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A molecular phylogeny of African kestrels with reference to divergence across the Indian Ocean

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Abstract

In this paper we examine the evolutionary relationships of kestrels from mainland Africa, Indian Ocean islands and related areas. We construct a molecular phylogeny of African kestrels, using approximately 1.0 kb of mitochondrial cytochrome *b* sequence. Our molecular results support an Old World origin for typical kestrels and an ancient divergence of kestrels into the New World, and indicate a more recent radiation of kestrels from Africa via Madagascar towards Mauritius and the Seychelles. Phylogenetic placement of the Australian kestrel suggests a recent origin from African kestrel stock. We compare evolutionary relationships based on kestrel plumage pattern and morphology to our molecular results for the African and Indian Ocean kestrels, and reveal some consistency with the different island forms. We apply a range of published avian cytochrome *b* substitution rates to our data, as an alternative to internal calibration of a molecular clock arising from incomplete paleontological information. We align these divergence estimates to the geological history of Indian Ocean island formation inferred from potassium–argon dating methods. The arrival of kestrels on Mauritius appears consistent with the cessation of volcanic activity on Mauritius. The estimated time and route of divergence of the Seychelles kestrel from Madagascar may be compatible with the emergence of smaller islands during Pleistocene sea level fluctuations.

1. Introduction

Classically, kestrels are small, long-winged, long-tailed, short-toed falcons. The kestrel group contains 13 species (Village, 1990), whose global distribution comprises a single New World species (American kestrel *Falco sparverius*) and 12 Old World species (Boyce and White, 1987). Of these, the Mauritius (*Falco punctatus*), Madagascar (*Falco newtoni*), Seychelles (*Falco araea*), and Australian kestrels (*Falco cenchroides*) are all regarded as distinct species endemic to their respective islands. The greater kestrel (*Falco rupicoloides*) is restricted to southern and eastern parts of Africa, whereas the lesser kestrel (*Falco naumanni*) and common kestrel (*Falco tinnunculus*) have more extensive ranges across both the African and Asian continents (Village, 1990). The presence of the majority of taxa on the African continent (10 species) has been suggested as evidence for an African origin for the kestrel group (Boyce and White, 1987). However, the absence of a kestrel fossil record (Olsen, 1985) has meant that assumptions of kestrel divergence within and from the African continent have not been easy to confirm.

The majority of kestrels (10 of the 13 species) have been placed within a subgenus *Tinnunculus* (Brown and Amadon, 1968; Cade, 1982) that describes those species of classical kestrel form and brown barred dorsal

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plumage (Fig. 1 shows their global distribution). Three additional species (grey kestrel *Falco ardioacius*, Madagascan barred kestrel *Falco zoniventris*, and Dickinsons' kestrel *Falco dickinsoni*) have been placed in a separate subgenus *Dissodectes* due to their distinctly different morphology and grey-coloured plumage (Brown and Amadon, 1968; Snow, 1978); these species are not dealt with in our study.

The finer subdivisions within the *Tinnunculus* kestrels remain unresolved. For example, some authorities (Brown and Amadon, 1968; Cade, 1982) suggest that all *Tinnunculus* kestrels could be described as a single super species, implying that geographical separation alone prevents the island forms from interbreeding, whereas Jones (1987) describes the morphology of the Mauritius kestrel and Seychelles kestrel as too divergent for inclusion within a single, large super-species. Both the greater kestrel and the lesser kestrel are also included by Brown and Amadon (1968) and Cade (1982) as *Tinnunculus* kestrels, but their morphology suggests that they are highly divergent forms (Jones, 1987), which might warrant a more distinct taxonomic position. We use mitochondrial (mt) cytochrome *b* DNA sequence data to examine the evolutionary relationships of these African kestrels. We include four African subspecies of the common kestrel, from a total of 10 Old World subspecies which make up a *F. tinnunculus* species complex that spans a wide distribution across African and Eurasian continents (Village, 1990).

Some ideas surrounding relationships between *Tinnunculus* kestrels have focused on biogeographical radiation and subsequent isolation as the most parsimonious explanation for current kestrel diversity (Jones, 1987; Village, 1990). Hence, those kestrel species endemic to oceanic islands (in this case, Mauritius and Seychelles) have been popular candidates for resolving their evolutionary history from morphological similarity. However, morphology has served to confuse the placement of some of these island forms within the *Tinnunculus* kestrels. For example, the Mauritius kestrel is unusual because it possesses shorter, broader, more rounded wings and longer tarsi and toes, features which have been interpreted as adaptations to forest-living (Jones, 1987). Temple (1977) considered the Mauritius form to be sufficiently specialised to prevent speculation beyond an African origin. Further uncertainty surrounding the evolutionary history of these island forms stems from a comparison of morphometrics across *Falco* species by Kemp and Crowe (1991). Their cluster and factor analysis of morphometric dimensions was able to group together most *Tinnunculus* kestrels, but indicated the Seychelles kestrel as a distinct outlier from both the *Tinnunculus* and *Dissodectes* kestrel subgenera.

Plumage has also confounded ideas on relationships between the *Tinnunculus* kestrels. Within this group, the degree of plumage dimorphism between the sexes is variable (Village, 1990), and the analysis is complicated by the existence of two phases (a light and a dark phase) of the Madagascan kestrel, and a lack of sexual plumage dimorphism in the Indian Ocean species (Village, 1990; Watson, 1981). The plumage is clearly dimorphic in the common, Australian and lesser kestrel, but in some species both sexes share a typical female or juvenile-type plumage (i.e., Mauritius, greater, moluccan kestrel (*Falco moluccensis*), fox kestrel (*Falco alopex*) and the light phase Madagascar kestrel), whereas both sexes of the Seychelles kestrel and the dark phase Madagascar kestrel resemble a male-type plumage (the moluccan and fox kestrels are not included in our study). Furthermore, shared plumage traits have driven speculation about the colonisation route from Africa across the Indian Ocean. On Madagascar, the dark phase resembles the Seychelles kestrel, and the light phase resembles the Mauritius kestrel (Benson and Penny, 1971; Benson, 1967; Jones and Owadally, 1985; Siegfreid and Frost, 1970; Watson, 1981).

