Conservation Genetics (2006) 7:551–562
DOI 10.1007/s10592-005-9066-9

Three genetically divergent lineages of the Oryx in eastern Africa: Evidence for an ancient introgressive hybridization

Charles Masembe[1,*], Vincent B. Muwanika[1], Silvester Nyakaana[1], Peter Arctander[2] & Hans R. Siegismund[2]
[1]*Institute of Environment & Natural Resources, Molecular Biology Laboratory, Makerere University, P.O. Box 7298, Kampala, Uganda;* [2]*Institute of Biology, Department of Evolutionary Biology, University of Copenhagen, Universitetsparken 15, DK-2100, Copenhagen, Ø Denmark (*Corresponding author: Fax: +256-41-530134; E-mail: cmasembe@muienr.mak.ac.ug)*

Received 9 May 2005; accepted 7 October 2005

Key words: introgressive hybridization, mitochondrial DNA, *Oryx beisa*, population structure

Abstract

Phylogeographic and population genetic studies using sequence information are frequently used to infer species boundaries and history; and to assess hybridization and population level processes. In this study, partial mitochondrial DNA (mtDNA) control region (423 bp) and cytochrome *b* sequences (666 bp) of *Oryx beisa* sampled from five isolated localities in its entire current range in Africa were analyzed to investigate the extent of genetic variation and differentiation between populations. We observed high nucleotide diversity at the control region in the total sample (6.3%) but within populations, it varied considerably ranging from 1.6% to 8.1%. Population pairwise genetic differentiation was generally significantly high (ranging from $F_{ST} = 0.15$, $P < 0.01$ to $F_{ST} = 0.54$, $P < 0.001$). In the total sample, 29 and 12 haplotypes were observed in the control region and the cytochrome *b* data sets respectively. For both data sets, the haplotypes cluster into three distinct clades (sequence divergence ranged from 6.0%–12.9% to 0.8%–1.0% for the control region and cytochrome *b* sequences, respectively) that do not correspond to sampling locations. Two of these clades are found in the same localities (Samburu and Marsabit), which represent the *O.beisa beisa* subspecies, whereas the last clade represents the fringe-eared oryx (*O. beisa callotis*). We interpret these findings in terms of an ancient hybridization and introgression between two formerly isolated taxa of *Oryx beisa*.

Introduction

Most species have their populations genetically subdivided to various levels. Depending on the degree of subdivision, the outcome after the mixing of formerly isolated populations may either result in introgression or in incipient speciation when the populations are sufficiently diverged. For this reason, the implications of mixing of two or more originally separate populations has recently attracted attention of both evolutionary and conservation biologists (e.g. Dowling and Secor 1997; Arnold et al. 1999; Stone 2000). Hybridization may be due to a low efficiency of the specific mating recognition system (Paterson 1993; Randler 2002). This illustrates incomplete sexual isolation, and may lead to considerable evolutionary changes (Grant and Grant 1994; Arnold et al. 1999), which indirectly have implications for species management and conservation programs. The mixing of genes from different species may alter evolutionary processes and

change the speed of speciation between these species (Grant and Grant 1994; Arnold 1997; Dowling and Secor 1997; Stone 2000). With introgression, such hybridization could be responsible for the decline or the extinction of numerous species.

In Africa, evidence of hybridization and introgression in natural populations has been detected in several large mammals like the kob (Birungi and Arctander 2000) and sable antelope (Pitra et al. 2002). In these species, genetically divergent lineages have been documented co-existing in the same geographical area with no observable morphological differences in individuals within the population. There is therefore considerable interest in population structure of taxa especially in areas of apparent overlap.

Hybridization has probably happened in several other taxa including the African Oryx. Based on external characteristics, these taxa have been placed at varying taxonomic levels with some of them being designated species while others in eastern Africa have subspecies status. Currently, the antelope genus Oryx contains four species, namely *O. gazella, O. dammah, O. leucoryx*, and *O. beisa*, of which the latter is divided into two subspecies *O. beisa beisa*, and *O. beisa callotis*, (Kingdon 2001). The East African Oryx (*Oryx beisa*), was formerly distributed in the Horn of Africa from the Red Sea littoral to Somalia and south to arid central Tanzania. Its most westerly limit being Jebel Lafon in Southern Sudan and Karamoja in North East Uganda (Kingdon 2001) (Figure 1). This Oryx is now limited to Kenya and Tanzania, and is conservation-dependent (East 1998). In Kenya it is found in the northern unprotected rangelands; and Marsabit, Samburu and Tsavo National parks. In Tanzania it is restricted to the north and north-East of the country in small to moderate numbers. Poaching and competition from livestock pose the biggest challenges to its survival. Effective protection against poaching and competition from livestock however occurs in only a few parts of the species current distribution (East 1998). Apart from populations in protected areas, there are no known efforts geared towards protection of populations outside protected areas. The Oryx in Kenya is particularly vulnerable since most of its remaining populations occur outside protected areas (East 1998).

Oryx beisa is classified into two subspecies based on external morphological characteristics (Kingdon 2001) as; (i) *Oryx beisa beisa* which has plain ears and is found North of River Tana, and (ii) *Oryx beisa callotis* (fringe-eared oryx), found south of River Tana. As a result there is no overlap in the distribution range of the two subspecies. *O. b. callotis* has at times been referred to as a subspecies of *Oryx gazella* (Gemsbok) which is a southern African antelope (Gatesy et al. 1992; Kingdon 2001). However, to date there is lack of substantive molecular data to validate this antelope group placement.

In this paper we used partial mtDNA control region and cytochrome *b* sequence data to investigate genetic diversity, genetic divergence and phylogenetic relationships in populations of *O. b. beisa* and *O. b. callotis* across their remaining geographical home range. In particular, we focus on how well defined the two groups are and whether there is evidence of gene flow among them.

Materials and methods

Sample collection

Samples were collected from Burko (BU, n = 19) and Monduli (MN, n = 16) forest reserves in Tanzania; Ziwani (ZI, n = 4) in Tsavo-East National Park; Marsabit (MAB, n = 3) and Samburu National Reserve (SA, n = 18) in Kenya (Figure 1). Samples were collected from skins (89%) and fresh droppings (11%). In the field, samples were stored in 25% Dimethyl-Sulfoxide (DMSO) (Amos and Hoelzel 1991) saturated with sodium chloride (NaCl) at ambient temperature, and at −80 °C in the laboratory.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted using the DNeasy protocol for animal tissues (QIAGEN) according to the manufacturer's instructions or by phenol/chloroform extraction after treatment with sodium dodecyl sulfate and proteinase K (Sambrook et al. 1989). There was no difference in the quality of DNA between droppings and tissues samples.

