Recent diversification in African greenbuls (Pycnonotidae: *Andropadus*) supports a montane speciation model

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SUMMARY

It is generally accepted that accentuated global climatic cycles since the Plio-Pleistocene (2.8 Ma ago) have caused the intermittent fragmentation of forest regions into isolated refugia thereby providing a mechanism for speciation of tropical forest biota contained within them. However, it has been assumed that this mechanism had its greatest effect in the species rich lowland regions. Contrary evidence from molecular studies of African and South American forest birds suggests that areas of recent intensive speciation, where mostly new lineages are clustered, occur in discrete tropical montane regions, while lowland regions contain mostly old species. Two predictions arise from this finding. First, a species phylogeny of an avian group, represented in both lowland and montane habitats, should be ordered such that montane forms are represented by the most derived characters. Second, montane speciation events should predominate within the past 2.8 Ma. In order to test this model I have investigated the evolutionary history of the recently radiated African greenbuls (genus *Andropadus*), using a molecular approach. This analysis finds that montane species are a derived monophyletic group when compared to lowland species of the same genus and recent speciation events (within the Plio-Pleistocene) have exclusively occurred in montane regions. These data support the view that montane regions have acted as centres of speciation during recent climatic instability.

1. INTRODUCTION

The most significant geological changes that affected the biota of Africa during the Tertiary were: (i) a general drying of northern Africa and a drastic contraction of forest cover, after the continent 'collided' with Asia in the early Miocene and the Tethys Sea was closed (Axelrod & Raven 1978); and (ii) formation of swells and rifting events in eastern Africa during the Neogene age, causing the isolation of the eastern lowland forests (coastal) from the main Guinea–Congolian rainforest block (Lovett 1993; Coppes 1994). The montane regions in eastern Africa, associated with these geological events, are continuous along the transition between the Congo Basin and the Albertine Rift mountains of eastern Zaire, Rwanda, Burundi and western Uganda, but otherwise are a discontinuous circle of inselbergs following clockwise from the Albertine Rift, the Kenya Highlands, the Eastern Arc crystalline fault-blocks in Tanzania and the Malawi Rift Mountains, and locally along the Ufipa plateau and Lake Tanganyika (figure 1). This circle is partly connected with the mountain scarps of east Southern Africa and the Ethiopian highlands. Within these montane regions live an evolutionary complex and highly endemic montane forest biota.

Over the last three decades, the prevailing explanation for the origin of the extraordinarily high biodiversity of tropical lowland forests was the 'refuge theory' (Haffer 1969, 1974; Diamond & Hamilton 1980; Mayr & O'Hara 1986; Crowe & Crowe 1982; reviews in Prance 1982; Whitmore & Prance 1987). The theory assumes that species evolved by isolation in forest areas which remained stable despite global ecoclimatic changes (Milankovitch cycles) that caused a general global cooling, with large glacial peaks (or arid periods in the tropics (deMenocal 1995)) during the last 2.8 Ma of which the ultimate 0.9 Ma have been the most accentuated (see Bartlein & Prentice 1989; Bennett 1990; Hooghiemstra *et al*. 1993). Thus, since it is assumed that climatic cyclic changes are an important driving mechanism for speciation, this affect may be expected to be most apparent during the Plio-Pleistocene. However the species richness peaks within lowland forests are largely made up by species of pre-Pliocene age (Fjeldså 1994) suggesting that this model cannot be used to account for the high diversity of lowland species. Although the refuge theory, as it applies to lowland forest biota, has come under continued criticism (e.g. Amorim 1991; Croizat 1976; Hackett 1993) it can perhaps be applied to montane habitats more readily (Fjeldså 1994; Vrba 1993).

Fjeldså (1994) and Fjeldså & Lovett (1997) evaluated the evolutionary importance of the accentuated ecoclimatic changes of the Plio-Pleistocene on the diversification of tropical forest birds using primarily

Figure 1. Map of East African ring of mountains. Blackened areas indicate highland regions with montane forest. Light grey areas indicate lowland rainforest. Montane regions referred to in the text are indicated. Distribution of *Andropadus tephrolaemus* cover all montane regions; *Andropadus muasukuensis* is present in Albertine Rift and Eastern Arc Mountains with isolates present in Kakamega forest of Kenya Highlands and Mount Rungwe of Malawi Rift; *Andropadus milanjensis* is present in Malawi Rift and Eastern Arc only.

the DNA divergence data provided by Sibley & Alqhuist (1990). Regions with a predominance of recently radiated (or 'new') species (less than 6 Ma old) are montane including the mountains of the East African circle and from elevated areas with savanna/forest mosaics. Contrary to the prediction from the classic refuge theory (Haffer 1969) the Guinea–Congo lowland forest birds represent relatively deep (old—more than 6 Ma) branches while 80% of lowland new species extend their range outside these forests making any prediction about their origin speculative. Thus, these authors suggest the lowland rainforests are largely areas

where species of potentially diverse origins accumulate according to carrying capacity over long time periods, whereas active centres of diversification are predominantly found in adjacent forest/savanna mosaics and, in particular, montane regions. Tropical montane regions contain heterogenous topography, and an associated vegetation of varying susceptibility to climatic change and are often regions of tectonism and volcanism. Such potential for temporal and spatial variation in habitat may well provide the conditions necessary to promote rapid divergence between non-continuous populations through isolation by local extinctions and shifting balance equilibria (Garcia-Ramos & Kirkpatrick 1997; Roy *et al.* 1997*a*,*b*).

