Molecular phylogeography and evolutionary history of the greater kudu (*Tragelaphus strepsiceros*)

by

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DECLARATION

I declare that the thesis, which I hereby submit for the degree of Doctor of Philosophy (Zoology) at the University of Pretoria is my own work and has not previously been submitted by me for a degree at another university.

James Shikuku Sakwa

October, 2001.

SUMMARY

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The greater kudu (*Tragelaphus strepsiceros*) is a large spiral-horned antelope that occurs in sub-Saharan Africa. The species is predominantly a browser and inhabits a diverse range of habitats including savanna woodland, scrub and open forests. The geographical distribution extends from south-eastern Chad, northern Central African Republic (CAR), through eastern Africa, to southern Africa. Throughout its range the species is threatened by habitat loss, fragmentation, diseases and hunting for trophy. Consequently, many populations have reduced numbers and are at great risk of local extinction.

In the absence of evidence from comprehensive studies, strategies for conservation and management of many species are often based on subspecies designations despite the fact that the original descriptions were based on few samples and morphological characters that vary extensively. To develop appropriate conservation and management measures, it is imperative to obtain information on population structure, historical demography and evolutionary history of the species. The information generated is used to define units for conservation of the species. In this study, the objective was to investigate population structure and evolutionary history of the greater kudu by analysing mitochondrial DNA (mtDNA) control region sequences and examining size variation in eight microsatellite loci. The mtDNA control region sequences were examined using a combined approach that included phylogeographic, nested clade and mismatch frequency distribution analyses. It was anticipated that use

of the two types of genetic markers with contrasting patterns of inheritance and mutation would enhance the understanding and interpretation of the evolutionary history of the species throughout its range. The results were used to evaluate subspecies taxonomy, draw inferences on historical demography and provide information relevant for conservation and management of *T. strepsiceros*.

Intraspecific variation in the mtDNA was examined in 94 samples from 12 locations and revealed low to medium levels of nucleotide diversity. The average nucleotide diversity was 2.7% (0.3% to 2.9%). The average sequence divergence between populations was 2.3% (0.0% to 5.7%). Eight microsatellite loci were analysed in 203 samples representing 13 locations. The number of alleles scored from these loci was 7-12 while the mean heterozygosity was 70.4% (66% to 76%). Microsatellite data showed shallow phylogeographic structure and the average measure of genetic differentiation Φ_{ST} was 0.046. Comparisons of allelic variation across all populations revealed that the Eastern Cape had lower allelic diversity and showed significant differences in allele frequency distribution suggesting a genetic bottleneck in the population's evolutionary past.

The combined analyses suggest that the greater kudu originated from Namibia and spread southwards before colonising other parts of its modern range. The results revealed weak geographic partitioning at the regional level, but showed two genetically distinct groups at the continental level. The first group comprised of populations from Namibia, Kimberley and the Eastern Cape from South Africa, while the second comprised of the remaining populations. The results suggest a single evolutionary significant unit (ESU) with two management units (MUs). In the long term, conservation efforts should focus on maintaining demographic connectivity over broad geographical areas within each MU in order to approximate the natural dispersal patterns of the species.

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LIST OF ABBREVIATIONS

AMOVA	Analysis of molecular variance
bp	base pairs (nucleotides)
BP	Before present
d.f.	Degrees of freedom
D-loop	Displacement loop found in the mtDNA control region
dNTPS	Deoxynucleotide triphosphates
DTT	Dithiothreitol
EDTA	Ethylenediaminetetra-acetic acid
F_{ST}	Wright's measure of population genetic differentiation
HCl	Hydrochloric acid
H_E	Expected heterozygosity
HKY85	Hasegawa-Kishino-Yano (1985) model of DNA substitution
H_O	Observed heterozygosity
HWE	Hardy-Weinberg Equilibrium
IAM	Infinite alleles model of mutation
IUCN	International union for conservation of nature
kb	Kilobase pairs
LIS	Low ionic strength buffer
$MgCl_2$	Magnesium chloride
MHC	Major histocompatibility complex
mtDNA	Mitochondrial DNA
Mya	Million years ago
NaCl/DMSO	Sodium chloride/Dimethyl sulphur dioxide
N_E	Effective population size
$N_{E(F)}$	Effective population size in females
ng	Nanogram
PAGE	Polyacrylamide gel electrophoresis
PAUP	Phylogenetic analysis using parsimony
PCR	Polymerase chain reaction
PHYLIP	Phylogenetic inference package
rRNA	Ribosomal RNA
SCI	Safari Club International
SMM	Stepwise model of mutation
TAE	Tris-acetic-acid-EDTA buffer

TBE	Tris-boric acid-EDTA buffer
TE	Tris-EDTA buffer
TPM	Two-phase model of mutation
tRNA	Transfer RNA

CHAPTER 1

General Introduction

1.1 Background

The greater kudu (*Tragelaphus strepsiceros*) is a large spiral-horned antelope endemic to sub-Saharan Africa. The species exhibits sexual dimorphism where males stand 1.95-2.45 meters high and weigh 190-315 kg, while females are 1.80-2.35 meters high and weigh 120-215 kg (Kingdon 1982). Social organisation is based on the female unit, where herds are small (approximately 10 individuals) and consists of several adult females and their offspring (Allen-Rowlandson 1980). Sexual maturity in males and females occurs after two years and young males leave maternal units to join loosely formed bachelor groups. Adult males are mostly solitary however, during the mating season they form loose associations with female groups. The greater kudu are not territorial but have separate home ranges for males and females. Maternal home ranges are about four square kilometres in size; male home ranges are approximately 11 square kilometres, are known to overlap, and include home ranges of several maternal groups (Allen-Rowlandson 1980). Mating occurs during the dry season and females return to the same refuge every dry season (Kingdon 1982). The greater kudu are predominantly browsers (Wilson 1965) and are a highly adaptable species capable of utilising a diverse range of habitats (Allen-Rowlandson 1980). They are found in savanna woodland, scrub and open forests where they prefer hilly terrain. They also occur in semi-arid zones where they are confined to thickets along water courses (Smithers 1983). In captivity, greater kudu have been known to live for up to 20 years (Jones 1982).

1.2 Geographical distribution

The geographical range of the greater kudu is sub-Saharan and extends from south-eastern Chad, northern Central African Republic (CAR), through southern Sudan, Ethiopia, eastern Africa, to southern Africa (reviewed in Ansell 1971). Although the distribution is fairly continuous, isolated populations are found in Kimberley and the Eastern Cape in South Africa (Skinner & Smithers 1990) and south-eastern Chad, northern CAR and Sudan (East 1996) (Fig. 1).