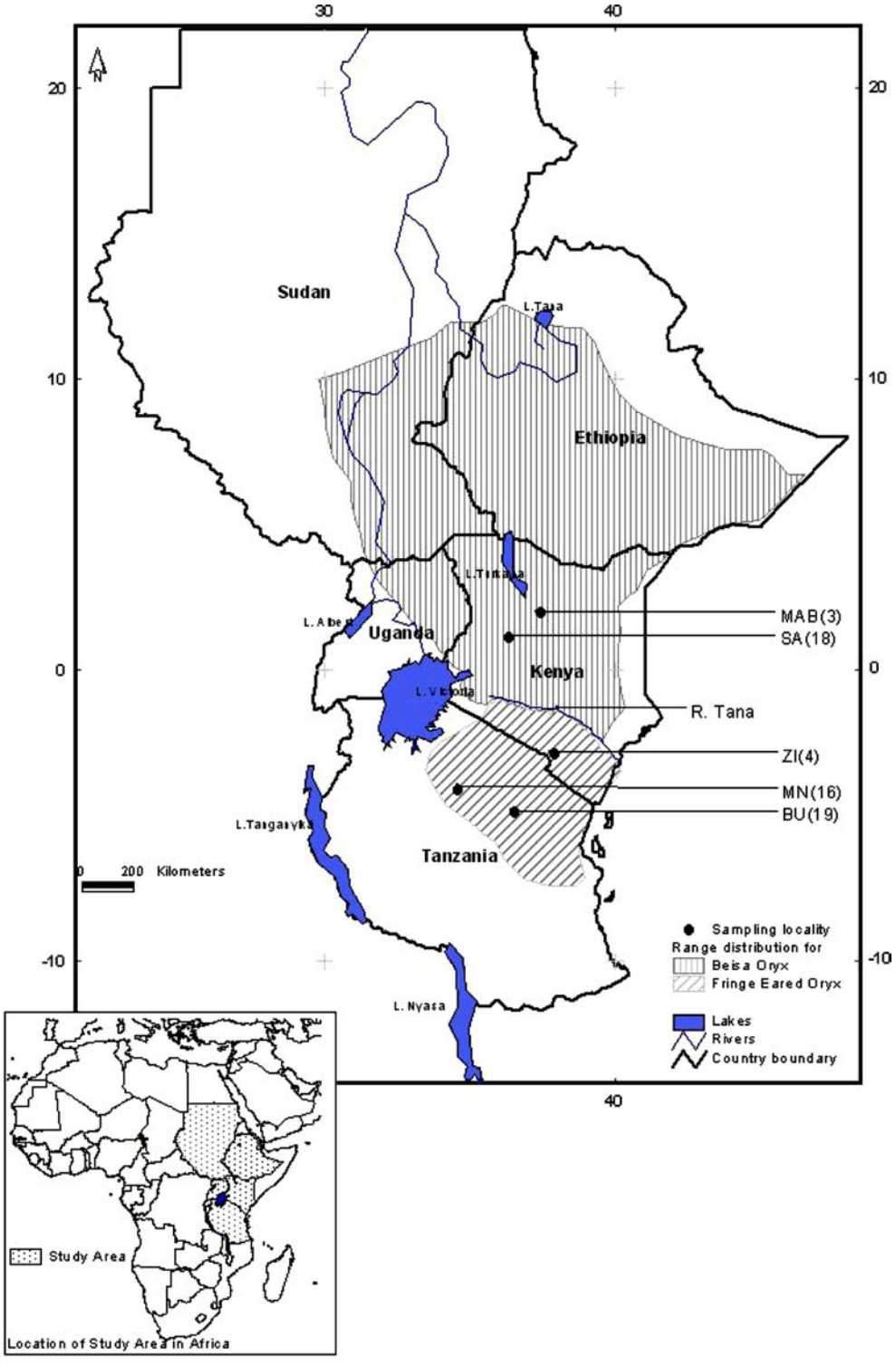

Figure 1. A Schematic map showing the original distribution of the East African oryx (*Oryx beisa*) and the sampling localities.

A fragment of the mtDNA control region (d-loop) was PCR amplified using the primer pair MT4 (Arnason et al. 1993) and B16168H (Simonsen et al. 1998). The PCRs were accomplished using the following conditions: one cycle of denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 40–60 s, and a final extension step at 72 °C for 10 min. Negative controls were included in each set of amplifications to check for contamination.

The PCR was performed in a 50 μl reaction volume containing approximately 2–5 ng of genomic DNA, 50 pmol of each primer, 1× PCR reaction buffer (Boehringer Mannheim GmbH), 50 pmol deoxynucleotide triphosphates (dNTPs), 14 μl of sterile double distilled water and 0.8 units of *Taq* DNA polymerase (Roche), using a Hybaid Express thermocycler. The double-stranded PCR product was purified using Qiaquick PCR purification columns (Qiagen). In all experiments, both heavy and light strands were sequenced following the dideoxy-chain termination method (Sanger et al. 1977) with the BigDye terminator cycle sequencing kit (Applied Biosystems) according to the manufacturer's instructions. The products were electrophoresed using 4% polyacrylamide gels on an ABI 377 (Applied Biosystems).

Preliminary analysis of mtDNA control region revealed 29 haplotypes clustering in three distinct clades. It is highly unlikely that sequences of particular individuals are similar at the control region and yet differ significantly at a coding gene such as cytochrome *b*. Therefore cytochrome *b* gene was amplified for representative individuals for each clade using primer pairs HL14725 and HH15149; HL14979 and HH15752; and HL15609 and HH15915 (Kocher et al. 1989; Irwin et al. 1991) to generate overlapping fragments (for primer pair sequences and reaction conditions see Table 1). Apart from the annealing temperature, similar parameters to those used in d-loop experiments were used for the cytochrome *b* gene.