Up until now, no molecular study has addressed the phylogenetics of a restricted African montane avian group composed of many neo-endemics. I present molecular data on one such group. The genus *Andropadus* is represented by 11 obligate forest dwelling species of which four species (*A. tephrolaemus*, *A. masukuensis*, *A. milanjensis* and *A. montanus*) are strictly montane and all except *A. montanus*, which is confined to Mount Cameroon, are represented in large portions of the montane circle of East Africa with wide range overlap between the species (figure 1). Other *Andropadus* species are widely distributed lowland taxa which are mostly sympatric.

Based upon a phylogenetic analysis, this paper examines two predictions stemming from Fjeldså's (1994) hypothesis: (i) that the predominant region of recent speciation is montane and would therefore be represented by the most derived characters, and (ii) that montane speciation has occurred within the time period of the recent and most accentuated climatic changes (i.e. within the past 2.8 Ma (deMenocal 1995)) based upon genetic distance estimates.

2. MATERIALS AND METHODS

Blood and feather samples were taken from mist-netted specimens over a three-year period. Blood and feathers were stored in a DMSO/NaCl solution (Seutin *et al.* 1991). *Phyllastrephus flavostriatus* was chosen as an outgroup for this analysis since the two genera are regarded as close sister groups (Keith *et al.* 1992). At least two samples per species per locality were analysed.

(*a*) *DNA extraction and sequencing*

DNA was prepared and sequenced as in Roy (1997). For this study I chose to compare sequence data from the two mitochondrial DNA (mtDNA) genes, *Cytochrome b* (*Cyt b*) and *NADH2* (*ND2*). Primer pairs L14841 and H15149 (Kocher *et al.* 1989), and L15546 and H15915 (Edwards *et al.* 1991) were used to sequence and compare 575 base pairs (bp) of the *Cyt b* gene. Primers for amplifying the mtDNA *ND2* gene, (H5578 and L5215; Hackett 1996) were also used to obtain and compare 296 bp of sequence for phylogenetic analyses.

(*b*) *Data analysis*

Sequences were analysed as in Roy (1997). Briefly, aligned sequences (Gilbert 1992) were analysed using PAUP (version 3.1.1; Swofford 1991) in order to infer phylogenetic relationships, and CS3 (Siegismund, unpublished program) was used to compare substitution patterns. Analysis of trees was achieved by MacClade (version 3.03, Maddison & Maddison 1993). Parsimony analysis was conducted using the 'branch and bound' option of the PAUP computer program. Initial parsimony analysis weighted all characters equally and analysed sequences of *Cyt b* and *ND2* separately. Further analyses took into account levels of observed saturation (multiple substitutions at a single site) at third codon positions, since these sites have the highest substitution rate in these two genes (Hackett 1996; Seibold & Helbig 1995). In order to detect saturation, scatter plots were constructed that compared time versus pairwise transitions and transversions. I used amino acid substitution as an indirect measure of time of divergence between two taxa on the *x*-axis, since these are likely to have a linear relationship over the timescale dealt with here (i.e. up to 30 Ma (Irwin *et al.* 1991)). Saturation was thought to have occurred when DNA substitutions level off as amino acid differences increase. When saturation was determined to have occurred, third position transitions were then down-weighed by 10 and 20. These figures were used because pairwise comparisons of taxa found that there were, on average, 16.6 and 19.6 third position transitions in those cases where only one third position transversion was counted for *Cyt b* and *ND2*, respectively.

Both gene sequences were combined to provide a total evidence data set. In order to assess possible conflicts between the phylogenetic information of both data sets a homogeneity test was run, based on the Michevich–Farris index (Michevich & Farris 1981). This was done by randomly partitioning characters from the combined data set into new data partitions of the same size and number as was present originally (i.e. 575 and 296 bp) 500 times. The distribution of combined lengths of the most parsimonious trees from each of the randomly generated partitions was then assessed and compared to the length of the original combined data set. If the original tree was greater than 95% of the randomly generated partition trees then the two data sets were regarded as being less congruent than would be expected by chance alone. To assess the degree of character support for each node in the phylogeny, bootstrap analysis (Felsenstein 1985) was undertaken.