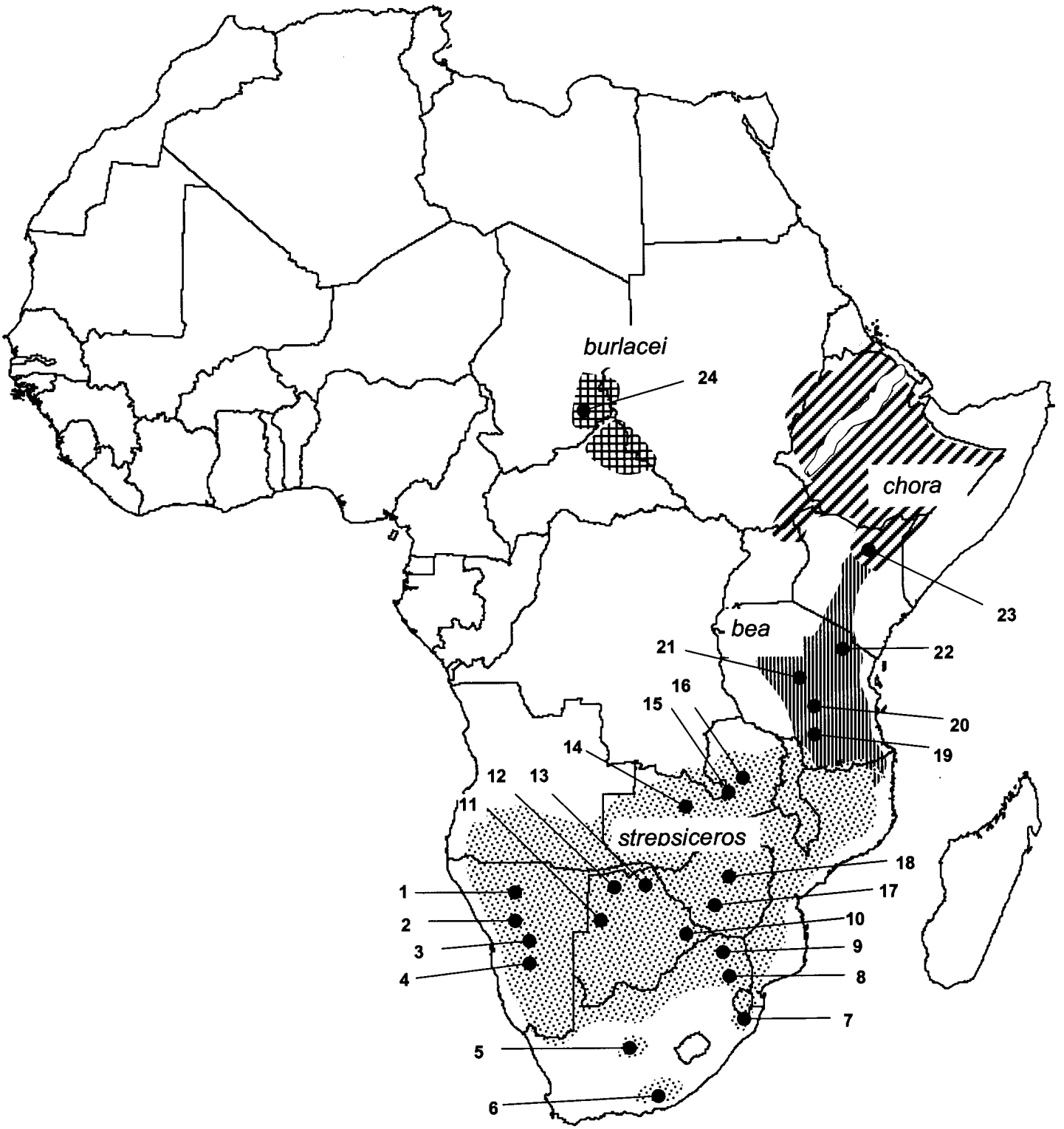


Fig. 1. Geographic distribution of the four subspecies in the greater kudu (*Tragelaphus strepsiceros*) indicated with different shading patterns (Ansell 1971). Numbers indicate the locality of samples used in this study and correspond to those shown in Table 1.

1.3 Fossil record

The greater kudu are relatively well represented in the fossil record compared to other African bovid species. The earliest appearance of greater kudu fossils date from the lower Pleistocene, approximately two Mya (Gentry 1978). The fossil record is based on measurements from horn cores and occurs at several sites across Africa. The sites include Olduvai Gorge (Leakey 1965) and Peninj in Tanzania (Gentry 1978), Koobi Fora from East Turkana Kenya (Harris 1976), Makapansgat Limeworks in South Africa (Gentry 1978) and Shungura and Mursi Formations in Omo Ethiopia (Gentry 1978). Paleontological data from Olduvai Gorge and Makapansgat Limeworks indicate that the fossil forms from these sites were larger than extant greater kudu and may have belonged to a different subspecies or species. The record from Shungura and Mursi in Omo Ethiopia show that the fossil forms were smaller than present day greater kudu. These differences may reflect adaptation to environments that were markedly different from present.

The widespread occurrence of greater kudu fossils roughly corresponds to current geographic distribution, particularly from north-eastern to southern Africa. The time of appearance of the fossils coincide with a period of rapid speciation in bovid evolution as suggested by Vrba (1985). This period was characterised by alternating moist and dry conditions, which induced changing patterns of vegetation types and may have influenced, in particular, the spread of the savanna vegetation in sub-Saharan Africa. The expansion and contraction of savanna vegetation type may have ultimately influenced the origin and subsequent expansion of greater kudu populations throughout its range.

Although the current geographical distribution of the greater kudu does not extend to north Africa, an earlier but unconfirmed fossil record dating from the upper Pliocene (approximately three million years ago) was found at Mansoura in Algeria (Gentry 1978). The greater kudu have, however, not been recorded from later paleontological deposits in northern Africa.

1.4 Taxonomy

The greater kudu belongs to the tribe *Tragelaphini* of the subfamily *Bovinae*. The tribe consists of nine extant species in three genera, *Tragelaphus, Taurotragus* and *Boocerus.* The genus *Tragelaphus* comprises of the bushbuck (*T. scriptus*), greater kudu (*T. strepsiceros*), lesser kudu (*T. imberbis*), mountain nyala (*T. buxtoni*), nyala (*T. angasii*) and sitatunga (*T. spekii*). *Taurotragus* includes the common eland (*Taurotragus oryx*) and derby eland (*Taurotragus derbianus*), while the monotypic *Boocerus* is represented by the bongo, *Boocerus euryceros* (Ansell 1971). The validity of these genera has been questioned by Van Gelder (1977) who proposed a single genus *Tragelaphus* based on the evidence of hybridisation between the bongo and sitatunga (Tijskens 1968), and between the greater kudu and eland (Jorge, Burtler & Benirschke 1976). Several studies based on fossil record (Vrba 1987), allozymes (Georgiadis 1990) and cytochome b sequence variation (Matthee & Robinson 1999a) concur with the suggestion of a single genus.

Within *T. strepsiceros* there is no consensus on the number of subspecies with criteria such as the number of stripes, colour and horn length in males underpinning the taxonomy. Ansell (1971) noted that populations found in the north of the range (particularly in Chad) are pale coloured, have smaller horns and fewer stripes (up to 5) on the back, while populations found in the south of the range have darker colour, longer horns and more stripes (up to 12).