The kestrel fossil record is insufficient to provide insight into the group's evolutionary history (Olsen, 1985). Existing molecular evidence has provided partial information on deeper phylogenetic relationships within the genus *Falco* (Seibold et al., 1993), and support for the current taxonomic distinction between *Dissodectes* and *Tinnunculus* kestrels (Olsen et al., 1989). However, relationships within *Tinnunculus* have not been resolved. In this paper, we apply the resolution afforded by mtDNA cytochrome *b* sequence to the *Tinnunculus* kestrel group, to examine their history of evolutionary divergence within, and radiation from, the African continent. We select the cytochrome *b* gene to sample genetic sequence in kestrels for several reasons. First, there is a higher probability of congruence of a mtDNA haplotype tree with the actual species tree, rather than a tree resolved using a nuclear gene, due to the smaller effective size of the mtDNA genome (Moore, 1995). Second, the resolving power of cytochrome *b* has been suggested as being at its optimum at the level of species and below, and may be the most suitable choice of gene for birds, due to their tendency to have lower rates of genic divergence across taxonomic levels, relative to other vertebrate groups (Moore and DeFilippis, 1997). We interpret our results in relation to kestrel morphology, and the geography and geological evolution of the Indian Ocean islands.

2. Materials and methods

Fresh blood samples or feathers were obtained from the following species and subspecies; Mauritius kestrel (*F. punctatus*), Seychelles kestrel (*F. araea*), Madagascar kestrel (*F. newtoni*), lesser kestrel (*F. naumanni*), greater kestrel (*F. rupicoloides*), common kestrel (*F. t. tinnun-*

culus), Canary Islands subspecies (*F. t. canariensis* from Tenerife Island, and *F. t. dacotiae* from Fuerteventura Island and Lanzarote Island), Central African subspecies (*F. t. rufescens*), and South African subspecies (South African rock kestrel *F. t. rupicolus*). Specimen information for all individuals from which DNA was sequenced is given in Table 1. Material was stored in a standard Tris:EDTA:SDS storage buffer. Total DNA was extracted in 5.0 ml volumes using proteinase *K* digestion, phenol:chloroform purification, NaCl extraction and ethanol precipitation. Methods followed Ausubel et al. (1989), but were modified for avian blood from Miller et al. (1988). The protocol was modified for feather samples by reducing extraction volumes to 1.0 ml. DNA was suspended in 500 μl 10 mM Tris–HCl, 1 mM EDTA (TE) buffer (pH 8.0), and stored at −20 °C.

PCR was used to amplify the whole of the cytochrome *b* gene (1.1 kb) as a single fragment. Amplifications (25 μl volume) were performed containing 3.0 mM NH_4 buffer (Gibco, UK), 1.5 mM $MgCl_2$, 100 μM of each dNTP, 0.5 pmol of each primer, and 0.75 U of *Taq* polymerase (Gibco, UK). For all species, except the Seychelles and Madagascar kestrel, the primers used were L14990 and H16065 (Desjardins and Morais, 1990). Species-specific PCR primers were designed for the Seychelles and Madagascar kestrels using the primer design software MacVector 4.5.3 (Eastman Kodak); SeyL, 5′CCTACTAGGAATCTGCCTAGCCACC3′; SeyH, 5′CGGTAAGGGGGAGGAGGATTAG3′ (corresponding to positions 15,000–15,024 and 15,981–16,003 of the chicken (*Gallus gallus*) mtDNA genome [Desjardins and Morais, 1990], approximate product size 0.95 kb).

PCR products were purified using the Geneclean Kit (Anachem, UK). Between 25 and 50 ng/μl of template DNA was used in each 10 μl volume sequencing reaction (PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit, Applied Biosystems). The sequencing primers used were L-14996 (5′ACATCTCAGCATG ATGAAAYTTYGG3′; R. Thomas, personal communications), L-15212 (Baker et al., 1995), L-15656, and H15914 (Helm-Bychowski and Cracraft, 1993). Species-specific sequencing primers were designed for the Seychelles and Madagascar kestrels; Sey1, 5′CACTAC ACAGCAGACACAACTC3′; Sey2, 5′CCAACTTAT TCTCAGCAATCCC3′ (located at positions 15,056–15,077 and 15,336–15357, respectively, of the chicken mtDNA genome, Desjardins and Morais, 1990). The

Table 1
Falco samples for which DNA sequence was obtained

Taxon name[a]	I.D./Band No.[b]	Origin	Location information	Sample type	Archive[h]
F. rupicoloides	5H03439	Wild	30 35′S 24 04′E	Blood	ZSL 5951
F. rupicoloides	5H03441	Wild	30 51′S 23 58′E	Blood	ZSL 5952
F. naumanni	5-68263[c]	Captive	Johannesburg Zoo	Blood	ZSL 5933
F. naumanni	D-1651	Captive	Johannesburg Zoo	Blood	ZSL 5934
F. t. rupicolus 1	5H03443	Wild	30 40′S 24 02′E	Blood	ZSL 5941
F. t. rupicolus 2	5H03444	Wild	30 37′S 23 58′E	Blood	ZSL 5942
F. t. rupicolus 3	5H03446	Wild	31 05′S 23 58′E	Blood	ZSL 5943
F. t. rupicolus 4	5H03447	Wild	30 38′S 24 00′E	Blood	ZSL 5944
F. t. rufescens	NMK 1349	Wild	Abyssinia, Ethiopia	Dried skin	ZSL 5962[i]
F. newtoni	TZ 20[d]	Captive	Tsimbazaza Zoo	Feather	ZSL 5961
F. punctatus	596407	Wild	Morne Seche, Mauritius	Blood	ZSL 5982
F. punctatus	594389	Wild	Tatamaka, Mauritius	Blood	ZSL 5981
F. araea	D-26164	Wild	Mahe, Seychelles	Blood	ZSL 5965
F. cenchroides	100-22307	Wild	Perth, W. Australia	Feather	ZSL 6-I[j]
F. t. tinnunculus 1	A-2001.8.1[e]	Captive	British Museum	Muscle	ZSL 6-I
F. t. tinnunculus 2	A-2001.8.2	Captive	British Museum	Muscle	ZSL 6-I
F. t. tinnunculus 3	A-2001.8.3	Captive	British Museum	Muscle	ZSL 6-I
F. t. dacotiae 1	F-18891[f]	Captive	Fuerteventura Is.	Blood	ZSL 5971
F. t. dacotiae 2	Unbanded[g]	Captive	Lanzarote Is.	Blood	ZSL 5972
F. t. canariensis	238/1998[f]	Captive	Tenerife Is.	Blood	ZSL 5978