All sequences have been submitted to GenBank with accession numbers AF003391-003471.

3. RESULTS

(*a*) *Analysis of sequence data*

Sequence analyses indicate a large variation in sequence divergence among the *Andropadus* genus (up to 18% for *Cyt b* and 21% for *ND2*, found between *A. masukuensis* and *A. importunus*). Such large values are extreme for intrageneric comparisons (e.g. Hackett 1996; Arctander *et al.* 1996). In particular *A. importunus* and *A. curvirostris* were the most divergent, and were overall as genetically distant (based upon base substitutions) to the montane *Andropadus* group as the outgroup was. This led to several inconsistencies in the analysis, however branch topologies were not altered by their exclusion, rather bootstrap support was somewhat increased (data not shown). Intraspecific comparisons were also highly variable, some as high as 13% divergence in *Cyt b* and 18% for *ND2* (e.g. *A. t. fusciceps* and *A. t. tephrolaemus*). Such differences provide clear support for a reconsideration of the present taxonomic ranking and order in the genus *Andropadus* (Roy 1997).

Saturation plots for both genes show a levelling off of transitions at third position, after about five amino acid

substitutions for *Cyt b* and about three amino acid substitutions for ND2 (figure 2), confirming that *ND2* has a faster DNA substitution rate than *Cyt b* (Desjardins & Morais 1990). Analysis of saturated data points found that they were all between lowland and montane and outgroup comparisons (figure 3). Transversions at third position do not appear to be saturated in either gene (figures 2 and 3).

To circumvent *a priori* fears over base composition variance across taxa (Collins *et al.* 1994, Lockhart *et al.* 1994), I compared the frequencies of all four bases in a matrix combining both genes and assessed the distribution with a chi-squared test. No differences in base composition were found across taxa ($p = 1$, χ^2, 78 d.f.) and subsequent phylogenetic inference was considered not to be influenced by this aspect.

(*b*) *Phylogenetics*

Individual phylogenetic analysis of the two genes was carried out using a weighting regime of 10:1 (see §2), since this produced the most resolved tree for both genes. Further weight (i.e. 20:1) did not change either trees' topology. Consensus trees from each gene were similar, leaving only a few nodes within *A. m. roehli* unresolved. Bootstrap analysis of these data sets provided good support for nodes represented in figure 4. A combined data set (total evidence) was found to have an insignificant amount of incongruence when analysed by a partition homogeneity test ($p = 0.82$). This data set produced two most parsimonious trees without weighing third position transition. A consensus tree was identical to that in figure 4, except a terminal node within *A. m. roehli* was unresolved. Further analysis using a 10:1 weighting regime resulted in one most parsimonious tree, and its analysis using 1000 bootstrap replicates resulted in a well resolved tree with all major nodes between subspecies having from 62 to 100% support (figure 4). This analysis however could not resolve the placement of *A. curvirostris* or *A. importunus*. Weighting the transversions 20-fold did not change the topology of the tree further.

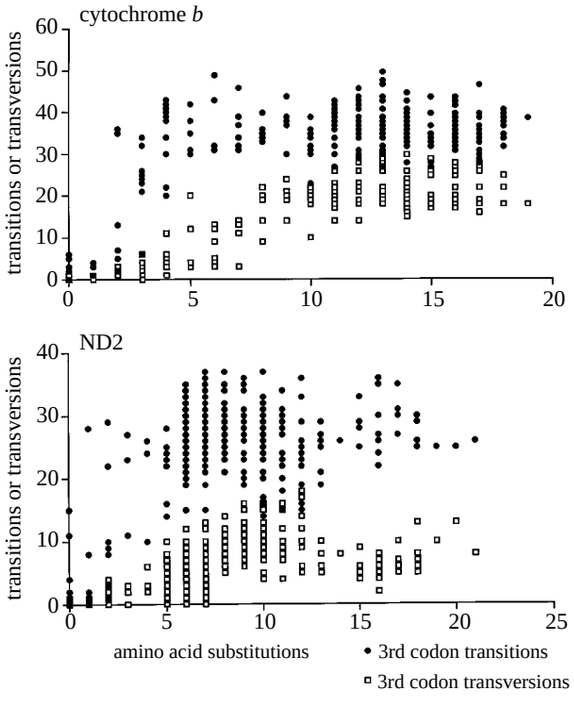

Figure 2. Saturation curve of third position transitions and transversions versus amino acid substitutions per pairwise comparison of taxa, for both *Cyt b* and *ND2*.