Table 1. Primers and annealing temperatures used to amplify the different cytochrome *b* fragments in the East African Oryx

Name of primer	Sequence	Annealing temperature °C
HL14725	5′-TGATATGAAAAA CCATCGTTGT-3′	50
HH15149	5′-CTCAGAATGATA TTTGTCCTCA-3′	
HL14979	5′-GACGTCAACTA CGGCTGAAT-3′	50
HH15752	5′-GGTTGTCCTCCA ATTCATGT-3′	
HL15609	5′-CTACGATCCATCC CAAACAAACT-3′	50
HH15915	5′-CAGTACCTCCGG TTTACAAGAC-3′	

Names of primers indicate the heavy (H) or light (L) strand and the position of the 3′ end of the primer according to the numbering system of the human mitochondrial DNA sequence (Anderson et al. 1981).

Sequence analyses

Mitochondrial DNA control region sequences were analysed and aligned using programs Sequencher (Gene Codes), BioEdit (Hall 2005) and ClustalX (Thompson et al. 1997). Indels were introduced to minimise transversions. For the cytochrome *b* gene, the sequences after alignment were translated into amino acid sequences using the vertebrate mitochondrial genetic code as implemented in MEGA *version* 2.1 software (Kumar et al. 2001).

Genetic variation and population structure

The nucleotide diversity index (π) was calculated (Nei 1987, equation 10.5) to estimate within population genetic diversity using the computer program DnaSP *version* 4.0 (Rozas et al. 2003). Exact tests of population differentiation (based on segregating sites and haplotype frequencies assuming a null hypothesis of panmixia), population pairwise comparisons, and analysis of molecular variance (AMOVA: Excoffier et al. 1992) were estimated to infer population genetic structure using the program ARLEQUIN *version* 2.000 (Schneider et al. 2000). For the AMOVA analysis, populations were grouped into designated morphological subspecies according to Kingdon (2001).

Phylogenetic analyses

Phylogenetic relationships between haplotypes were estimated in two ways:

(a) We used the approach outlined by Huelsenbeck and Crandall (1997) to test alternative models of evolution, employing PAUP* 4.0b10 (PPC) (Swofford 2001) and MODELTEST 3.06 (Posada and Crandall 2001) using the hierarchical likelihood ratio test (hLRT) for the goodness of fit. This was then enforced to calculate maximum likelihood distances which were used to estimate an unrooted quartet puzzle tree using the computer program TREE-PUZZLE 5.2 (Schmidt et al. 2002). These analyses were done with indels included and when entire positions with indels excluded. Reliability of nodes defined by this tree was assessed using 10000 quartet-puzzling steps.

(b) Alternative models of evolution were estimated as in (a) above. Given the relatively slow rate of cytochrome *b* evolution compared to the control region, it was possible to align cytochrome *b* haplotypes with an outgroup. The Gemsbok sequence AF249973 (Matthee and Davis 2000) was used as outgroup. Consequently a rooted tree was estimated using the quartet puzzling method (Strimmer and Haeseler 1996) implemented in the computer program TREE-PUZZLE 5.2 (Schmidt et al. 2002) with 10000 steps under the maximum likelihood criterion.

Results

MtDNA control region sequence characteristics

Fifty-eight mtDNA control region sequences were generated, spanning a 423 base pair fragment of the left domain. A total of 140 segregating sites were observed in the total sample defining 29 haplotypes (Figure 2). Sequences of these haplotypes have been submitted to GenBank (accession numbers DQ138211-DQ138239). Haplotypes ZI01, BU02, BU04 and BU18 were shared between localities that fall within single subspecies ranges. The remaining haplotypes were singletons and subspecies specific (Figure 2).

For phylogenetic analysis, the best likelihood scores were obtained with the HKY + G model (Hasegawa et al. 1985). The parameters specified by MODELTEST were used for further analyses. The substitution rate matrix used was (A-C) = 1.000,

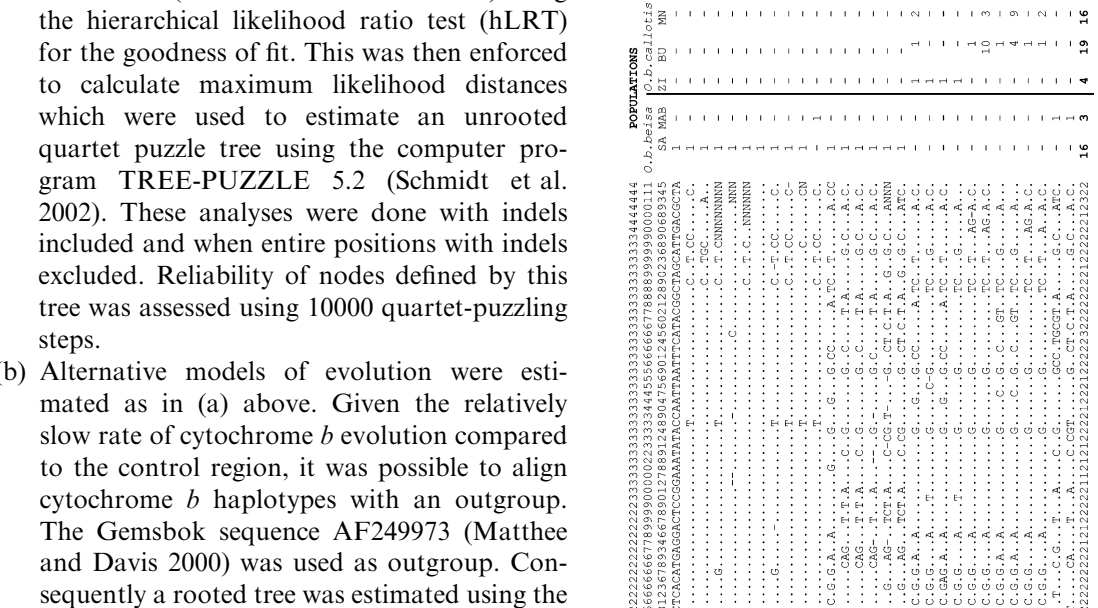

Figure 2. Distribution of the 29 observed d-loop haplotypes from a sample of 58 *Oryx beisa* sampled from Eastern Africa. The vertical numbers indicate the positions of the polymorphic sites relative to haplotype 1. A dash (–) represents an insertion/deletion introduced to optimise alignment. Haplotype names indicate the sampling localities as used in the text.