Haltenorth & Diller (1980) and Wilson & Reeder (1993) recognised one species (*T. strepsiceros*) subsuming all previous subspecies. Kingdon (1997) on the other hand accepted three subspecies; *T. s. strepsiceros* for east and southern Africa, *T. s. chora* for north-east Africa, including northern Kenya and *T. s. cottoni* for Chad, CAR and Sudan. Other classifications include Ansell (1971) with four subspecies (*T. s strepsiceros* found in southern Africa, *T. s. bea* in east Africa, *T. s. chora* in north-east Africa and *T. s. burlacei* in Chad, CAR and Sudan), and the SCI (1997) with five subspecies which are essentially similar to those described by Ansell (1971) but with the elevation of the population from the Eastern Cape to subspecies status. Individuals in this population are morphologically different from the other greater kudu in the subregion. They have few stripes on the back, are pale coloured and are on average small in size (SCI 1997). It is worth noting that the population from the Eastern Cape

has historically been isolated and the observed differences may reflect founder effects and adaptation to local conditions.

The morphological characters used to describe the subspecies exhibit extensive variation even among individuals within a subspecies (Ansell 1971) and are therefore not reliable indicators of diversity among greater kudu populations. It is therefore difficult to define the geographical limits or the intergrading zones of these geographic forms. In this study, the greater kudu are assumed to belong to *T. strepsiceros*, thus subsuming previous subspecies as described by Haltenorth & Diller (1980) and Wilson & Reeder (1993).

1.5 Phylogeography and management of populations

In the absence of evidence from phylogeographic studies, conservation and management strategies for many species are generally based on subspecies descriptions despite the fact that these classifications were based on few samples, limited geographic sampling and morphological characters that exhibit extensive individual variation (Avise et al. 1987). In order to develop appropriate conservation and management measures for the species, it is imperative to obtain information on population structure, geographic partitioning of genetic variation and evolutionary history of the species (Ryder 1986, Avise et al. 1987, Moritz 1994a). The information generated is used to define units of conservation within the species (Milligan et al. 1994). According to Moritz (1994b), long term conservation and management requires identification of evolutionarily significant units (ESUs), described as sets of populations distinguished by strong phylogenetic structuring of mtDNA variation (reciprocal monophyly of haplotypes) and significant divergence of nuclear alleles. Evolutionary significant units consist of historically isolated and thus independently evolving sets of populations with evolutionary potential for unique adaptive divergence (Moritz 1999). Movement of individuals between ESUs should be discouraged in order to avoid mixing populations with separate evolutionary heritage. In the short term, conservation and management strategies would include identifying management units (MUs), which are characterised by low levels of gene flow, and are described as sets of populations with significant divergence of allele frequencies within nuclear or mitochondrial DNA (Moritz 1994b). Correct identification of ESUs or MUs within a species depends upon use of a sampling design that covers the entire range of the species, and use of an adequate

number of nucleotides in the case of mtDNA and sufficient number of nuclear loci (Moritz 1994b).

1.6 Conservation status

The greater kudu form an important part of the game ranching industry and are hunted primarily for trophy as well as for meat and hides. Of the eighteen sub-Saharan countries in which the greater kudu occur (Fig. 1), hunting is permitted in sixteen of them (SCI 1997). Because of their substantial commercial value, and the increasing fragmentation of habitat, many populations have reduced numbers and are at greater risk of local extinction (Wade & McCauley 1988). In southern Africa, greater kudu have disappeared from parts of KwaZulu-Natal, eastern parts of the Northern Cape province and the Orange River valley. In southern Botswana where they occur, the greater kudu are considered uncommon (Smithers 1983) while in eastern Africa, they have almost disappeared from northern-eastern Uganda, parts of southern Sudan (SCI 1997) and have almost disappeared from Somalia (Ansell 1971). Apart from loss of habitat, fragmentation and human persecution, diseases have also impacted negatively on the size of greater kudu populations. During the later part of the 19th century, a severe outbreak of rinderpest adversely affected the status of populations throughout the range (Plowright 1982). The effects of subsequent epidemics in the 20th century were less severe, and some populations particularly in Kenya and Somalia have not yet recovered (Stuart & Stuart 1997). Although many greater kudu populations are under threat, the overall status throughout the range is considered satisfactory (East 1998). According to the IUCN (1996), the greater kudu is classified as a species under the lower risk category whose continued survival depends upon active conservation measures.

1.7 Choice of genetic markers

Two types of genetic markers with contrasting modes of inheritance and mutation were used in this investigation. Mitochondrial DNA (mtDNA) control region sequences and size variation at microsatellite loci were used to assess genetic variation in the greater kudu. Mitochondrial DNA is the most commonly used genetic marker for assessing phylogenetic relationships among closely related species and among closely related populations of the same species (Avise & Lansman 1983, Avise 1994, Smith & Wayne 1996). In animals, the mtDNA is a closed circular molecule of approximately 15-20 kb

in length. The molecule consists of 13 protein coding genes, 22 tRNA genes, 2 rRNA genes and a non-coding segment called the control region.

There are several reasons why mtDNA is commonly used in population genetics and molecular systematics studies. In vertebrates, the molecule is haploid and does not undergo recombination, is transmitted maternally and evolves 5-10 times faster than single copy nuclear genes (Hutchinson et al. 1974, Brown et al. 1979, Avise 1994, Smith & Wayne 1996). Within the mtDNA molecule, the control region has been particularly useful in phylogeographic studies because the region consists of sequence blocks that mutate 4-5 times faster than the entire mtDNA molecule (Brown et al. 1993). The mtDNA control region has therefore proven to be an effective marker for examining genetic variability at the intraspecific level (reviewed in Avise 1994). In bovid species, the control region sequences have been used to assess genetic variation in several species including cattle (*Bos sp.*) (Loftus et al. 1994), Grant's gazelle (*Gazella granti*) (Arctander et al. 1996a), impala (*Aepiceros melampus*) (Arctander et al. 1996b), buffalo (*Syncerus caffer*) (Simonsen et al. 1998), roan (*Hippotragus equinus*) and sable (*H. niger*) (Matthee & Robinson 1999b), and hartebeest (*Alcelaphus buselaphus*), topi (*Damaliscus lunatus*) and wildebeest (*Connochaetes taurinus*) (Arctander et al. 1999).

The main limitation of using mtDNA in phylogeographic studies is that the molecule is maternally inherited, therefore interpretation of the results reflect evolutionary processes that influence maternal lineages. For species where males disperse more than females, a complete picture of the genetic structure may be obtained by screening nuclear genes, which are bi-parentally inherited.

Two bi-parentally inherited markers commonly used in phylogeographic studies are the major histocompatibility complex (MHC) and microsatellite loci (reviewed in Smith & Wayne 1996). The MHC is one of three multigene families contained within the immunoglobin superfamily in metazoans (Klein 1986). Studies have shown that MHC genes play an important role in immune response to foreign pathogens and exhibit extraordinary allelic diversity (Klein 1987). In mammals the MHC is approximately 3500 kb long and consists of several hundred genes and the most frequently studied genes are found in class I and class II of the MHC (Klein 1987). Despite the high allelic diversity in the MHC, there is mounting evidence which shows that frequency

dependent selection have a significant effect on the molecular evolution of the mammalian MHC loci (Klein 1987). This presents a major drawback in the use of MHC loci in phylogeographic studies (Klein et al. 1993).