4. DISCUSSION

(*a*) *Highland–lowland relationships*

Fjeldså (1994) suggested that tropical forest lowland regions are characterized by old species, whose widespread redistribution has eradicated traces of their origins. However, based upon his conclusions, it is expected that the most recent speciation events within tropical avian groups has predominantly occurred in montane regions. The phylogenetic data presented here show that all the largely sympatric lowland forms (*A. curvirostris*, *A. virens* and *A. latirostris*) are separated by deep branches (figure 4). Although we cannot exclude the possibility of extinctions along these lineages, this result suggests that speciation events of lowland forms of *Andropadus* took place in the distant past. The montane group of *Andropadus* greenbuls, however, is a derived monophyletic clade relative to lowland forms, suggesting that recent differentiation of this genus has occurred exclusively in the mountains (Fjeldså 1994; Roy *et al.* 1997*b*).

(*b*) *Time of divergence*

In order to test whether the divergence times of montane species correlated with the major recent climatic periods of instability i.e. within the last 2.8 Ma (deMenocal 1995), I attempted to convert the genetic distance data to estimates of time. Several problems exist with assuming a molecular clock, such as non-linear and unequal evolutionary rates between lineages (see Li 1993; Hillis & Moritz 1990). Based upon mammalian *Cyt b*, Irwin *et al.* (1991) suggest the rates of 10% for third codon position substitutions and 0.5% for third codon position transversion substitutions, per Ma. I expected the former estimate to remain linear when comparing close taxa or until third position transitions become saturated, after which the latter estimate should be used. Arctander *et al.* (1996) has used a mammalian rate of 1% for third codon position substitutions per 0.05 Ma for considering avian radiations. It has been suggested that in general, mammalian mtDNA evolves faster than that of birds (Kessler & Avise 1985; Mindell *et al.* 1996), therefore these estimates may be low.

Using a molecular clock rate based upon both third base transversions alone, I calculated that the major vicariance event between the *A. masukuensis* group and *A. tephrolaemus* group (figure 4) occurred around 20 Ma ago (figure 3) perhaps with isolated ancestral populations on Mount Cameroon and the Albertine Rift, respectively (Roy 1997). I then went on to calculate the timing of the significant radiations seen within the major montane monophyletic clades, namely East African

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	*A. masukuensis roehli* Uluguru		1	1	0	0	0	2	1	2	2	9	10	10	9	10	10	10	10	10	11	11	11	14	14	15	12	14
2	*A. masukuensis roehli* Udzungwa	4		2	1	1	1	3	2	3	3	9	9	9	8	9	9	9	9	9	10	10	11	14	14	14	11	13
3	*A. masukuensis roehli* S. Pare	3	5		1	1	1	2	1	2	2	9	9	9	8	9	9	9	9	9	10	10	10	13	13	15	11	13
4	*A. masukuensis roehli* Nguru	1	4	2		0	0	2	1	2	2	9	10	10	9	10	10	10	10	10	11	11	11	14	14	15	12	14
5	*A. masukuensis roehli* N. E. Udzungwa	2	2	4	2		0	2	1	2	2	9	10	10	9	10	10	10	10	10	11	11	11	14	14	15	12	14
6	*A. masukuensis roehli* E. Usambara	2	4	1	1	2		2	1	2	2	9	10	10	9	10	10	10	10	10	11	11	11	14	14	15	11	14
7	*A. masukuensis kakamagae* Kakamega	22	23	24	23	23	23		2	3	3	10	9	9	10	11	11	10	10	10	10	10	11	14	14	14	11	13
8	*A. masukuensis masukuensis* Rungwe	13	15	14	13	14	12	19		2	2	9	9	9	10	10	10	10	10	10	10	10	11	15	15	16	13	14
9	*A. tephrolaemus tephrolaemus* Cameroon	18	19	19	18	18	18	21	18		0	9	10	10	9	10	10	10	10	10	10	10	11	14	14	14	11	14
10	*A. tephrolaemus tephrolaemus* Cameroon	18	19	19	18	18	18	21	18	0		9	10	10	9	10	10	10	10	10	10	10	11	14	14	14	11	14
11	*A. montanus* Cameroon	30	28	30	29	29	29	30	30	27	27		8	7	8	10	10	9	9	9	10	10	15	15	15	14	11	13
12	*A. tephrolaemus neumanni* Uluguru	32	31	32	33	33	33	29	27	29	29	27		1	2	7	7	6	6	6	5	5	10	10	9	11	10	11
13	*A. tephrolaemus chlorigula* Udzungwa	27	27	26	27	28	27	28	23	28	28	29	19		2	7	7	6	6	6	5	5	10	11	10	12	9	11
14	*A. tephrolaemus fusciceps* Rungwe	26	27	27	27	28	27	28	24	27	27	26	19	12		7	7	7	7	7	5	5	9	10	10	11	10	9
15	*A. tephrolaemus nigriceps* Mt. Meru	31	29	29	30	31	30	31	27	30	30	33	28	24	28		0	1	1	1	7	7	9	11	12	16	10	12
16	*A. tephrolaemus usumbarae* S. Pare	28	28	28	28	29	29	29	25	28	28	29	26	24	26	11		1	1	1	7	7	9	11	11	15	10	11
17	*A. tephrolaemus kikuyensis* Rwenzori	26	26	26	26	27	25	28	22	26	26	28	26	24	24	19	16		0	0	6	6	10	11	11	15	10	11
18	*A. tephrolaemus kikuyensis* Rwenzori	26	26	26	26	27	25	28	22	26	26	28	26	24	24	19	16	0		0	6	6	10	11	11	15	10	11
19	*A. tephrolaemus kikuyensis* Rwenzori	26	26	26	26	27	25	28	22	26	26	28	26	24	24	19	16	0	0		6	6	10	11	11	15	10	11
20	*A. milanjensis striifacies* Udzungwa	32	31	32	33	32	33	31	30	33	33	31	26	27	26	29	26	26	26	26		0	11	11	11	14	11	11
21	*A. milanjensis striifacies* Uluguru	32	31	32	33	32	33	31	30	33	33	31	26	27	26	29	26	26	26	26	0		11	11	11	14	11	11
22	*A. latirostris* Uganda	32	32	29	31	32	31	33	29	32	32	35	31	30	31	31	27	28	28	28	34	34		6	6	15	10	10
23	*A. virens* Uganda	29	28	29	29	29	29	31	32	33	33	33	28	29	31	30	27	31	31	31	32	32	30		1	14	12	9
24	*A. virens* Cameroon	30	28	30	30	29	30	34	33	34	34	33	30	29	31	32	30	33	33	33	30	30	32	7		14	13	9
25	*A. importunus* Arabuko	40	38	41	40	40	39	35	37	35	35	36	33	35	34	36	34	36	36	36	35	35	39	33	37		12	13
26	*A. curvirostris* Uganda	31	28	30	31	30	30	30	32	30	30	30	27	26	28	29	30	28	28	28	28	28	32	31	30	35		10
27	*P. flavostriatus* Tanzania	32	30	32	32	32	32	30	29	29	29	30	30	29	28	32	27	27	27	27	27	27	30	23	28	33	27	