(A-G) = 13.424, (A-T) = 0.339, (C-G) = 0.339, (C-T) = 13.424, (G-T) = 1.000. Base frequencies were: A = 0.381, C = 0.233, G = 0.134, T = 0.253. A transition–transversion ratio of 9 was observed. A gamma distribution (Uzzell and Corbin 1971; Yang 1994) shape parameter, $\alpha = 0.677$ was observed. This result indicates that a relatively large proportion of the nucleotide sites are experiencing substitutions and therefore presence of phylogenetic signal.

Cytochrome b sequence characteristics

The fragments generated correspond to positions (*a*) 1–409, (*b*) 410–850, and (*c*) 851–1136 of the *Oryx gazella* sequence (AF249973; Matthee and Davis 2000). None of these sequences were found to include stop codons, and all exhibited a strong third position codon mutational bias in accordance with expectations for coding sequences. All sequences were translated into protein sequences and all of them yielded the expected cytochrome *b* amino acids. Collectively, this evidence suggests that none of the generated cytochrome *b* sequences was a nuclear pseudogene.

Eight individuals (SA07, ZI02, SA10, BU06, BU19, BU01, SA18, and SA30; Accession numbers DQ138192- DQ138199) amplified successfully for all the three fragments, while 19 amplified for fragments *a* and *c*. Subsequent analyses were done after joining fragments *a* and *c* for all individuals to provide a total of 666 bp. Nucleotide substitutions were observed at 25 (3.8%) of the 666 sites, the majority of which were transitions (23 sites, 3.5%). Five, three, and 17 substitutions were at first, second and third codon positions respectively (Figure 3). Only five substitutions resulted in amino acid changes (Figure 3). Twelve haplotypes were observed, and these were submitted in GenBank under accession numbers (DQ138200-DQ138210.)

MtDNA control region nucleotide diversity and differentiation

The overall nucleotide diversity was 6.3%, and varied greatly from as low as 1.6–8.1% in Burko and Samburu populations respectively (Table 2). Population differentiation tests based on mtDNA haplotype frequencies and segregating sites were highly significant for all pairwise comparisons as

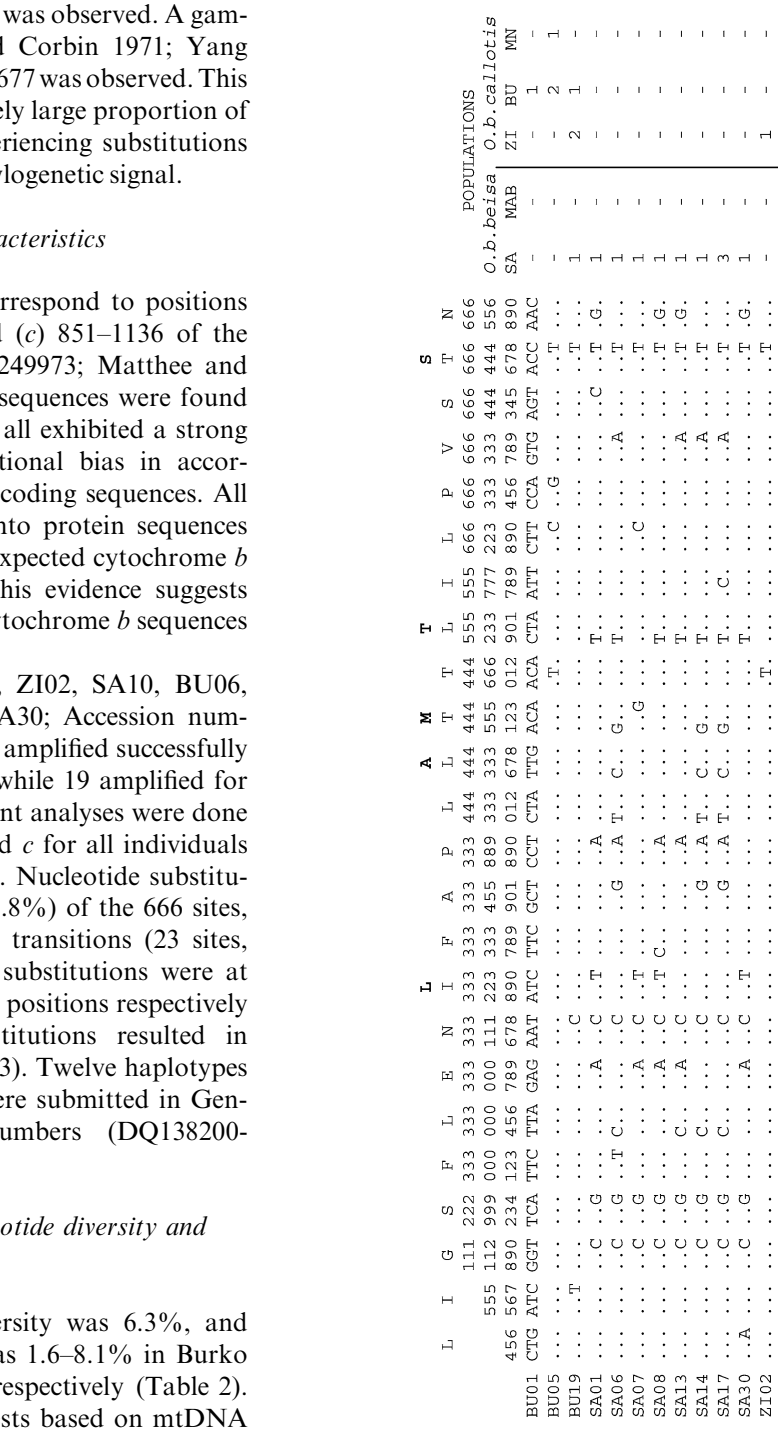

Figure 3. Alignment of partial cytochrome *b* haplotypes of *Oryx beisa* from eastern Africa. Positions where there are amino acid changes are shown by a super imposed amino acid.

Table 2. Pairwise population F_{ST} differentiation values and intra population nucleotide diversity on the diagonal. Population codes are as used in the text

	SA	ZI	BU	MN
SA	8.1			
ZI	0.36**	4.1		
BU	0.54***	0.37**	1.6	
MN	0.52***	0.27*	0.15*	1.9

* $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$.

shown in Table 2. Analysis of molecular variance revealed highly significant subdivision in the total sample ($F_{ST} = 0.589$, $P < 0.001$), among populations within subspecies ($F_{SC} = 0.078$, $P < 0.001$) and between the two subspecies ($F_{CT} = 0.554$, $P < 0.001$).