Microsatellites are short tandemly repeated sequence motifs that consist of repeat units 1-6 base pairs in length (Hamada et al. 1982). They are widely distributed in the eukaryotic nuclear genome, occurring approximately every 100 kb (Weber 1990). In mammals, the most common microsatellite motif is GT/CA, which occur approximately every 30 kb (Tautz & Renz 1984). Microsatellites are often highly polymorphic due to variation in the number of repeat units (Litt & Luyt 1989, Tautz 1989, Weber & May 1989) and variation among alleles is due to a gain or loss of a repeat unit. The changes in repeat unit is caused by an intramolecular mutation mechanism called DNA slippage (Schlötterer & Tautz 1992) and the most common mutation changes are single repeat units.

In order to understand the distribution and extent of the observed microsatellite length variation, several theoretical models are commonly used. Kimura & Crow (1964) proposed the infinite alleles model (IAM) and according to the model the number of possible alleles at a locus is enormous, making every new allele unique. Out of the infinite number of possible alleles, it is unlikely that a new allele will mutate to a state that is already present in the population. The expectations under the IAM model were verified by several empirical studies that looked at the distribution of allele frequencies at protein loci (Kimura 1968, Ohta 1976, Chakraborty et al. 1980). The second theoretical model is the stepwise mutation model (SMM). This model was introduced by Ohta and Kimura (1973) who noted that many protein loci had one frequent allele and the remaining alleles were distributed roughly in a symmetrical manner on either side of the frequent allele. The main difference between SMM and IAM is the assumption that there are only two adjacent states that an allele can mutate to in a single step as opposed to infinite number under IAM expectations. Under SMM, the evolutionary divergence between alleles is proportional to the number of mutational steps separating them (MacHugh 1994). The third mutation model is the two-phase mutation model (TPM), which was proposed by Direnzo et al. (1994). This model incorporates the mutational process of the SMM, but uses coalescence theory to predict the expected variance in repeat number under different mutational processes

and demographic histories. Of the three models, SMM is most widely used to describe microsatellite mutation.

Microsatellites have become the marker of choice for many types of genetic analysis including determination of parentage and kinship (Amos et al. 1993, Morin et al. 1994), population genetic structure (Bruford & Wayne 1993, reviewed in Bruford et al. 1996), forensics (Freqeau & Fourney 1993) and gene mapping (Litt et al. 1993). Factors that favour use of microsatellites in genetic analysis include high polymorphism, Mendelian inheritance, co-dominance and ease of use with cross species primers (Avise 1994, Bruford et al. 1996, Engel et al. 1996). The mutation rate found in microsatellite loci is higher (10^{-3} to 10^{-4} per locus per gamete per generation, Weber & Wong 1993) compared to the mitochondrial DNA control region (approximately 10^{-6} substitutions per site per generation, Avise 1994). The genetic partitioning detected from microsatellite loci therefore represents a more recent population history compared to that obtained from mtDNA control region. The combined use of analyses from mtDNA control region sequences, and size variation at microsatellite loci, provides a powerful approach to understanding the genetic structure and evolutionary history of a population.

In contrast to the use of mtDNA control region variation, few studies have employed size variation at microsatellite loci to investigate genetic substructure in African bovid species. The exceptions include cattle (*Bos sp.*) (Loftus et al. 1994), Grant's gazelle (*Gazella granti*) (Arctander et al. 1996a) and buffalo (*Syncerus caffer*) (Simonsen et al. 1998, O'Ryan et al. 1998, Van Hooft et al. 2000).

1.8 Previous study of genetic variation in the greater kudu

A previous genetic survey based on mtDNA control region sequence variation found two genetic groups in the greater kudu and suggested separate conservation measures for populations in Namibia (Nersting & Arctander 2001). This study, however, suffered from limited geographic sampling with regard to samples from South Africa, particularly the Eastern Cape region and south-western Chad (including northern CAR and Sudan).

In the present investigation, samples obtained from the geographic range of all the four subspecies as delimited by Ansell (1971) were used to investigate molecular genetic variation in the greater kudu using mtDNA control region sequences and microsatellite

loci. A combined approach that included phylogeographic, nested clade and mismatch frequency distribution analyses of mtDNA sequences and analyses of size variation at microsatellite loci was used not only to examine phylogeographic partitioning and evolutionary history in the greater kudu, but also to examine historical demographic processes. It was anticipated that the use of the two types of genetic markers with contrasting patterns of inheritance and mutation would enhance our understanding and interpretation of the evolutionary history of the greater kudu throughout its range.

Objectives of the study

The aim of the study was to characterise molecular genetic variation in the greater kudu using mtDNA control region sequences and size variation at microsatellite loci.

The specific objectives were to:

i) determine the amount of genetic variation in greater kudu populations throughout its contemporary range
ii) determine the extent of genetic structure and genetic partitioning in the species
iii) infer historical processes that have influenced current genetic patterns by using hierarchical analysis of the spatial distribution of the genetic variation.

The results from the analyses were used to make inferences on past demographic processes, clarify subspecies taxonomic classification and provide insights relevant to the conservation and management of the greater kudu (*T. strepsiceros*).

CHAPTER 2

Materials and Methods

2.1 Sample collection and Extraction of DNA

2.1.1 Sample collection

The statistical accuracy necessary to determine the genetic structure and evolutionary history of a population is influenced by the number of samples as well as the number of genetic markers used. Strategies used to obtain samples varied depending on the population in question. Samples were obtained from three regions: eastern, southern and central Africa (Fig. 1, Table 1). In eastern Africa, samples were obtained from four locations in Tanzania, three in Zambia and two in Zimbabwe. One sample from Samburu, Kenya was the single representative of the subspecies *T. s. chora*. In southern Africa, samples were obtained from four locations in Botswana, four locations in Namibia, and five locations in South Africa. Only four samples were obtained from the geographic area covering the range of the subspecies *T. s. burlacei*. The exact origin of these samples is, however, not known. According to SCI (1997), greater kudu of this subspecies are extremely rare possibly due to the arid conditions, and pressure from hunting for trophy.

Samples were obtained from fresh tissue, dried salted skins, museum collections (teeth and skins) and by remote skin biopsy darting (Karesh et al. 1987) and used for analyses. Samples obtained by skin biopsy darting, and from wildlife slaughter houses, were collected and stored in saturated NaCl/DMSO (Amos & Hoelzel 1991) for preservation before dispatch to the laboratory.

2.1.2 Extraction of DNA

For genomic DNA extraction, samples were divided into three categories: teeth, museum skins and fresh material. In order to maximise the amount of DNA obtained from each tooth and museum skin sample, two DNA extraction protocols were followed.

2.1.2.1 Extraction of DNA from teeth samples

To minimise chances of contamination, sample preparation and DNA extraction from each tooth sample was conducted in an isolated area: extractions were done in a laminar flow hood. A modified protocol by Hagelberg (1994) was followed where each tooth sample was washed with concentrated HCl for 20 minutes to remove debris and

Table 1. Locality, country of origin and sample size of *Tragelaphus strepsiceros* specimens collected in this study.