Figure 3. Percentage sequence divergence between third codon positions in *Cyt b*. Below the diagonal are figures for the total substitutions, above it are figures for transversions only. Thick-lined boxes are montane comparisons, thin-lined boxes are clades that are estimated to have radiated within the last three million years.

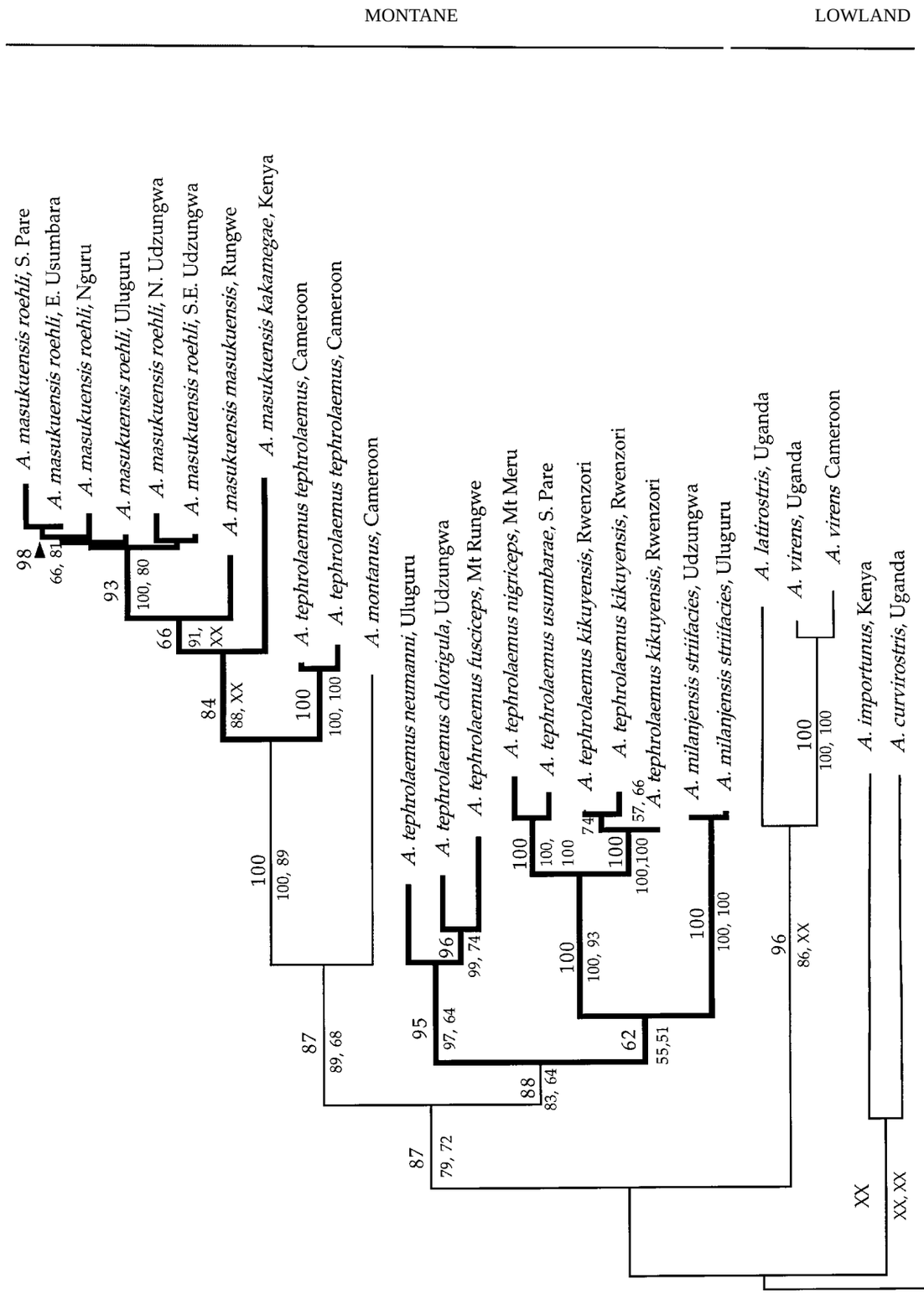

Figure 4. The single most parsimonious phylogenetic tree of 26 *Andropadus* species and subspecies using sequences of *Cyt b* (575 bp) and *ND2* (296 bp) combined. Species and regional distribution are indicated. Thick bold branches are montane radiations estimated to have occurred within the past 3 Ma. Sequence data was analysed by the 'branch and bound' search option of PAUP 3.1.1 (Swofford 1993), downweighting third position transition to transversion by 10:1, the tree has a consistency index excluding uninformative characters of 0.473. Bootstrap values are indicated above the branch line. Bootstrap values of trees from *Cyt b* and *ND2* genes (both downweighted third position transition by 10:1) individually analysed are given below the line (respectively). Only bootstrap support above 50% is shown and XX indicates either that the node gained a bootstrap value below 50% and it was unresolved, or that a different branching order was supported for individual genes. Branch lengths are proportional to amount of change along that branch.

A. masukuensis and both East African *A. tephrolaemus* groups (figures 3 and 4). Using the measure based upon third total substitutions, *A. masukuensis* populations have on average diverged within the last 1 Ma, with an explosive radiation within the Eastern Arc within the past 0.5 Ma (figures 3 and 4). Surprisingly, *A. t. tephrolaemus* from Cameroon may have diverged from East African *A. m. masukuensis* only 2 Ma ago. Eastern Arc *A. tephrolaemus* subspecies have diverged within the past 2.8 Ma (figures 3 and 4), with an average divergence within this group of 1 Ma. The two major clades of *A. tephrolaemus* from the Eastern Arc (*A. t. neumanni/A. t. chlorigula/A. t. fusciceps* and *A. t. usumbarae/A. t. nigriceps/A. t. kikuyensis*) each radiated approximately 12 Ma ago (figures 3 and 4) possibly from the Albertine Rift region. These figures may be halved if we employ the alternative clock used by Arctander *et al.* (1996).