Phylogenetic relationships

MtDNA control region

The quartet puzzle analyses gave trees with the same topology. Figure 4 shows the phylogenetic relationships of the observed haplotypes. There are three distinct clades A, B and C comprised of haplotypes that coincide with sampling localities. With the exception of a single haplotype from Samburu (SA10), haplotypes sampled from 39 individuals in the range of *O.b. callotis* comprise a distinct lineage (Clade B). This clade comprises haplotypes sampled from Ziwani, Monduli and Burko which fall within the range of *O.b. callotis*. Clades A and C are comprised of haplotypes sampled exclusively from Marsabit and Samburu which fall in the range of *O.b. beisa*. Samburu and Marsabit were therefore found to consist of haplotypes falling in two distinct clades (Figure 4). One haplotype found in only one individual (SA10) sampled from *O.b. beisa* range clades with *O. b. callotis*. The net sequence divergence between A and C was 12.0%; B and C was 10.4% while that between A and B was 6.0%.

Cytochrome b

The best likelihood scores were obtained with the HKY + G model (Hasegawa et al. 1985). The substitution rate matrix used was (A-C) = 1.000, (A-G) = 18.665, (A-T) = 1.000, (C-G) = 1.000, (C-T) = 18.665, (G-T) = 1.000. The base frequencies were: A = 0.327, C = 0.289, G = 0.131, T = 0.254. A transition–transversion ratio of 17 and a gamma distribution shape parameter, $\alpha = 0.074$ were observed. Phylogenetic reconstruction revealed three clades (Figure 5) with the same topology as the d-loop analysis. Haplotype SA10 still groups with *O. b. callotis*. The existence of two Oryx lineages in the Samburu and Marsabit populations is still evident in the cytochrome *b* results (clades C and A in Figure 5).

Discussion

Phylogenetic relationships among the East African Oryx

Our phylogenetic analyses (Figures 4, 5) contradict some aspects of the current morphological subspecies groupings of the East African Oryx (see Kingdon 2001). They show that the East African Oryx comprises three genetically distinct and highly divergent groups. This is supported by both control region and cytochrome *b* sequence data. One of the groups (clade B) coincides with the geographical range of the subspecies, *O. b. callotis*. However, the other two groups co-occur in the same localities within the geographical range of one subspecies, *O. b. beisa*. There are several possible explanations for the co-occurrence of highly divergent clades in the same locality within a presumably single subspecies:

First, it could be evidence for the presence of Nuclear mitochondrial DNA sequences (Numts). Numts occur in many eukaryotes, as they have been reported in vertebrates, invertebrates, yeast, and plants (Tsuzuki et al. 1983; Louis and Haber 1991; Arctander 1995; Bensasson et al. 2001; Jensen-Seaman et al. 2004). Inadvertent PCR amplification of the nuclear copy of a mtDNA gene can confound phylogenetic interpretations if it is assumed that the amplified product is the authentic mitochondrial product (e.g. Jensen-Seaman et al. 2004). Although it cannot be ruled out, presence of Numts in this study is an unlikely explanation for co-existence of two clades because the cytochrome *b* sequence in this study translated into the expected amino acids without any stops. Besides both control region and cytochrome *b* data sets were concordant.

Second, it could indicate the presence of a previously unknown cryptic subspecies. This is,