ID	Locality	Country	Sample Size	Type of Sample	Source
1	Etosha, Omaruru & Hobatere	Namibia	9	Skin biopsy	Nesting & Arctander 2001
2	Otjiwarongo	Namibia	15	Dry Skins	Marlon Beyer
3	Mt. View, Ovita	Namibia	9	Skin biopsy	Nesting & Arctander 2001
4	Corona, Abbabis	Namibia	18	Skin biopsy	Nesting & Arctander 2001, Marlon Beyer
5	Kimberley	South Africa	4	Teeth	Mofenyi Taxidermy
6	Eastern Cape	South Africa	23	Tissue	Michael Dorfling
7	KwaZulu-Natal	South Africa	5	Dry Skins	Lifeform Taxidermy
8	Mpumalanga	South Africa	8	Dry Skins	Lifeform Taxidermy
9	Limpopo	South Africa	25	Dry Skins	Nigel Fairhead and K. Hecker
10	Mokolodi	Botswana	7	Skin biopsy	Debbie Peake
11	Ghanzi	Botswana	20	Dry Skin	Debbie Peake
12	Okavango	Botswana	18	Dry Skins	Debbie Peake
13	Chobe	Botswana	14	Skin biopsy	Nesting & Arctander 2001
14	Kafue	Zambia	1	Skin biopsy	Nesting & Arctander 2001
15	Luangwa	Zambia	3	Skin biopsy	Nesting & Arctander 2001
16	Chitambo	Zambia	5	Dry Skins	Dieter Ochsenbein
17	Bulawayo	Zimbabwe	10	Dry Skins	Dieter Ochsenbein
18	Shangani	Zimbabwe	6	Skin biopsy	Nesting & Arctander 2001
19	Lukwati	Tanzania	9	Dry Skins	Nico van Rooyen taxidermy
20	Ikiri-Rungwa, Kizingo	Tanzania	12	Skin biopsy	Nesting & Arctander 2001
21	Ugalla West, Wembere, Ugalla	Tanzania	15	Skin biopsy	N. Georgiadis, Nesting & Arctander 2001
22	Arusha, Burko, Maasai, Makau	Tanzania	20	Dry Skins	Nesting & Arctander 2001
23	Samburu	Kenya	1	Skin biopsy	Nesting & Arctander 2001
24	Chad	Chad	4	Museum skins	Brussels Museum.

dirt from the surface. Distilled water was used to rinse each tooth before drying on a blotting paper. Each sample was drilled, the powder put in a solution containing 2 mL of 0.5m EDTA and 0.05g of DTT to dissolve for 12 hours. Samples were centrifuged and EDTA removed. The standard phenol/chloroform procedure as described in Sambrook et al. (1989) was used to extract DNA.

2.1.2.2 Extraction of DNA from museum skins and fresh material

DNA from museum skin samples was extracted in a laminar flow hood to minimise chances of contamination. The procedure followed a modification of the protocol for animal tissues as described from the DNeasy Tissue Kit Handbook (QIAGEN 1999). For fresh material, a standard phenol/chloroform DNA extraction protocol as described by Sambrook et al. (1989) was followed.

2.2 Mitochondrial DNA

2.2.1 Samples used for mtDNA analysis

A total of 94 greater kudu samples obtained from 12 localities were used in this aspect of the investigation (Fig. 1, Table 2).

2.2.2 Choice of primers for mtDNA control region

In greater kudu, the 5' end of the control region was amplified via PCR (Mullis et al. 1986, Saiki et al. 1988) using universal primers L15926 5' – ACA CTG GTC TTG TAA ACC - 3' located in the $tRNA^{pro}$ gene (Kocher et al. 1989), and H16499 5' – CTT GAA GTA GGA ACC AGA T- 3', located in the conserved sequence block (Southern et al. 1988). Because of the poor quality and low yield of DNA from teeth and museum skin samples, three sets of internal primers (Table 3) were constructed following standard guidelines (Sambrook et al. 1989). These greater kudu specific primers were used for PCR amplification and resulted in 100-200 bp fragments.

Table 2. Geographic origin and sample size of greater kudu specimens sequenced for the mtDNA control region. ID refers to localities in Fig. 1.

ID	Locality	Code	Country	Samples size	Type of Sample
2	Otjiwarongo	NTJ	Namibia	13	Dry Skins
5	Kimberley	SKM	South Africa	4	Teeth
6	Eastern Cape	SEC	South Africa	18	Tissue
7	KwaZulu- Natal	SKZ	South Africa	5	Dry Skins
8	Mpumalanga	SMP	South Africa	8	Dry Skins
9	Limpopo	SLM	South Africa	6	Dry Skins
10	Mokolodi	BOM	Botswana	7	Dry Skins
11	Ghanzi	BOG	Botswana	8	Dry Skin
12	Okavango	BOK	Botswana	8	Dry Skins
16	Chitambo	ZAM	Zambia	4	Dry Skins
17	Bulawayo	ZIM	Zimbabwe	9	Dry Skins
24	Chad	CHD	Chad	4	Museum skins

Table 3. Sequences of three sets of internal primers used to amplify and sequence samples from teeth and museum skins.

	Primer name and sequence		
SET 1	L1	5' - ATTAAATGCCCCATGCTTAT	- 3' (FORWARD)
	2H	5' - TTGCTTATATGCATGGGG	- 3' (REVERSE)
SET 2	L2	5' - GACATAATATGTATATAG	- 3' (FORWARD)
	2H1	5' - CCCTGACGAAAGAACCAGATG	- 3' (REVERSE)
SET 3	L3	5' - AATCGTGGGGGTAGCTATTT	- 3' (FORWARD)
	H16499	5' - CTTGAAGTAGGAACCAGAT	- 3' (REVERSE)

2.2.3 Amplification and sequencing of the mtDNA control region

Polymerase chain reactions were performed in a 9600 Perkin Elmer Thermal Cycler in a 50 µL reaction volume using 20 ng of target DNA and 0.8 units of Taq DNA polymerase (Southern Cross Technologies), 2.5 mM $MgCl_2$, 200 µM dNTPs, 1X of PCR reaction buffer[a] and 50 pmol of each primer. The following cycling conditions were used: 94 °C for 5 minutes, 94 °C for 30 seconds, 50 °C for 30 seconds, 72 °C for 30 seconds (30 cycles) and 72 °C for 3 minutes. Amplified PCR products were visualised in a 0.8% agarose gels (Southern Cross Technologies), excised from the gel and purified using the High Pure PCR Purification Kit (Roche diagnostics). Purified PCR samples were quantified by ultra violet absorbance spectrophotometry and DNA concentrations of 100-150 ng for each sample were used to prepare 10 µL reaction volumes using 3.2 pmol of primer and quarter reaction for tissue samples or half reaction for teeth samples. Cycle sequencing reactions were performed in a 9600 Perkin Elmer Thermal Cycler that generated DNA products with labelled extensions (PE Biosystems). These DNA products were precipitated using ethanol following the Perkin Elmer protocol and separated on an ABI PRISM 377 DNA automated sequencer (PE Biosystems). Each sample was sequenced in the forward and reverse directions. Every tooth and museum skin sample was sequenced six times using the three sets of primers.