Lowland taxa comparisons based upon total substitutions are prone to error due to saturation of third codon position transitions (figure 2). For this reason I used the rate of 0.5% third codon position transversion per 1 Ma. Based upon this rate of divergence, lowland forms diverged from montane groups between 18 and 32 Ma ago. Lowland lineages treated in this analysis would have diverged from each other between 12 and 28 Ma ago.

These estimates are based upon gene divergence which we assume reflects the time since divergence of two ancestral populations. However gene divergence estimates are likely to always overestimate the sought after population vicariance dates due to ancestral genetic variation (see Edwards 1997). In addition, the possibility of substitution rate differences among lineages may also produce an error window in these calculations, making the dates provisional pending more rigorous statistical analysis.

Speciation of forest biota is thought to have been driven by climatic cycles that have been pronounced within the past 2.8 Ma. The magnitude of these cyclic changes are thought to have increased over time, those of the Quaternary being the greatest. The results presented here suggest that montane radiations between all regions has been extensive since approximately 3 Ma ago. Further, the majority of terminal nodes radiating between isolated mountain areas appears to have occurred well within the Pleistocene, thereby supporting the climatically induced speciation model (deMenocal 1995; Fjeldså 1994; Haffer 1974). This complex pattern of phylogenetic and ecological relationships (Roy 1997) indicate a dynamic and cyclical process of rapid expansion of novel (derived) forms into 'new' montane areas followed by subsequent isolation and divergence of populations.