[a] DNA sequence was obtained for several individuals for some taxa, for which sample information for each individual is included.

[b] Identification numbers consist of band numbers for wild caught birds, or collection numbers for samples obtained from captive birds from zoological collections or rehabilitation centres, or museum collections where study specimens have been deposited.

[c] Johannesburg Zoological Gardens, Jan Smuts Avenue, Parkview 2193, Johannesburg, South Africa.

[d] Parc Botanique et Zoologique de Tsimbazaza, Antananarivo 101, Madagascar.

[e] Specimens were originally obtained from UK captive-breeding stock, and are currently registered and held at the British Museum of Natural History, Tring, UK.

[f] Centro de Rehabilitación de Fauna Silvestre, Vivero Forestal Tafira Baja, 35017 Tafira, Las Palmas de Gran Canaria, Spain.

[g] Centro de Recuperación de Fauna Silvestre, Carretera de La Esperanza km 0.4, La Laguna, Sta. Cruz de Tenerife, Spain.

[h] Archive accession numbers for DNA samples held at the Blood and Tissue Bank, Institute of Zoology, Zoological Society of London, UK.

[i] Validation authority; G. Amutete, Department of Ornithology, National Museums of Kenya, PO Box 40658, Nairobi, Kenya.

[j] Validation authority; P. Pain, Eagles Heritage Centre, Margaret River, Western Australia.

sequencing products were analysed on a semi-automated DNA sequencer (ABI Prism 377). Accuracy of the sequence obtained for each individual was confirmed by aligning multiple overlapping fragments from forward and reverse sequencing reactions, such that each portion of the gene was sequenced 3–4 times.

Sequences were edited and aligned using the multiple sequence alignment program Sequencher (Gene Codes, 1998; version 3.1.1). Positioning for correct reading frame was verified by alignment with chicken mtDNA sequence positions 14,995–16,020 (Desjardins and Morais, 1990). The sequenced nucleotide data were also aligned with the following GenBank sequences; peregrine falcon (*F. peregrinus* AF090338), western redfooted falcon (*F. vespertinus* FVU83311), and American kestrel (FSU83306). To confirm correct reading-frame positions, the nucleotide sequence was translated using MacClade 3.0.4 (Maddison and Maddison, 1992).

The peregrine falcon and western redfooted falcon were chosen as outgroups for their different relative affinities to the ingroup taxa. Brown and Amadon (1968) regard the taxonomic position of the redfooted falcons to be close to the *Dissodectes* kestrels, a placement that is supported by electrophoretic analysis of feather proteins (Olsen et al., 1989). Alternatively, some authorities place them outside of the kestrel group (Village, 1990), and others posit a distinct subgenus *Erythropus* (Suschkin, 1905). The peregrine falcon is regarded as more distantly related to the *Tinnunculus* kestrels than the western redfooted falcon (Brown and Amadon, 1968; Cade, 1982), but both species are falcons with a wide geographic distribution; the western redfooted falcon occurs across Africa and Asia, and the peregrine falcon ranges across both the Old and New World (Brown and Amadon, 1968; Cade, 1982). The American kestrel was included as an ingroup taxon since recent taxonomic authority recognises this species as a *Tinnunculus* kestrel (Brown and Amadon, 1968; Cade, 1982; Village, 1990), and when phylogenetic analyses were repeated specifying the American kestrel as either an ingroup or outgroup taxa, tree topology and branch length remained unchanged.

Phylogenetic analysis was performed using PAUP* (Swofford, 1997), and included parsimony, distance, and maximum likelihood methods of phylogeny reconstruction. Under parsimony analysis, branches were collapsed if the maximum length was zero. Gaps in the sequence were treated as missing data, and character-state optimisation used accelerated transformation. We used a heuristic search method, and starting-trees were obtained by stepwise-addition using simple addition sequence. A single tree was held at each step. The criteria for tree-swapping used a tree-bisection–reconnection algorithm. Bootstrap analysis (Felsenstein, 1985, 1988) for 1000 replicates using equal weighting of characters provided confidence estimates for groups contained within the most parsimonious trees (the consensus method used was 50% majority-rule). Parsimony trees were compared using the pairwise test of Kishino and Hasegawa (1989) within PAUP* (Swofford, 1997). Distance analyses specified the substitution model of