[a]10X PCR reaction buffer consists of 500 mM KCl, 100 mM Tris-HCl and 1.0% Triton X-100.

For each sample, a consensus sequence was obtained by aligning sequences from forward and reverse primers in the program Sequence Navigator. Consensus sequences for all samples were aligned using the program CLUSTAL X, a multiple sequence alignment program (Thompson et al. 1997).

2.3 Microsatellite DNA

Microsatellites are short segments of DNA in which specific repeats of 1-6 bases recur tandemly. Due to high variability and relative ease of scoring, microsatellites are widely used for many types of genetic analysis including population studies, determination of parentage and kinship (Jarne & Lagoda 1996).

For animals with gender-biased dispersal patterns, population structure derived from maternal genes is considerably different from one deduced from bi-parentally inherited genes (Avise 1994). In the greater kudu, females are thought to be philopatric (Kingdon 1982), suggesting that males may be responsible for long distance dispersal of genes.

2.3.1 Samples used for microsatellite DNA analysis

A total of 203 greater kudu samples obtained from 13 locations were used for microsatellite analysis (Fig. 1, Table 4).

2.3.2 Assembly of a panel of microsatellite loci

Several studies have shown that the flanking sequence and chromosomal location of most microsatellite markers are often conserved in related species, allowing cross-species PCR amplification (Schlötterer at al. 1991, Primmer et al. 1996, Engel et al. 1996). The success in using heterologous PCR primers eliminates the need to develop new sets of primers for each species. In an attempt to identify polymorphic loci in the greater kudu, a panel of 21 dinucleotide microsatellite loci was assembled for screening. Of the 21 loci, 17 were originally isolated in cattle (*Bos sp.*) and four in sheep (*Ovis aries*) (Table 5). These loci were selected because they were polymorphic (at least five alleles) in cattle and in other species such as buffalo (*Syncerus caffer*), oryx (*Oryx leucoryx*), goat *(Capra hircus*) and sheep. Primer sequences were obtained from published literature and each locus was tested for PCR amplification using three greater kudu samples.

Table 4. Geographic origin and sample size of greater kudu specimens used for microsatellite analysis. ID refers to localities in Fig. 1.

ID	Geographic origin	Code	Country	Sample Size	Type of Sample
2	Otjiwarongo	NTJ	Namibia	15	Dry Skins
4	Corona, Abbabis	NCO	Namibia	18	Skin biopsy
6	Eastern Cape	SEC	South Africa	23	Tissue
8	Mpumalanga	SMP	South Africa	7	Dry Skins
9	Limpopo	SLM	South Africa	25	Dry Skins
11	Ghanzi	BOG	Botswana	20	Dry Skin
12	Okavango	BOK	Botswana	18	Dry Skins
15, 16	Luangwa, Chitambo	ZAM	Zambia	5	Skin biopsy
17, 18	Bulawayo, Shangani	ZIM	Zimbabwe	16	Dry Skins
19	Lukwati	TLK	Tanzania	9	Dry Skins
20	Ikiri-Rungwa, Kizingo	TRU	Tanzania	12	Skin biopsy
21	Ugalla West, Wembere, Ugalla	TAB	Tanzania	15	Skin biopsy
22	Arusha, Burko, Maasai, Makau	TAR	Tanzania	20	Dry Skins

Table 5. Microsatellite loci selected for the initial screening of polymorphism in the greater kudu (*Tragelaphus strepsiceros*).

Locus	Amplification primer 5'-3'	Polymorphism in other species	No. of alleles in other species	Reference[a]
ILSTS5	GGAAGCAATGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	Buffalo	14	Kemp et al. 1995.
AGLA293	GTCTGAAATTGGAGGCAATGAGGC CCCAAGACAACTCAAGTCAAAGGACC	Buffalo	11	Georges & Massey 1992.
BM4025	TCGAATGAACTTTTTTGGCC CACTGACTATGTGACTTTGGGC	Buffalo	10	Bishop et al. 1994.
BL1080	TTCTGAATGCACCCTTGTTTAG CTGGGCAACTAACTAATCCTGG	Sheep	9	Smith et al 1997.
BMS772	TTGTGCAATCAAGTGGTAACTG CTCACTAAGATGCCTGGTGATC	Sheep	9	Stone et al. 1995.
BMS1004	TTAAAAGTCAGAAAGGGAAGCC CTCGACCTCACATACTCAAAGC	Sheep	9	Stone et al. 1995.
BR2936	GAGCCTTGTGGGCTACAGTC GAAGATTGCAAATGGAAAGACC	Sheep	9	Bishop et al. 1994.
INRA144	TCGGTGTGGGAGGTGACTACAT TGCTGGTGGGCTCCGTCACC	Sheep	8	Eggen et al.1994.
OARHH64[b]	CGTTCCCTCACTATGGAAAGTTATATATGC CACTCTATTGTAAGAATTTGAATGAGAGC	Oryx	7	Henry et al. 1993.
TGLA48	AAATGTTTTATCTTGACTACTAAGC ACATGACTCTGCCATAGAGCAT	Buffalo	7	Georges & Massey 1992.
BMS1237	GTTTTCACTAGCACCCTGTGG CCCAGTTAACCCTAGAGTCGG	Sheep	6	Stone et al 1995.

Table 5 (continued).

Locus	Amplification primer 5'-3'	Polymorphism in other species	No. of alleles in other species	Reference[a]
CSSM18	ATGCGTCCTAGAAACTTGAGATTG GAAATCATCTGGTCATTATCAGTG	Sheep	6	Moore et al. 1994.
MAF50[b]	GTAGACTACTCATGAAAATCAGGTCTTAGG GGGACATGCAGCTATACACTTGAG	Oryx	6	Swarbrik et al. 1992.
OARCP26[b]	GGCCTAACAGAATTCAGATGATGTTGC GTCACCATACTGACGGCTGGTTCC	Oryx	6	Ede et al.1995
TGLA73	GCTTCTTTCTCTTTAAATTCTATATGG GAGAGGAGAATCACC TAGAGAGGC	Buffalo	6	Georges & Massey 1992.
RBP3	CTATGATCACCTTCTATGCTTCC CCCTAAATACTACCATCTTAGAAG	Oryx	6	MacHugh et al. 1997.
BM3215	TGCATCAACTAAGCCACACTG TTACTCGCTGGTTTTCTGGG	Goat	5	Stone et al. 1995.
ETH225	GATCACCTTGCCACTATTTCCT ACATGACAGCCAGCTGCTACT	Sheep	5	Fries et al. 1993.
MAF46[b]	AAATACCCTATAAGGCACAGTACCAC CACCATGGCCACCTGGAATCAGG	Oryx	5	Swarbrick, Dietz et al. 1992.
BMC3224	CCATCACTGCTATTCTACCTCC CACAGCCAATTTCTGATTTCA	Sheep	5	Kappes et al. 1997.
OARFC304[b]	CCCTAGGAGCTTTCAATAAAGAATCG CGCTGCTGTCAACTGGGTCAGGG	Oryx	5	Buchanan & Crawford 1993.