(*c*) *A model and mechanism for the evolution of montane avifauna*

Montane regions in the tropics are highly heterogenous and contain many areas that may be stable in terms of climate and vegetational cover throughout periods of shifting global climate, and would, in a sense, act as small refuges (*sensu* Brown & Ab'Saber 1979). Along the montane circle, it is assumed that the ecoclimatically most stable parts are in upper Zaire and on some east-facing escarpments of the Eastern Arc Mountains, which are under direct climatic influence from the Indian Ocean (Lovett 1993). Associated with these stable regions are clusters of newly evolved avian species and relictual forest bird species (Fjeldså 1994; Roy *et al.* 1997*b*) suggesting that points of stability are areas which induce biotic diversification. Because the assumed stable areas are small, the populations of animals and plants that would live in them would themselves be of small size. This would lead to rapid divergence from parent populations due to the speed of the fixation of alleles and founder effect (Avise 1994).

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REFERENCES

Amorim, D. S. 1991 Refuge model simulations: testing the theory. *Revta Bras. Ent.* **35**, 803–812.

Arctander, P., Folmer, O. & Fjeldså, J. 1996 The phylogenetic relationships of Berthelot's pipit *Anthus berthelottii* illustrated by DNA sequence data, with remarks on the genetic distance between rock and water pipits, *A. spinoletta*. *Ibis* **138**, 263–272.

Avise, J. C. 1994 *Molecular markers, natural history and evolution*. New York: Chapman & Hall.

Axelrod, D. I. & Raven, P. H. 1978 Late Cretaceous and Tertiary vegetation history of Africa. In *Biogeography and ecology of Southern Africa* (ed. M. J. A. Werger), pp. 77–130. The Hague: Dr W. Junk Publications.

Bartlein, P. J. & Prentice I. C. 1989 Orbital variations, climate and paleoecology. *Trends Ecol. Evol.* **4**, 195–199.

Bennet, K. D. 1990 Milankowitch cycles and their effects on species in ecological and evolutionary time. *Paleobiology* **16**, 1121.

Brown Jr., K. S. & Ab'Saber, A. N. 1979 Ice-age forest refuges and evolution in the neotropics: correlation of paleoclimatological, geomorphological and pedological data with modern biological endemism. *Paleoclimas* **5**, 130.

Collins, T. M., Kraus, F. & Estabrook, G. 1994 Compositional effects and weighting of nucleotide sequences for phylogenetic analysis. *Syst. Biol.* **43**, 449–459.

Coppes, Y. 1994 East side story: the origin of humankind. *Sci. Am.* **270**, 62–69.

Croizat, L. C. 1976 Biogeografia analitica y sintética panbiogeogrpahy de las Américas. *Bull. Acad. Ciencias Fisicas, Matématicas y Naturales* **35**, 1–890.

Crowe, T. M. & Crowe, A. A. 1982 Patterns of distribution, diversity and endemism in Afro-tropical birds. *J. Zool.* **198**, 417–442.

deMenocal, P. B. 1995 Plio-Pleistocene African climate. *Science* **270**, 53–58.

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

Diamond, A. W. & Hamilton, A. C. 1980 The distribution of forest passerine birds and Quaternary climatic change in Africa. *J. Zool.* **191**, 379–402.

Edwards, S. V. 1997 Relevance of microevolutionary processes to higher level molecular systematics. In *Avian molecular evolution and systematics* (ed. D. Mindell). pp. 251–278. San Diego: Academic Press.

Edwards, S. V., Arctander, P. & Wilson, A. C. 1991 Mitochondrial resolution of a deep branch in the genealogical tree for perching birds. *Proc. R. Soc. Lond.* B **243**, 99–107.

Felsenstein, J. 1985 Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **79**, 783–791.

Fjeldså, J. 1994 Geographical patterns of relict and young species of birds in Africa and South America and implications for conservation priorities. *Biodiversity Conserv.* **3**, 107–226.

Fjeldså, J. 1997 Geographical patterns of neoendemic and relict species of Andean forest birds: the significance of ecologically stable areas. *Mem. NY Bot. Gard.* (In the press.)

Fjeldså, J. & Lovett, J. 1997 Geographical patterns of phylogenetic relicts and phylogenetically subordinate species in tropical African forest. *Biodiversity Conserv.* (In the press.)

Garcia-Ramos, G. & Kirkpatrick, M. 1997 Genetic models of adaptation and gene flow in peripheral populations. *Evolution* **51**, 21–28.

Gilbert, D. G. 1992 SeqApp, a biosequence editor and analysis application. Apple Computer, Inc.

Hackett, S. J. 1993 Phylogenetic and biogeographic relationships in the neotropical genus *Gymnopithys* (Formicariidae). *Wilson Bull.* **105**, 301–315.

Hackett, S. J. 1996 Molecular phylogenetics and biogeography of tanagers in the genus Ramphocelus (Aves). *Molec. Phyl. Evol.* **5**, 368–382.