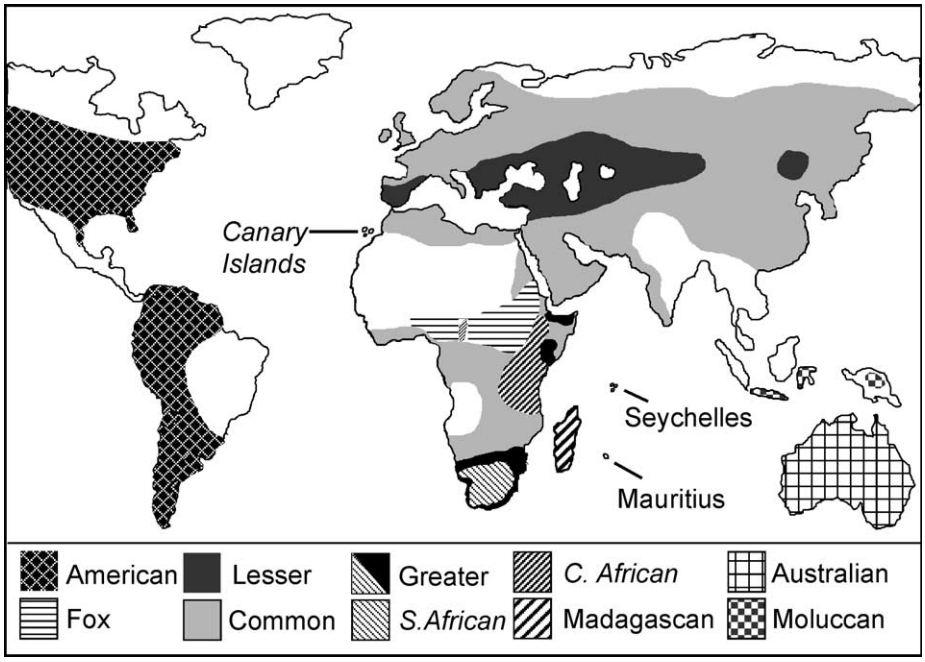

Fig. 1. Distribution of the *Tinnunculus* kestrels after Brown and Amadon (1968). Distributions shown for the greater, lesser, and fox festrel are sympatric with that shown for the common kestrel. The four subspecies of the common kestrel included in this study are labelled in italics. DNA sequence data were not obtained for the moluccan kestrel or fox kestrel.

Hasegawa et al. (1985), 'HKY85 model,' since this model of sequence evolution mostly closely accounts for the qualities known to exist within our data (Moore and DeFilippis, 1997; Yoder et al., 1996). The HKY85 model parameters can recognise unequal base frequencies, different substitution rates for transitions and transversions, a substitution rate that can be set to vary between sites, and can account for the possibility of multiple substitutions at the same site (Hasegawa et al., 1985). Heuristic searches under this model specified a gamma shape parameter of 0.283 to describe rates for variable sites. Missing data were distributed proportionally to unambiguous changes. Analysis by distance incorporated both substitution types, and a function objective that adhered to a model of minimum evolution. Negative branch lengths were set to zero when calculating tree scores. Starting trees for branch-swapping were derived by neighbour-joining, and tree-swapping used the tree-bisection–reconnection algorithm. Distance analysis was performed using UPGMA (Sneath and Sokal, 1973) and neighbour-joining algorithms. Bootstrap re-sampling specified 1000 replicates.

Maximum likelihood analysis incorporated the HKY85 algorithm, which could be set to enable the substitution rate to differ between sites. The distribution of rates was described by a gamma distribution (Yang, 1996), divided into four rate categories represented by the mean; the shape parameter estimated from our data was 0.283. Acknowledgment that our data conformed to the general behaviour of cytochrome *b* evolution, and displayed a degree of rate heterogeneity, negated the use of less complex models for further analysis. Starting trees were obtained via stepwise addition. To assess the degree of agreement of a molecular clock to our data, trees were generated under maximum likelihood both with and without a molecular clock enforced, and the likelihood scores were evaluated (Kishino and Hasegawa, 1989; Swofford, 1997).

The absence of a reliable kestrel fossil record prevents a calculable estimate of a local cytochrome *b* substitution rate for alignment of our molecular data to dated geological events. Although a substitution rate of 2.0% per million years (%/MY) has been calculated for mammals (Brown et al., 1979, 1982; Irwin et al., 1991; Kocher et al., 1989) and applied to some avian studies (Klicka and Zink, 1997, 1998, but see Arbogast and Slowinski, 1998), evidence is conflicting as to whether birds may have slower substitution rates relative to other groups (Johns and Avise, 1998; Mindell et al., 1996). As an alternative solution, we calculated a range of divergence times for selected nodes within our kestrel phylogeny using three estimates of avian cytochrome *b* substitution rates, locally calibrated for other species using fossil records (0.7–1.7%/MY, Krajewski and King, 1996; 2.0%/MY, Moore et al., 1999), or geological dating of island-formation (1.6%/MY, Fleischer et al., 1998). The higher end of this range is similar to the substitution rate obtained for mammals (2.0%/MY, Brown et al., 1979; Irwin et al., 1991; Kocher et al., 1989) that was applied by Randi (1996) and Klicka and Zink (1997) to avian taxa (see Arbogast and Slowinski, 1998). We applied this range of substitution rates (0.7–1.7%/MY, Krajewski and King, 1996; 1.6%/MY, Fleischer et al., 1998; 2.0%/MY, Moore et al., 1999) to obtain a range of divergence times for particular kestrel species, and we aligned these dates to the geological history of the Indian Ocean.

3. Results

DNA sequences obtained for all taxa in this study have been deposited in GenBank (Accession Numbers AF279465–AF279478). Table 2 shows the uncorrected nucleotide and amino acid cytochrome *b* distances. The majority of the highest sequence divergence values in Table 2 involves the two outgroup taxa (range; 7.79–11.10%), with a single exception for the comparison between the American and greater kestrel (12.06%). The uncorrected amino acid distances across all taxa were on average 2.4 times lower than nucleotide sequence divergence, indicating that the majority of the sequence variation is at the third codon position.

