associated with pheasants, such as elaborate male ornamentation and a promiscuous mating system, evolved independently in different galliform taxa.

We gratefully acknowledge the help of A. Risser and E. Lewins of the San Diego Zoo, who, with the permission of R. Van Bocxstaele of the Royal Zoological Society of Antwerp, supplied us with blood from *Afropavo*. S. Lattimer generously allowed us to collect blood samples from other galliform species. We would like to thank P. O. Lewis for his comments on an earlier version of this manuscript and M. A. Nelson assisting us in the translation of Italian. DNA sequencing was performed in the NSF-supported (RIMI program grant HRD-9550649) Molecular Biology Facility at the University of New Mexico.

REFERENCES

Akishinonomiya, F., Miyake, T., Takada, M., Ohno, S. & Kondo, N. 1995 The genetic link between the Chinese bamboo partridge (*Bambusicola thoracica*) and the chicken and junglefowls of the genus *Gallus*. *Proc. Natn. Acad. Sci. USA* **92**, 11053–11056.

Arctander, P. 1995 Comparison of a mitochondrial gene and a corresponding nuclear pseudogene. *Proc. R. Soc. Lond.* B **262**, 13–19.

Berbee, M. L. & Taylor, J. W. 1993 Dating the evolutionary radiations of the true fungi. *Can. J. Bot.* **71**, 1114–1127.

Chapin, J. P. 1936 A new peacock-like bird from the Belgian Congo. *Rev. Zool. Bot. Afr.* **29**, 1–6.

Crowe, T. M. & Kemp, A. C. 1986 African historical biogeography as reflected by galliform and hornbill evolution. In *Acta XIX Congressus Internationalis Ornithologici* (ed. H. Ouellet), pp. 2510–2518. University of Ottawa Press.

Dayhoff, M. O., Schwartz, R. M. & Orcutt, B. C. 1978 A model of evolutionary change in proteins. In *Atlas of protein sequence and structure* (ed. M. O. Dayhoff), pp. 345–352. Silver Springs, MD: National Biomedical Research Foundation.

de Boer, L. E. M. & van Bocxstaele, R. 1981 Somatic chromosomes of the Congo Peafowl (*Afropavo congensis*) and their bearing on the species' affinities. *Condor* **83**, 204–208.

Desjardins, P. & Morais, R. 1990 Sequence and gene organization of the chicken mitochondrial genome: a novel gene order in higher vertebrates. *J. Mol. Biol.* **212**, 599–634.

Felsenstein, J. 1993 *PHYLIP (phylogeny inference package)*, version 3.5c. Department of Genetics, University of Washington, Seattle, WA.

Fitch, W. M. & Margoliash, E. 1967 Construction of phylogenetic trees. *Science* **155**, 279–284.

Ghigi, A. 1949 Sulla posizione sistematics di *Afropavo congensis* Chapin. *Mem. R. Accad. Sci. Ist. Bologna. Cl. Sci. Fis.* **5**, 35–40.

Gysels, H. & Rabaey, M. 1962 Taxonomic relationships of *Afropavo congensis* Chapin 1936 by means of biochemical techniques. *Bull. Soc. R. Zool. Anvers* **26**, 72–85.

Helm-Bychowski, K. M. & Wilson, A. C. 1986 Rates of nuclear evolution in pheasant-like birds: evidence from restriction maps. *Proc. Natn. Acad. Sci. USA* **83**, 688–692.

Hillis, D. M., Mable, B. K. & Moritz, C. 1996 Applications of molecular systematics: the state of the field and a look to the future. In *Molecular systematics* (ed. D. M. Hillis, C. Moritz & B. K. Mable), pp. 515–543. Sunderland, MA: Sinauer Associates, Inc.

Howell, N. 1989 Evolutionary conservation of protein regions in the protonmotive cytochrome *b* and their possible roles in redox catalysis. *J. Mol. Evol.* **29**, 157–169.

Hulselmans, J. L. J. 1962 The comparative myology of the pelvic limb of *Afropavo congensis* Chapin 1936. *Bull. Soc. R. Zool. Anvers* **26**, 24–69.

Johnsgard, P. A. 1986 *The pheasants of the world*. Oxford University Press.

Kim, J. 1996 General inconsistency conditions for maximum parsimony: effects of branch lengths and increasing numbers of taxa. *Syst. Biol.* **45**, 363–374.