[a] refers to the paper where primer sequences were first published.
[b] refers to the four microsatellite loci isolated in sheep (*Ovis aries*). The remaining 17 loci were isolated in cattle (*Bos sp.*).

2.3.3 *PCR amplification of microsatellite loci*

Polymerase chain reactions were carried out using 15 µL total reaction volumes in a 9600 Perkin Elmer Thermal Cycler. To determine the optimal temperature, a range of annealing temperatures, starting at 50 °C was used for each primer pair. The following cycling conditions were used: 94 °C for 3 minutes, 94 °C for 15 seconds, 50-60 °C for 30 seconds, 72 °C for 30 seconds (10 cycles), then 89 °C for 15 seconds, 50 °C for 30 seconds, 72 °C for 30 seconds (25 cycles) and a final extension at 72 °C for 20 minutes. For $MgCl_2$, a variety of concentrations between 1.5 mM and 2.5 mM were used until a concentration that resulted in optimal amplification was found. Reaction conditions are shown in Table 6.

Table 6. Reagents and reaction volumes used for PCR amplification of microsatellite loci

Reagent	Volume	Final Concentration
10X PCR Reaction Buffer	1.6 µL	1 X
dNTPs mix (25mM each nucleotide)	1.6 µL	200 µM (each nucleotide)
Primer 1 (10 µM)	0.4 µL	4 pmol
Primer 2 (10 µM)	0.4 µL	4 pmol
$MgCl_2$ (25 mM)	0.6 -1.5 µL	1.5 mM – 2.5 mM
Taq DNA Polymerase (5 units/µL)	0.1 µL	0.5 units
dH_20	8.6 µL	
Genomic DNA template (20 ng/µL)	1.0 µL	20 ng

The amplified PCR products were electrophoresed through 2% agarose gels with 0.5 µg of ethidium bromide using TAE buffer and visually inspected under ultra violet (UV) light. Amplification conditions that resulted in a single band were assumed to be optimal, while those that produced two bands were tentatively taken to indicate the presence of a heterozygote as long as the two bands were not more than 50 bp apart. Conditions that resulted in 3-5 bands were selected for further optimisation.

Of the 21 loci, six were discarded for the following reasons; four loci (AGLA293, BM4025, BMS772 and TGLA48) resulted in no PCR product while two (ILSTS5 and INRA144) resulted in heavy background smears. The remaining 15 loci with optimised PCR conditions were selected for use in screening for polymorphism. A panel of 10

greater kudu samples taken from five geographic locations of the range was used. Of the 10 samples, two were taken from each of the following locations: Tanzania (Arusha), Botswana (Ghanzi), South Africa (Eastern Cape), Namibia (Otjiwarongo) and Zimbabwe (Shangani). Screening for polymorphism was performed using the GelStar Nucleic Acid Gel Stain protocol (BioWhittaker Molecular Applications). Several fluorescence labelled primers obtained from other research groups were also used to screen for polymorphism. Amplified PCR products from fluorescence labelled primers were resolved directly on an ABI PRISM 377 DNA sequencer (PE Biosystems).

2.3.4 Screening for polymorphism using GelStar Nucleic Acid Gel Stain

Each of the 15 loci was amplified in 10 greater kudu samples using optimal PCR conditions. Polymerase chain reaction products generated were electrophoresed through 2% agarose gels, excised from the gel and purified using the High Pure PCR Purification Kit (Roche diagnostics). Purified PCR products were mixed with 40 µL of a low ionic strength buffer (LIS) and denatured at 97 °C for three minutes. LIS buffer comprising of 10g saccharose, 0.01g of bromophenol blue and 0.01g xylene cyanol in 100 ml of distilled water was used as a loading dye and as a matrix to prevent single stranded DNA from re-annealing at room temperature (Maruya et al. 1996). The mixture was loaded, using a syringe, on a vertical 8% PAGE gel placed in TBE buffer (Sambrook et al. 1989). A DNA ladder was included in the lanes and the products electrophoresed at 120 V for four hours at room temperature. After electrophoresis, a mixture comprising of 10 ml of 1X TE, 10 mL glycerol and 5 µL GelStar Nucleic Acid Gel Stain was poured onto the gel and left to incubate for 45 minutes at room temperature in a dark room. The GelStar stain is a light sensitive fluorescent dye that provides a fast and effective way of detecting differences in allele sizes (BioWhittaker 2000). After staining, the gel was placed under UV light and PCR products were inspected for polymorphism.

2.3.5 Selection of polymorphic microsatellite primers

Of the 15 microsatellite primer sets optimised for PCR amplification, a panel of nine loci were selected and labelled with fluorescent dye (Table 7). These loci were selected because of the level of polymorphism, the quality of the signal and possibility of co-loading the loci. The remaining six loci (BL1080, BM3215, BMS1004, BR2936, MAF50 and TGLA73) were monomorphic in the test samples.

To facilitate co-loading of multiple loci in one lane, the nine polymorphic microsatellites were divided into two categories based on allele size range and fluorescence dye used. The first category consisted of five loci (RPB3, BMS1237, CSSM18, OARHH64 and OARCP26), while the second had four loci (ETH225, MAF46, OARFC304 and BMC3224). Amplified PCR products were pooled for each category and 0.5 µL was added to a loading mix which comprised of 1.5 µL formamide, 0.25 µL loading buffer, 0.25 µL of Genescan-500 TAMRA (PE Biosystems). The resulting mixture was denatured at 97 ^{0}C for 3 minutes and loaded on ABI PRISM 377 DNA automated sequencer (PE Biosystems) for analysis. The nine polymorphic microsatellite loci were genotyped in all 203 greater kudu samples.

2.3.6 Scoring of microsatellite alleles

In order to remove bias in the scoring of alleles, two samples of known allele size were used as reference in each gel run. The greater kudu samples were scored according to the reference and relative to each other. After scoring, alleles were designated using the program GENOTYPER 2.02 (DNA fragment analysis software, PE Biosystems) employing the 3rd order least squares size calling method. The information generated was exported to a spreadsheet program where allele designations were converted from fractional values to whole numbers by grouping together alleles that are likely to contain the same microsatellite repeat. The scored alleles were used in subsequent analyses.

Table 7. Microsatellite loci used to assay genetic variation in the greater kudu (*Tragelaphus strepsiceros*).