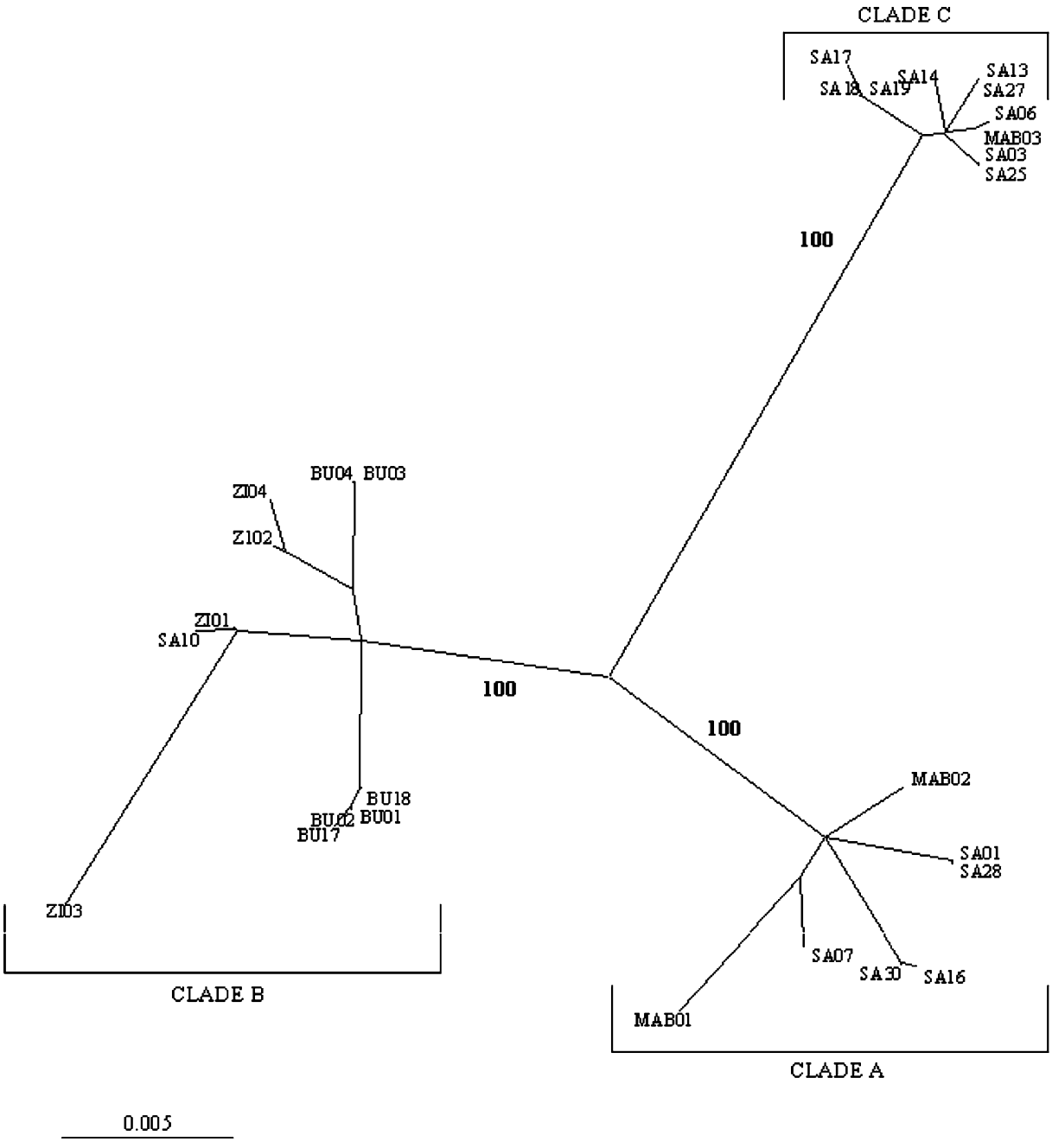

Figure 4. A Quartet puzzling tree of mtDNA control region haplotypes of the East African Oryx. Numbers along branches indicate the reliability of nodes after 10000 quartet-puzzling steps. Haplotype names indicate the sampling localities as used in the text.

however, an unlikely explanation in the Oryx because although cryptic species have been reported in many taxa such as fish (e.g. Gleeson et al. 1999), springsnails (e.g. Liu et al. 2003) and rotifera (e.g. Gomez et al. 2002), it has not been widely documented in large mammals. In addition, although morphological characters may be prone to convergent evolution, there are no field observations that have reported morphological variation in the Oryx from areas included in this study.

The third and probably a more likely explanation for the co-existence of two divergent

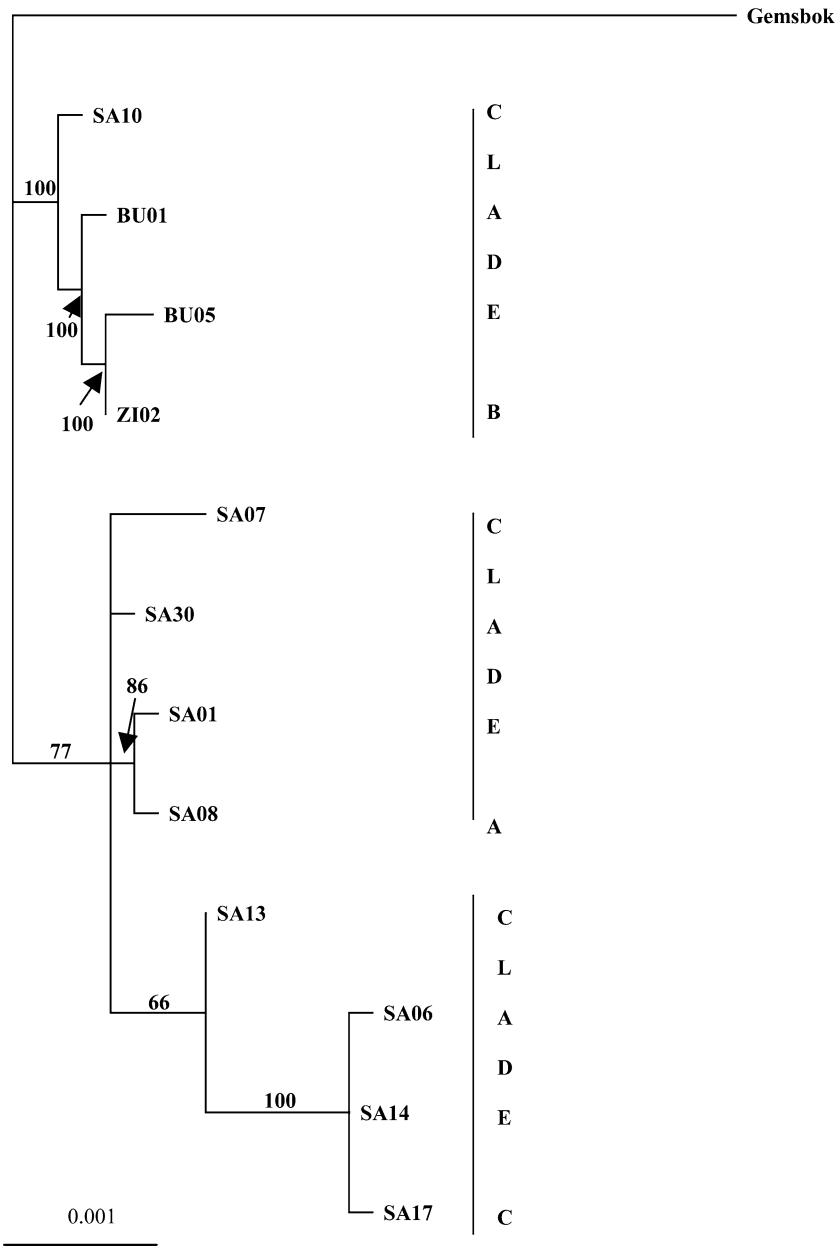

Figure 5. A Quartet puzzling tree of partial cytochrome *b* sequences of the East African Oryx. Haplotype names indicate the sampling localities as used in the text.

lineages in the same localities in a presumably same subspecies is introgressive hybridization. Fossil records show the First Appearance Date (FAD) of the Oryx lineages in the Shungura formation to be 2.52 Mya, in the Late Pliocene- early Pleistocene (Vrba 1995). Past climatic history, topography and environmental factors all interact and exert important influences on the geographical patterns and levels of observed genetic differentiation among species (Avise 1994). The Plio-Pleistocene climatic changes (Hamilton 1982; Bonnefile 1985; deMenocal 1995) could have shaped the phylogenetic relationships we observe today in the East African Oryx. One possible scenario is that

three Oryx taxa evolved in isolation, followed by secondary contact between two of them (clades A and C), either by range expansion or migration due to climatic fluctuations. The presence of two divergent lineages within *O.b.beisa* is suggestive of past introgressive hybridisation.

Patterns of diversity and population structure

The overall level of nucleotide diversity was high (6.3%), with the Samburu population standing out with the highest nucleotide diversity reported so far for this antelope (8.1%). This is due to the presence of two genetically divergent lineages co-existing in the Samburu National Reserve compared to the populations which have a single lineage (*O.b.callotis*) in Burko and Monduli populations. Generally, mitochondrial diversity in the East African Oryx is comparable to that reported among several closely related antelope species in Africa (e.g. Arctander et al. 1996; Birungi et al. 2000; Pitra et al. 2002) and other large mammals of Africa (e.g. Nyakaana et al. 2002; Muwanika et al. 2003). There is significant and strong differentiation between subspecies and a lower but still significant differentiation between the studied populations of the fringe-eared oryx.