The base composition at each codon position varied in accordance with the expected constraints of vertebrate cytochrome *b* (Moore and DeFilippis, 1997). A test for homogeneity of base frequencies ($\chi^2_{48} = 5.072$, $P = 1.0$) suggested that base composition was unlikely to have produced phylogenetic bias (Lockhart et al., 1992). The rates of accumulation of transition and transversion substitutions also conformed to the general behaviour of cytochrome *b* evolution (Li and Graur, 1991; Yoder et al., 1996), When substitution rates within the data were aligned across Jukes–Cantor corrected distances (Jukes and Cantor, 1969), a linear relationship confirmed the presence of a consistent phylogenetic signal across all genetic distances, suggesting a minimal effect from saturation on the sequence data. Across a total 998 bases, the data held 43, 8, and 190 variable characters, respectively, for first, second, and third positions, of which 17, 3, and 89, respectively, were parsimony informative (Swofford, 1997).

3.1. Phylogenetic analysis

Parsimony, distance, and maximum likelihood analysis produced comparable tree topologies, but with some differences noted below. Parsimony analysis produced two trees which differed only in the positioning of the Mauritius kestrel, placing it either within the Indian Ocean group (Fig. 2a), or as a separate sister taxa to the *F. tinnunculus* species complex (Fig. 2b). Tree length and

Table 2
Uncorrected nucleotide distances (below) and amino acid distances (above)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	*F. peregrinus*		0.0266	0.0133	0.0239	0.0298	0.0326	0.0326	0.0298	0.033	0.0333	0.0333	0.0333	0.0305	0.0299	0.0179	0.019	0.0214
2	*F. sparverius*	0.0997		0.0239	0.0266	0.0327	0.0326	0.0326	0.0268	0.03	0.0303	0.0303	0.0303	0.0274	0.0269	0.0209	0.0222	0.0214
3	*F. vespertinus*	0.0779	0.0901		0.0213	0.0327	0.0356	0.0356	0.0327	0.036	0.0364	0.0364	0.0364	0.0335	0.0328	0.0209	0.0222	0.0245
4	*F. naumanni*	0.111	0.1128	0.0911		0.0238	0.0267	0.0267	0.0238	0.027	0.0273	0.0273	0.0273	0.0244	0.0239	0.0179	0.019	0.0153
5	*F. rupicoloides*	0.1042	0.1206	0.0978	0.0803		0.0149	0.0208	0.0179	0.015	0.0152	0.0212	0.0212	0.0183	0.0179	0.0179	0.0066	0.0092
6	*F. t. rupicolus 3*	0.106	0.1125	0.0946	0.0828	0.0397		0.0059	0.003	0.006	0	0.0061	0.0061	0.0305	0.003	0.0209	0.0098	0.0122
7	*F. t. rufescens*	0.104	0.1104	0.0925	0.0809	0.0434	0.0094		0.003	0.012	0.0061	0.0061	0.0061	0.0305	0.003	0.0209	0.0131	0.0122
8	*F. t. tinnunculus*	0.1021	0.1076	0.0907	0.0791	0.0425	0.0084	0.001		0.009	0.003	0.003	0.003	0	0	0.0179	0.0099	0.0092
9	*F. t. rupicolus 1*	0.1052	0.1128	0.0953	0.0862	0.0411	0.004	0.0137	0.0127		0.0061	0.0121	0.0121	0.0915	0.0091	0.021	0.01	0.0123
10	*F. t. rupicolus 2*	0.106	0.1116	0.0943	0.0839	0.0407	0.001	0.0109	0.0098	0.005		0.0061	0.0061	0.0305	0.0033	0.0212	0.0101	0.0124
11	*F. t. canariensis*	0.103	0.1106	0.0914	0.0795	0.0431	0.0105	0.0029	0.0019	0.0147	0.0118		0.0061	0.0305	0.0033	0.0212	0.0134	0.0124
12	*F. t. dacotiae 1*	0.1041	0.1097	0.0904	0.0785	0.0442	0.0096	0.0038	0.0029	0.0137	0.0109	0.0048		0.0305	0.0033	0.0212	0.0134	0.0124
13	*F. t. dacotiae 2*	0.1026	0.1083	0.0909	0.0789	0.0434	0.0087	0.001	0	0.0128	0.0099	0.0019	0.0029		0	0.0183	0.0101	0.0094
14	*F. cenchroides*	0.1055	0.1091	0.0921	0.0803	0.0416	0.0066	0.0047	0.0038	0.0107	0.0079	0.0057	0.0048	0.0039		0.0179	0.0099	0.0092
15	*F. newtoni*	0.1026	0.1091	0.0941	0.0795	0.0465	0.0351	0.0351	0.0342	0.0361	0.0376	0.0347	0.0357	0.0348	0.0341		0.0099	0.0092
16	*F. punctatus*	0.0965	0.1089	0.0927	0.0829	0.0412	0.0292	0.0326	0.0305	0.0285	0.0287	0.031	0.0301	0.0265	0.0265	0.0314		0.0032
17	*F. araea*	0.0965	0.1073	0.0895	0.0788	0.0479	0.0388	0.0368	0.0358	0.0386	0.04	0.0361	0.0372	0.0363	0.0357	0.0172	0.0355	

the number of unambiguous events were identical for both resolutions (length = 346; transitions = 194; transversions = 34). Both trees were compared using the pairwise test of Kishino and Hasegawa (1989) to assess if either tree gave a significantly better representation of the data. In this case there was no significant difference between the two trees at the $P < 0.05$ level. Bootstrap support (Fig 2a) was high for the positions of the lesser kestrel, greater kestrel, close grouping of the Madagascar and Seychelles kestrel, and the South African rock kestrel as a sister taxon to the remaining taxa of the *F. tinnunculus* species complex. A notable result is the high support for the position of the Australian kestrel within the *F. tinnunculus* species complex. The low bootstrap support for the Indian Ocean and common kestrel groups as being sister groups was due to the unresolved position of the Mauritius kestrel under parsimony.