Haffer, J. 1969 Speciation in Amazonian forest birds. *Science* **165**, 131–137.

Haffer, J. 1974 Avian speciation in tropical South America. *Publ. Nuttal Ornithol. Club* **14**, 1–390.

Hillis, D. M. & Moritz, C. 1990 *Molecular systematics*. Massachusetts: Sinauer Associates Inc.

Hooghiemstra, H., Melica, J. L., Berger, A. & Shackleton, N. J. 1993 Frequency spectra and paleoclimatic variability of the high-resolution 30–1450 ka Funza I pollen record, Eastern Cordillera, Colombia. *Quaternary Sci. Rev.* **12**, 141–156.

Huelsenbeck, J. P. & Hillis, D. M. 1993 Success of phylogenetic methods in the four taxon case. *Syst. Biol.* **42**, 247–264.

Irwin, D. M., Kocher, T. D. & Wilson, A. C. 1991 Evolution of the cytochrome b gene of mammals. *J. Molec. Evol.* **32**, 128–144.

Keith, S., Urban, E. K. & Fry, C. H. 1992 *The birds of Africa, IV.* London: Academic.

Kessler, L. G. & Avise, J. C. 1985 A comparative description of mitochondrial differentiation in selected avian and other vertebrate genera. *Molec. Biol. Evol.* **2**, 109–125.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X. & Wilson, A. C. 1989 Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natn. Acad. Sci. USA* **86**, 6196–6200.

Li, W. H. 1993 So, what about the molecular clock hypothesis? *Curr. Opin. Genet. Devel.* **3**, 896–901.

Lockhart, P. J., Steel, M. A., Hendy, M. D. & Penny, D. 1994 Recovering evolutionary trees under a more realistic model of sequence evolution. *Molec. Biol. Evol.* **11**, 605–612.

Lovett, J. C. 1993 Climatic history and forest distribution in eastern Africa. Eastern Arc moist flora. In *Biogeography and ecology of the rain forests of eastern Africa* (ed. J. C. Lovett & S. K. Wasser), pp. 23–29, 33–55. Cambridge University Press.

Maddison, W. P. & Maddison, D. R. 1993 *MacClade: analysis of phylogeny and character evolution, version 3.04.* Sunderland, MA: Sinauer.

Mayr, E. & O'Hara R. J. 1986 The biogeographic evidence supporting the Pleistocene forest refuge hypothesis. *Evolution* **40**, 55–67.

Michevich, M. F. & Farris, J. S. 1981 The implications of congruence in *Menidia*. *Syst. Zool.* **30**, 351–370.

Mindell, D. P., Knight, A. & Baer, C. 1996 Slow rates of molecular evolution in birds and the metabolic rate and body temperature hypothesis. *Molec. Biol. Evol.* **13**, 422–426.

Prance, G. T. (ed.) 1982 *Biological diversification in the tropics*. New York: Columbia University Press.

Prigogine, A. 1987 Disjunctions of montane forest birds in the Afrotropical region. *Bonn Zool. Beitr.* **38**, 195–207.

Roy, M. S. 1997 Phylogeny and evolutionary history of montane greenbuls (*Andropadus*, Pycnonotidae). *Ibis*. (Submitted.)

Roy, M. S., Torres-Mura, J.-C. & Hertel, F. 1997*a* Evolution and history of the hummingbirds of the Juan Fernandez Islands of Chile. *Ibis* **140**, 56–64.

Roy, M. S., Da Silva, J., Arctander, P., Garcia-Moreno, J. & Fjeldså, J. 1997*b* The role of montane regions in the speciation of South American and African birds. In *Avian molecular evolution and systematics* (ed. D. Mindell). pp. 325–343. San Diego: Academic Press.

Seibold, I. & Helbig, A. J. 1995 Evolutionary history of New and Old World vultures inferred from nucleotide sequences of the mitochondrial *cytochrome b* gene. *Phil. Trans. R. Soc. Lond.* **B350**, 163–178.

Seutin, G., White, B. N. & Boag, P. T. 1991 Preservation of avian blood and tissue samples for DNA analysis. *Can. J. Zool.* **69**, 82–90.

Sibley, C. G., & Alqhuist, J. E. 1990 *Phylogeny and classification of birds: a study in molecular evolution*. New Haven, CT: Yale University Press.

Swofford, D. L. 1991 PAUP: phylogenetic analysis using parsimony, version 3.1. Champaign, IL: Center for Biodiversity of the Illinois Natural History Survey.

Vrba, E. S. 1993 Mammal evolution in the African Neogene and a new look at the Great American Interchange. In *Biological relationships between Africa and South America* (ed. P. Goldblatt), pp. 393–432. New Haven, CT/London: Yale University Press.

Whitmore, T. C. & Prance, G. T. 1987 *Biogeography and Quaternary history in tropical America*. 214 pp. Oxford, UK: Clarendon Press.

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