Conservation implications

Empirical investigations of intraspecific outbreeding and subsequent introgressive hybridization in natural populations of large mammals are not common but a recent study by Pitra et al. (2002) reported such a phenomenon among conspecific populations of the Sable antelope *Hippotragus niger*. In order to reduce the risk of outbreeding depression, conservationists commonly agree to prevent mating between distantly related individuals from geographically distinct conspecific populations or subspecies (Arnold 1992; Roques et al. 2001). In contrast to this, our data (this study) and that of Pitra et al. (2002) suggest that intraspecific outbreeding actually happens in nature when individuals from distinct populations/lineages meet. Our data further indicates that crossing geographically distinct populations of a wild species seems to have beneficial effects by increasing genetic variability, although we do not know about possible detrimental effects on the reproductive fitness of the progeny caused by outbreeding depression when the two taxa originally met (but see Greig 1979; Coulson et al. 1999; Marshall and Spalton 2000; Pitra et al. 2002). There is no suggestion that this ancient introgressive hybridization has left any signs of outbreeding depression in *O. beisa* of today. Indeed a recent study suggests that there are no fixed chromosomal differences among the three species of Oryx occurring in East Africa, North Africa, and the Middle East (Kumamoto et al. 1999). Karyotypes of these taxa are indistinguishable, which reflects the more recent isolation of their populations.

Our mtDNA data further suggests the presence of two genetically distinct groups that coincide with subspecies' ranges in the East African Oryx. Although the concept of Evolutionarily Significant Unit (ESU; Ryder 1986) should be interpreted with caution (Moritz 1994), the sequence divergence indicates that the two groups have been historically isolated and may have distinct evolutionary potential. Therefore conservation efforts could be directed towards preserving genetic integrity of each group. We also observed significant differentiation among populations within the fringe-eared oryx subspecies (where this test was possible, Table 2) indicating limited dispersal. In the event of a local extinction, these results imply that it might be difficult for natural recolonization through migration to occur.

Acknowledgements

This work is part of a PhD study by Charles Masembe conducted under the Wildlife Genetics Project, a collaborative project between Makerere University Institute of Environment and Natural Resources (MUIENR), Uganda and the University of Copenhagen, Denmark. Financial support was provided by the Danish International Development Agency (DANIDA) and the Danish Natural Science Research Council. We would like to thank Wildlife authorities and Park staff from Kenya and Tanzania for their support in collecting samples. We are grateful to two anonymous reviewers for their comments on a previous version of the manuscript.

References

Amos W, Hoelzel AR (1991) Long-term preservation of whale skin for DNA analysis. In: *Genetic Ecology of Whales and Dolphins*. (ed. Hoelzel AR) Report of the International Whaling Commission. *Special issue*, **13**, 99–104.

Anderson S, Bankier AT, Barell BG, de Bruijn MH, Coulson AR, et al. (1981) Sequence and organisation of the human mitochondrial genome. *Nature* **290**, 457–465.

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

Arctander P, Kat P, Simonsen BT, Siegismund HR (1996) Population genetics of Kenyan Impalas—Consequences for conservation. In: *Molecular Genetics in Conservation* (eds. Smith TB, Wayne RK), pp. 399–412. Oxford University Press, Oxford.

Arnason U, Gullberg A, Johnsson AE, Ledje C (1993) The nucleotide sequence of the mitochondrial DNA molecule of the grey seal, *Halichoerus grypus*, and a comparison with mitochondrial sequences of other true seals. *J. Mol. Evol.*, **37**, 323–330.

Arnold ML (1992) Natural hybridization as an evolutionary process. *Annu. Rev. Ecol. Syst.*, **23**, 237–261.

Arnold ML (1997) Natural Hybridization and Evolution. Oxford.

Arnold ML, Bulger MR, Burke JM, Hempel AL, Williams JH (1999) Natural hybridization: how low can you go and still be important?. *Ecology*, **80**, 371–381.

Avise JC (1994) *Molecular Markers, Natural History and Evolution*, Chapman and Hall, New York, London.

Bensasson D, Zhang DX, Hartl DL, Hewitt GM (2001) A census of mitochondrial pseudogenes: molecular trouble-makers or helpful witnesses from the past. *Trends Ecol. Evol.*, **16**, 314–321.

Birungi J, Arctander P (2000) Large sequence divergence of mitochondrial DNA genotypes of the control region within populations of the African antelope, kob (*Kobus kob*). *Mol. Ecol.*, **9**, 1997–2008.

Bonnefile R (1985) Evolution of continental vegetation: The palaeobotanical record from East Africa. *Suid Afrikaanse Tydskrif vir Natuurwetenskap en Tegnologie*, **81**, 267–270.

Coulson TN, Albon SD, Slate J, Pemberton JM (1999) Microsatellite loci reveal sex-dependent responses to inbreeding and outbreeding in red deer calves. *Evolution*, **53**, 11960–19512.

deMenocal BP (1995) Plio-Pleistocene African climate. *Science*, **270**, 53–58.

Dowling TE, Secor CL (1997) The role of hybridization and introgression in the diversification of animals. *Annu. Rev. Ecol. Syst.*, **28**, 593–619.

East R (1998) African antelope database 1998. IUCN/SSC Antelope Specialist Group Report.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application of human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Gatsey J, Yelon D, DeSalle R, Vrba E (1992) Phylogeny of the Bovidae (Artiodactyla, Mammalia), based on mitochondrial ribosomal DNA sequences. *Mol. Biol. Evol.*, **9**, 433–446.

Gleeson DM, Howitt RLJ, Ling N (1999) Genetic variation, population structure and cryptic species within the black mudfish, *Neochanna diversus*, and endemic galaxiid from New Zealand. *Mol. Ecol.*, **8**, 47–57.

Gomez A, Serra M, Carvalho GR, Lunt DH (2002) Speciation in ancient cryptic species complexes: evidence from the molecular phylogeny of *Brachionus plicatilis* (Rotifera). *Evolution*, **56**, 1431–1444.

Grant PR, Grant BR (1994) Hybridization of bird species. *Science*, **256**, 193–197.