Phylogeny reconstruction by distance analysis using the HKY85 model (minimum evolution score; 0.480) was most consistent with the phylogeny in Fig. 2b, but with two differences. First, distance analysis placed the greater kestrel as a sister taxon to the *F. tinnunculus* species complex, and second, the Mauritius kestrel did not group with the other Indian Ocean kestrels, but was placed as ancestral both to them and the greater kestrel by two short internodal branch lengths. Bootstrap support was high for the position of the lesser kestrel (100%), and the Madagascar and Seychelles kestrels (99%), whereas the remaining groups within the topology remained unresolved. However, the positions of the unsupported internodes were concurrent with the shortened internodal branch lengths within the tree topology under distance analysis. Distance analysis using UPGMA produced a similar arrangement to the parsimony tree in Fig. 2b. Neighbour-joining determined the position of the Mauritius kestrel within the Indian Ocean group as unresolved, and placed the greater kestrel as a sister taxon to the *F. tinnunculus* species complex.

Tree topology under maximum likelihood analysis was consistent with parsimony tree 1 (see Fig. 2a). The tree in which the molecular clock was not enforced did not have a significantly greater likelihood than the tree in which it was. The difference in likelihood was assessed using the χ^2 approximation (Kishino and Hasegawa, 1989) for twice the difference in log likelihood ($\log(L)$ clock enforced = 3080.183; $\log(L)$ clock not enforced = 3071.7642; $\chi^2_{15} = 16.837$; NS).

A range of estimated divergence times were calculated from the branch lengths of the maximum likelihood tree for which a molecular clock was enforced, using estimates of cytochrome *b* substitution rates obtained for other avian taxa (Fleischer et al., 1998; Krajewski and King, 1996; Moore et al., 1999). Fig. 3 compares this range of divergence estimates to the geological history of the Indian Ocean.

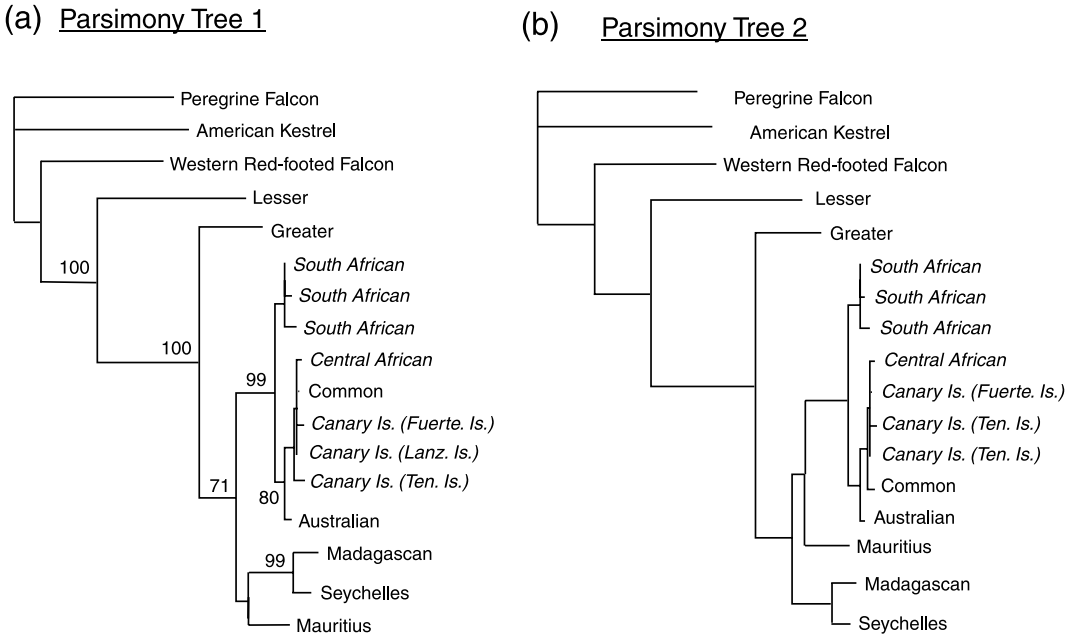

Fig. 2. Trees obtained from maximum parsimony analysis. (a) Mauritius, Seychelles, and Madagascar kestrel form an Indian Ocean clade. (b) Indian Ocean clade becomes separated, due to different position of the Mauritius kestrel.

The range of plausible divergence values from Fig. 3 indicates that the divergence of African kestrel stock into the Indian Ocean took place between 0.9–2.6 million years ago (MYA). The branching order suggests that, whereas colonisation of the Seychelles by kestrels took place via Madagascar relatively recently (between 0.3 and 1.0 MYA), Mauritius is likely to have been colonised much earlier. The short internal branch length for the Mauritius kestrel implies that Mauritius was colonised by kestrels very soon after their arrival on Madagascar. However, the poorly resolved branching order shown by some phylogenetic analysis for the Mauritius kestrel indicates that the possibility of a direct colonisation from the African continent cannot be excluded. The calculated ranges for the two Indian Ocean nodes in Fig. 3 do overlap, but we note that the range of possible values is more than doubled by our inclusion of the slowest substitution rate obtained for avian cytochrome *b* of 0.7% (Krajewski and King, 1996). This is a comparatively slow substitution rate that may be attributable to life history differences (Krajewski and King, 1996). A full interpretation of the phylogeny is limited by an unresolved chronology of events suggested by some analysis (Fig. 2a and b), which can only identify the colonisation of Mauritius as an independent event from that of Madagascar and the Seychelles. However, the range of divergence times applied in Fig. 3 support the statement that the Mauritius kestrel has had a longer history of island isolation than the Seychelles kestrel.

The phylogeny indicates the South African rock kestrel as the most genetically divergent of the currently recognised African subspecies of the *F. tinnunculus* species complex that were sampled. The South African form is the most distinctive of all the races of *F. tinnunculus* in plumage characters (Jones personal communications). The South African rock kestrel is more divergent than the Australian kestrel species, whose position alongside some of the recently diverged common kestrel subspecies indicates a very recent (0.1–0.4 MYA) radiation. The very early divergence of the American kestrel from the remainder of the *Tinnunculus* kestrels is contrary to the ideas of Boyce and White (1987), who suggest that the expansion of kestrels into the New World was a comparatively recent event relative to the group's phylogenetic history.