Kishino, H. & Hasegawa, M. 1989 Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *J. Molec. Evol.* **29**, 170–179.

Kornegay, J. R., Kocher, T. D., Williams, L. A. & Wilson, A. C. 1993 Pathways of lysozyme evolution inferred from the sequences of cytochrome *b* in birds. *J. Molec. Evol.* **37**, 367–379.

Kumar, S., Tamura, K. & Nei, M. 1993 *MEGA: molecular evolutionary genetics analysis, version 1.01*. University Park, PA 16802: The Pennsylvania State University.

Lowe, P. R. 1938 Some preliminary notes on the anatomy and systematic position of *Afropavo congensis* Chapin. In *Proc. of the 9th Int. Ornithological Congr.* (ed. J. Delacour), pp. 219–230. Rouen, France.

Mainardi, D. 1963 Immunological distances and phylogenetic relationships in birds. In *Proc. of the 13th Int. Ornithological Congr.* (ed. C. G. Sibley), pp. 103–114. Lawrence, KS: American Ornithologists' Union and Allen Press.

Marshall, H. D. & Baker, A. J. 1997 Structural conservation and variation in the mitochondrial control region of fringilline finches (*Fringilla* spp.) and the greenfinch (*Carduelis chloris*). *Molec. Biol. Evol.* **14**, 173–184.

Morain, S. A. 1984 *Systematic and regional biogeography*. New York: Van Nostrand Reinhold Company.

Moreau, R. E. 1966 *The bird faunas of Africa and its islands*. New York: Academic Press.

Mourer-Chauviré, C. 1989 A peafowl from the pliocene of Perpignan, France. *Palaeontology* **32**, 439–446.

Mourer-Chauviré, C. 1992 The Galliformes (Aves) from the Phosphorites du Quercy (France): systematics and biostratigraphy. In *Science Series, Natural History Museum of Los Angeles County, no. 32* (ed. K. E. J. Campbell), pp. 67–95.

Nelson, M. A., Kang, S., Braun, E. L. (and 23 co-authors) 1997 Expressed sequences from conidial, mycelial and sexual stages of *Neurospora crassa*. *Fungal Genet. Biol.* (In the press.)

Nunn, G. B. & Cracraft, J. 1996 Phylogenetic relationships among the major lineages of the birds-of-paradise (Paradisaeidae) using mitochondrial DNA gene sequences. *Molec. Phylo. Evol.* **5**, 445–459.

Olson, S. L. 1974 *Telecrax* restudied: a small eocene guineafowl. *Wilson Bull.* **86**, 246–250.

Olson, S. L. 1985 The fossil record of birds. In *Avian biology*, vol. 8 (ed. D. S. Farner, J. R. King & K. C. Parkes), pp. 79–252. Orlando, FL: Academic Press.

Randi, E., Fusco, G., Lorenzini, R. & Crowe, T. M. 1991 Phylogenetic relationships and rates of allozyme evolution within the Phasianidae. *Biochem. Syst. Ecol.* **19**, 213–221.

Saitou, N. & Nei, M. 1987 The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molec. Biol. Evol.* **4**, 406–425.

Székely, T. & Reynolds, J. D. 1995 Evolutionary transitions in parental care in shorebirds. *Proc. R. Soc. Lond.* B **262**, 57–64.

Swofford, D. L. 1993 *PAUP: phylogenetic analysis using parsimony.* Formerly distributed by Illinois Natural History Survey, Champaign, IL.

Taibel, A. M. 1961 Analogie fisio-etologiche nel settore riproduttivo tra *Afropavo* Chapin e *Penelope* Merrem. *Natura* **5**, 57–64.

Tamura, K. & Nei, M. 1993 Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molec. Biol. Evol.* **10**, 512–526.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. 1994 CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalities and weight matrix choice. *Nucl. Acids Res.* **22**, 4673–4680.

Verheyen, R. 1956 Contribution de l'anatomie et a la systematique des Galliformes. *Bull. Inst. Roy. Sci. Nat. Belgique* **32**, 1–24.

Wakeley, J. 1996 The excess of transitions among nucleotide substitutions: new methods of estimating transition bias underscore its significance. *Trends Ecol. Evol.* **11**, 158–163.

Yang, Z. 1994 Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. *J. Molec. Evol.* **39**, 306–314.

Received 6 May 1997; accepted 25 June 1997