Greig JC (1979) Principles of genetic conservation in relation to wildlife management in Southern Africa. *S. A. J. W. Res.*, **9**, 57–78.

Hall TA (2005) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Ibis Therapeutics, A division of Isis Pharmaceuticals, 1891 Rutherford Road, Carlsbad, CA 92008, 760–603–2375.

Hamilton AC (1982) *Environmental History of East Africa: a Study of the Quaternary*, Academic Press, London.

Hasegawa M, Kishino K, Yano T (1985) Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.*, **22**, 160–174.

Huelsenbeck JP, Crandall KA (1997) Phylogeny estimation and hypothesis testing using maximum likelihood. *Annu. Rev. Ecol. Syst.*, **28**, 437–466.

Irwin DM, Kocher TD, Wilson AC (1991) Evolution of the cytochrome b gene of mammals. *J. Mol. Evol.*, **32**, 128–144.

Jensen-Seaman M, Sarmiento IEE, Deinard AS, Kidd KK (2004) Nuclear Integrations of Mitochondrial DNA in Gorillas. *Am. J. Primatol.*, **63**, 139–147.

Kingdon J (2001) *The Kingdon Field Guide to African Mammals*, Academic Press, San Diego.

Kocher TD, Thomas WK, Meyer A, et al. (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA.*, **86**, 6196–6200.

Kumamoto AT, Charter SJ, Kingswood SC, Ryder OA, DS Gallagher DS (1999) Centric fusion differences among *Oryx dammah*, *O. gazella*, and *O. leucoryx* (Artiodactyla, Bovidae). *Cytogenet. Cell. Genet.*, **86**, 74–80.

Kumar S, Tamura K, Jakobsen IB, Nei M (2001) *MEGA2: Molecular Evolutionary Genetics Analysis Software*, Arizona State University, Tempe, Arizona, USA.

Liu PH, Hershler R, Clift K (2003) Mitochondrial DNA sequences reveal extensive cryptic diversity within a western American springsnail. *Mol. Ecol.*, **12**, 2771–2782.

Louis EJ, Haber JE (1991) Evolutionary recent transfer of a group I mitochondrial intron to telomere regions in *Saccharomyces cerevisiae*. *Curr. Genet.*, **20**, 411–415.

Marshall TC, Spalton JA (2000) Simultaneous inbreeding and outbreeding depression in reintroduced Arabian oryx. *Anim. Cons.*, **3**, 241–248.

Matthee CA, Davis SK (2000) Molecular insights into the evolution of the family Bovidae: a nuclear DNA perspective. *Mol. Biol. Evol.*, **18**, 1220–1230.

Moritz C (1994) Applications of mitochondrial-DNA analysis in conservation – a critical review. *Mol. Ecol.*, **3**, 401–411.

Muwanika VB, Nyakaana S, Siegismund HR, Arctander P (2003) Phylogeography and population structure of the common warthog (Phacochoerus africanus) inferred from variation in mitochondrial DNA sequences and microsatellite loci. *Heredity*, **91**, 361–372.

Nei M (1987) *Molecular Evolutionary Genetics*, Columbia University Press, New York.

Nyakaana S, Arctander P, Siegismund HR (2002) Population structure of the African savannah elephant inferred from mitochondrial control region sequences and nuclear microsatellite loci. *Heredity*, **89**, 90–98.

Paterson HEH (1993) *Evolution and the Recognition Concept of Species: Collected writings*, Johns Hopkins University Press, Baltimore, MD.

Pitra C, Hansen AJ, Lieckfeldt D, Arctander P (2002) An exceptional case of historical outbreeding in African sable antelope populations. *Mol. Ecol.*, **11**, 1197–1208.

Posada D, Crandall KA (2001) Selecting the best-fit model of nucleotide substitution. *Syst. Biol.*, **50**, 580–601.

Randler C (2002) Avian hybridization, mixed pairing and female choice. *Anim. Behav.*, **63**, 103–119.

Roques S, Sevigny JM, Bernatchez L (2001) Evidence for broadscale introgressive hybridization between two redfish (genus Sebastes) in the North-west Atlantic: a rare marine example. *Mol. Ecol.*, **10**, 149–165.

Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, **19**, 2496–2497.

Ryder OA (1986) Species conservation and systematics: the dilemma of subspecies. *TREE*, **1**, 9–10.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.*, **74**, 5463–5467.

Schmidt HA, Strimmer K, Vingron M, von Haeseler A (2002) TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. *Bioinformatics*, **18**, 502–504.

Schneider S, Roessli D, Excoffier L (2000) ARLEQUIN version 2.0: A software for population genetic data analysis. Genetics and Biometry Laboratory. University of Geneva.

Simonsen BT, Siegismund HR, Arctander P (1998) Population structure of African buffalo inferred from mtDNA sequences and microsatellite loci: high variation but low differentiation. *Mol. Ecol.*, **7**, 225–237.

Stone G (2000) Phylogeography, hybridization and speciation. *Trends Ecol. Evol.*, **15**, 354–355.

Strimmer K, Haeseler AV (1996) Quartet Puzzling: A Quartet Maximum-Likelihood Method for Reconstructing Tree Topologies. *Mol. Biol. Evol.*, **13**, 964–969.

Swofford DL (2001) *PAUP* 4.0b10 (PPC) – Phylogenetic Analysis Using Parsimony (*and Other Methods)*, Sinauer Associates, Sunderland, MA.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acids Res.*, **24**, 4876–4882.

Tsuzuki T, Nomiyama H, Setoyama C, Maeda S, Shimada K (1983) Presence of mitochondrial-DNA-like sequences in the human nuclear DNA. *Gene*, **25**, 223–229.

Uzzell T, Corbin KW (1971) Fitting discrete probability distributions to evolutionary events. *Science*, **172**, 1089–1096.

Vrba ES (1995) The fossil records of African antelopes (Mammalia, Bovidae) in relation to human evolution and paleoclimate In: *Paleoclimate and Evolution, with Emphasis on Human Origins* (eds. Vrba ES, Denton GH, Patridge TC, Burckle LH), pp. 385–424. Yale University Press, NewHaven Conn.

Yang Z, Goldman N, Friday A (1994) Comparison of models for nucleotide substitution used in maximum-likelihood phylogenetic estimation. *Mol. Biol. Evol.*, **7**, 1081–1090.