4. Discussion

All three analytical approaches produced trees of broadly similar topology, but with inconsistent positions for the Mauritius and greater kestrel. Although the short internode lengths (Lanyon, 1988; Moore and DeFilippis, 1997) mean that the exact joining point of the Mauritius kestrel is unresolved, there is very strong support for the finding that the Madagascan and Seychelles kestrels have a more recent common ancestor (Fig. 2). The geography of the Indian Ocean (Fig. 1) suggests that the most parsimonious explanation for the route of Indian Ocean kestrel radiation is most likely to be via geographically closest neighbours, implying that colonisation of Mauritius was via Madagascar. However, there is only a very short period, shown in Fig. 3, during which there was a common ancestor exclusively

for the Indian Ocean species (1.8–1.9 MYA). This suggests only a brief evolutionary history for the Mauritius kestrel ancestor on Madagascar, or multiple colonisations.

The recent history of the Mauritius kestrel differs from that of most other kestrel species, in that it documents a severe population bottleneck (Groombridge et al., 2000; Jones et al., 1995; Temple, 1986). One interpretation is that, as a consequence, the molecular sequence obtained here might not represent the species true ancestry. The DNA sequences obtained for the two Mauritius samples were identical, and each individual can be traced to a different historical lineage since the onset of the populations recovery. Although we cannot evaluate the full effect of this bottleneck on our molecular data, we note that in our genetic survey of the lesser and greater kestrel, both described by our results as highly distinct from the remaining species, no within-species divergence was observed.

The inherently large margins of error associated with estimated divergence times imply that they should be treated with a degree of caution (see Arbogast and Slowinski, 1998; Hillis et al., 1996; Klicka and Zink, 1998). Our inclusion of the relatively slow substitution rate of 0.7%/MY obtained for cranes (Gruiformes) by Krajewski and King (1996) broadened our calculated range of divergence times considerably, but this slower rate has been suggested as being a consequence of longer generation time (Krajewski and King, 1996). Kestrels may have relatively quick generation times; most species can breed when a year old, although many do not breed until older (Jones pers. comm.). Alternatively, inaccuracies underlying fossil dating may account for a portion of observed differences between substitution rates obtained for different avian taxa. Even withstanding our broad range of plausible divergence times based on available substitution rates for avian cytochome *b*, an alignment of our molecular divergence times to potas-

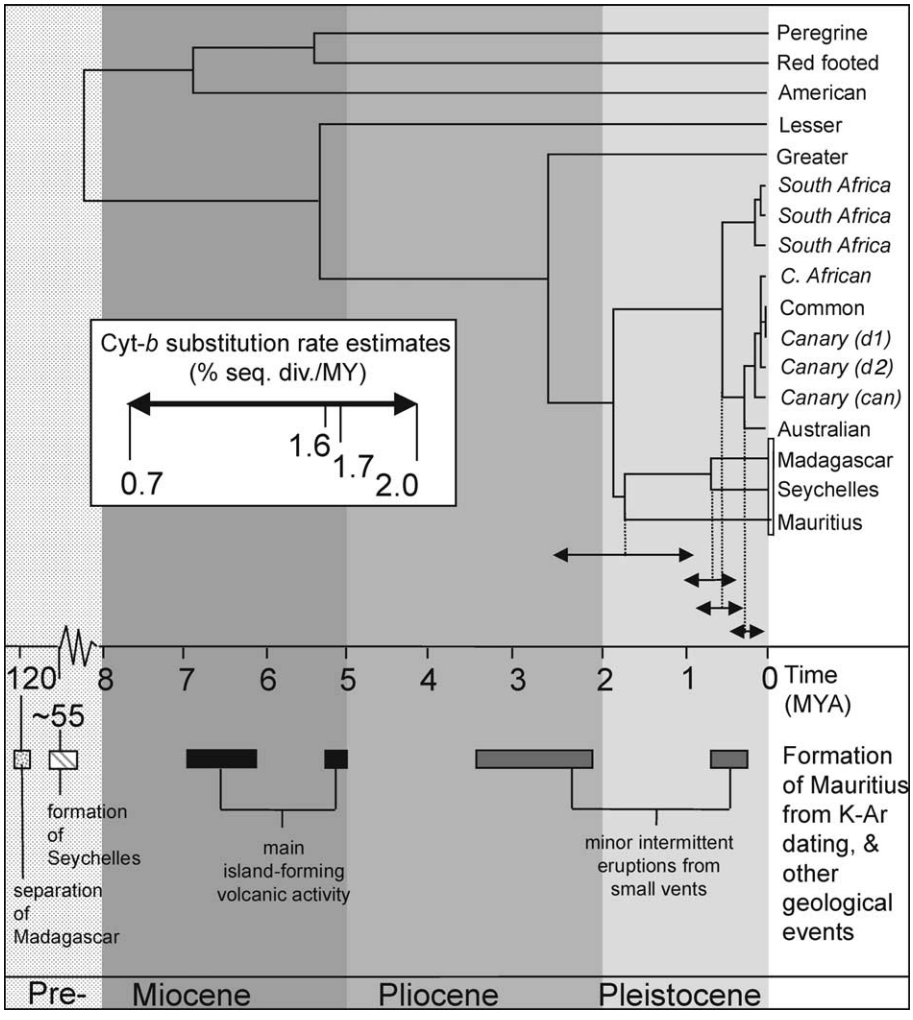

Fig. 3. Alignment of the maximum likelihood tree (with molecular clock enforced) to the volcanic history of Mauritius, and the geological formation of Madagascar and the Seychelles. Ranges of molecular divergence times calculated from branch lengths by applying a rate of between 0.7% and 2.0% sequence divergence per million years (see inset). Canary Islands subspecies of the common kestrel; *F. t. dacotiae* from Fuerteventura Is. (d1); *F. t. dacotiae* from Lanzarote Is. (d2); *F. t. canariensis* from Tenerife Is. (can); see Table 1.

sium–argon (K–Ar) dating of geological events (McDougall and Chamalaun, 1969) indicates that colonisation of Mauritius (1.9–2.6 MYA) appears to have coincided with the end of volcanic activity. Although there is evidence of more recent volcanic activities on Mauritius (see Fig. 3), these have been identified as low volume extrusions from small vents (McDougall and Chamalaun, 1969). Our molecular results suggest that the early colonising kestrel population was not extirpated by the most recent volcanic activities on Mauritius.

Recent theoretical work by Moore et al. (1999) suggests that cytochrome *b* sequence divergence is accurate as a predictor of time of divergence only up to about 5.0 MY before present (i.e., about 10% overall sequence divergence), and that predictions of dates beyond 5.0 MY are likely to be overestimated. We note that, for the nodes for which we calculate ranges of divergence time estimates, none of them exceed this boundary (Mauritius kestrel, 3.66%; Seychelles kestrel, 1.40%; South African rock kestrel, 1.17%; Australian kestrel, 0.56%; taken from branch lengths of the γ-HKY85 maximum likelihood tree, Fig. 3).

The molecular results indicate that the Seychelles were colonised from Madagascar between 0.3 and 1.0 MYA, which may seem surprisingly recent in view of the more ancient origin estimated for the Seychelles archipelago at 55–65 MYA (Norton and Sclater, 1979). However, climatic oscillations may have played a role in aiding kestrel dispersal to the Seychelles. Past changes in sea level have been driven by glacial cycles during the Pleistocene era (Stoddart, 1971; Berger, 1984), and mean global sea level has undergone a gradual net reduction of approximately 100 m during the last 2.0 MY (Stoddart, 1971; Stowe, 1987). The Aldabra, Farquhar, and Amirante island groups are eroded submerged platforms, formed as a result of volcanic leakage (Braithwaite, 1984), that lie between Madagascar and the Seychelles. The magnitude of sea level reduction is likely to have exposed these submerged platforms (Braithwaite, 1984), which may have enhanced the opportunity for kestrels to colonise the Seychelles from Madagascar. The Aldabran kestrel (*F. newtoni aldabranus*), which is regarded as a subspecies of the Madagascar kestrel (Benson, 1967; Benson and Penny, 1971), may be a relict of such dispersal.

Boyce and White (1987) suggest that the influence of rapid climate change (see Hewitt, 1996) might have contributed to the divergence of some kestrel taxa on the African continent during the Pleistocene period, a time when African climate became sensitive to remote changes in high-latitude climate (deMenocal, 1995). The associated habitat fragmentation (see Avise and Walker, 1998) could have initiated the divergence of the South African rock kestrel, which our phylogeny places as a distinctive sister taxon to the remaining taxa of the *F. tinnunculus* species complex. The genetic difference is comparable to several inter-species values, and our molecular result is compatible with observed differences in plumage and ecology. The plumage of the South African rock kestrel differs from that of the other subspecies of the *F. tinnunculus* complex (Cade, 1982; Clark, 1995) in that the females possess a plumage normally associated with male kestrels (i.e., a grey-coloured head and tail, and a spotted rather than barred ventral plumage). Compared with nominate *tinnunculus*, the South African rock kestrel has a shorter tail and tarsus (Cheke and Jones, 1987). There are also ecological differences; the South African rock kestrel is most commonly found in arid areas (Van Zyl, 1997), unlike their *F. t. tinnunculus* counterparts.

The Mauritius kestrel shows the highest level of adaptation for forest-dwelling compared to other kestrel species (Jones, 1987), the most striking feature being the short, rounded, broad wings, which are 17% shorter than those of the common kestrel (Jones, 1987), possibly to increase manoeuvrability under the forest canopy (McKelvey, 1978; Temple, 1977). The tarsi and toes of the Mauritius kestrel are also more elongated than those of the common kestrel (32% and 16% longer, respectively), presumably for snatching agile prey (arboreal lizards) off vegetation (Jones, 1987). The Seychelles kestrel shows similar but less pronounced adaptation for forest living, which may imply a comparatively shorter period of isolation compared to the Mauritius kestrel, and as such would be consistent with our molecular phylogeny. The Madagascar kestrel is found in open grasslands and is uncommon in forested habitats (Morris and Hawkins, 1998), which might suggest that the morphological adaptations for forest dwelling observed in the Seychelles and Mauritius kestrels may have evolved in both species on their respective islands.

Some aspects of our molecular phylogeny differ from the morphological phylogeny of Boyce and White (1987), which suggested a recent origin for the American kestrel. However, they did indicate that this species shared two characters with the more basal lesser kestrel; namely the presence of blue-grey coloured greater wing coverts and secondary feathers. Our molecular data reinforce the latter interpretation. Some of our molecular results support those of Boyce and White (1987), including a relatively early divergence of the lesser and greater kestrels, placement of the Indian Ocean kestrels as a sister group to the *F. tinnunculus* species complex, and a close relationship between the Australian and common kestrel. The close affinity of the Australian and common kestrel implies a recent divergence of the Australian kestrel, but over a relatively long distance. This result has been attributed to very recent Pleistocene glacial events forcing common kestrel stock southwards from Asia (Boyce and White, 1987), a hypothesis that is supported by our molecular data. The Australian kestrel

is separated geographically from the common kestrel by the moluccan kestrel, which Cade (1982) considers very close to, if not conspecific with, the common kestrel. These three taxa may represent clinal variation within the same species or super-species, an explanation that is consistent with our result for the Australian kestrel, and the spread of *Tinnunculus* kestrels across Europe to Asia and Australia.

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