Locus	Source	Fluorescent Dye	Annealing Temp (°C)	$MgCl_2$ Conc. (mM)	Number of alleles	Allele size range (base pairs)	Reference
BMC3224	Cattle	TET	50	1.5	8	182 - 188	Kappes et al. 1997
BMS1237	Cattle	TET	55	1.5	15	145 - 181	Stone et al 1995
CSSM18	Cattle	HEX	50	2.5	6	118- 128	More et al. 1994
ETH225	Cattle	HEX	50	1.5	12	141 - 167	Fries et al. 1993
MAF46	Sheep	HEX	55	2.0	14	82 - 116	Swarbrick, Dietz et al. 1992
OARCP26	Sheep	HEX	55	2.0	14	164 - 190	Ede, Pierson & Crawford 1995
OARFC304	Sheep	FAM	58	2.0	17	123 - 165	Buchanan & Crawford 1993
OARHH64	Sheep	TET	50	1.5	8	110 - 128	Henry et al. 1993
RBP3	Cattle	FAM	50	2.0	7	124 - 138	MacHugh et al. 1997

2.4 Statistical analysis

2.4.1 Mitochondrial DNA control region sequences

2.4.1.1 Choice of DNA substitution model

Mitochondrial DNA control region sequences were aligned using CLUSTAL X, (Thompson et al. 1997) and aligned sequences were used for further analysis. To select a model of DNA substitution that best fits the data, a maximum likelihood ratio test implemented in the program MODELTEST ver 3.0.4 (Posada & Crandal 1998) was used. Fifty-six DNA substitution models were tested in a pairwise comparison and significance of the likelihood scores obtained using the chi-square test. The HKY85 (Hasegawa et al. 1985) model with gamma correction (Gu & Zhang 1997) emerged as the best fit to the data at $p < 0.01$. This model was selected for estimating sequence divergences.

2.4.1.2 Phylogenetic relationships and choice of taxa

Phylogenetic relationships between haplotypes were reconstructed using neighbour joining (Saitou & Nei 1987) in the program PAUP ver 4.0b1 (Swofford 1998). For rooted phylogenetic trees the eland (*Taurotragus oryx*) was used as outgroup. The choice of the eland was based on evidence from previous studies that indicated a close phylogenetic relationship between eland and the greater kudu (Georgiadis et al. 1990, Matthee & Robinson 1999a). Confidence in the phylogenetic nodes was assessed using 1000 bootstrap replications (Felsenstein 1985).

2.4.1.3 Analysis of population genetic differentiation

To examine the extent of differentiation among populations, an analysis of molecular variance (AMOVA, Excoffier et al. 1992) was used. AMOVA is a hierarchical analysis in which the correlation among haplotype distances at various levels is used as F statistic analogues, designated as Φ statistics. Φ_{ST} is the correlation of random haplotypes within a population relative to that from a whole species. Φ_{CT} is the correlation of random haplotypes within a group of populations relative to the total population and measures the proportion of genetic variation among groupings of populations. Φ_{SC} measures the proportion of genetic variation among populations within a region. The significance of Φ statistics was tested using 1000 bootstrap replications as implemented in the program ARLEQUIN (Schneider et al. 1997).

Genetic distances between pairs of haplotypes were estimated as the proportion of nucleotide differences. Φ_{ST} values between pairs of populations were also calculated.

2.4.1.4 Haplotype and nucleotide diversity

Haplotype (H) and nucleotide diversity (π) indices provide information on the general demographic history of a population. Haplotype diversity varies between 0-1 whereas π ranges from 0% to 10% (zero for no divergence, to approximately 10% for very deep divergences) (Avise 2000). According to Grant & Bowen (1998), populations with low H and π may have experienced severe or prolonged bottlenecks in recent times, while populations with high H and π are associated with stable populations with large N_E (effective population size). High H and low π are indicative of rapid population growth from a bottlenecked ancestral population. Low H and high π are indicative of a severe but short bottleneck (Avise 2000).

Estimates of haplotype diversity within populations were obtained by calculating (H), using the equation:

$$H = n\,(1-\Sigma f_i^2)/(n-1)$$

where f_i is the frequency of the i^{th} mtDNA haplotype, and n is the number of individuals sampled (Nei & Tajima 1981).

The estimates for nucleotide diversity, π (the average number of differences between two DNA sequences at each nucleotide site) (Nei & Li 1979) were obtained using the program ARLEQUIN.

2.4.1.5 Mismatch frequency distribution analysis

The distribution of pairwise nucleotide differences among haplotypes in a population was used to draw inferences about historical demography of greater kudu populations. Population expansions and contractions have been shown to result in recognisable signatures in the patterns of molecular diversity (Harpending et al. 1998, Schneider & Excoffier 1999). This approach has been used in several studies for example human (Harpending et al. 1993, Harpending 1994) and hartebeest, topi and wildebeest (Arctander et al. 1999). Using mismatch distribution, sudden population expansions are expected to produce a star phylogeny with an even distribution of pairwise

differences leading to a unimodal distribution. Stable or constant size populations have multimodal or geometric distributions (Neigel & Avise 1986, Nee et al. 1996). The observed distribution was compared to the expected distribution and the departures, under the expansion hypothesis (Rogers & Harpending 1992), were tested using the chi-square test of goodness of fit in the program ARLEQUIN.

2.4.2 Nested clade analysis

Traditional methods used for investigating geographic subdivision in populations rely on F-statistics calculated from haplotype or allelic frequencies (Wright 1943, Slatkin 1981) where the frequencies are overlaid on a geographical distribution (Slatkin & Maddison 1989). These methods find association between haplotypes and the geographic locality but do not attempt to reveal the underlying causes of the associations. It is known that retention of ancestral haplotypes in sub-divided populations may lead to an F_{ST} value of less than one, implying gene flow even when dispersal is non existent (Templeton 1998).

Nested clade analysis uses genealogical information to infer the probable causes of the observed geographic associations by statistically evaluating the expected patterns a population exhibits under different models of population structure and historical events. The expected patterns are restricted range expansion, allopatric fragmentation and restricted gene flow via isolation by distance (Templeton et al. 1995). This approach has been used in various studies including the tiger salamander (*Ambystoma tigrinum*) (Templeton et al. 1995), buffalo (*Syncerus caffer*), impala (*Aepyceros melampus*) and the wildebeest (*Connochaetes taurinus*) (Templeton & Georgiadis 1996). For greater kudu, the application of nested clade analysis was used to determine historical factors influencing the observed genetic pattern and to provide insights relevant for long term conservation and management of the species.

2.4.2.1 Samples used in nested clade analysis

To adequately cover the species range, 180 greater kudu samples were used. Of the 180 samples, 86 were obtained from Nersting and Arctander (2001) (Fig. 1, Table 8). All sequences were aligned and a 400 bp segment of mtDNA control region used for nested clade analysis.

Table 8. Geographic origin and sample size of greater kudu specimens used for nested clade analysis. ID refers to localities in Fig. 1.

ID	Geographic origin	Country	Sample Size	Type of Sample
1	Etosha, Omaruru & Hobatere	Namibia	9	Skin biopsy
2	Otjiwarongo	Namibia	13	Dry Skins
3	Mt. View, Ovita	Namibia	9	Skin biopsy
4	Corona, Abbabis	Namibia	12	Skin biopsy
5	Kimberley	South Africa	5	Teeth
6	Eastern Cape	South Africa	18	Tissue
7	KwaZulu-Natal	South Africa	5	Dry Skins
8	Mpumalanga	South Africa	8	Dry Skins
9	Limpopo	South Africa	6	Dry Skins
10	Mokolodi	Botswana	7	Skin biopsy
11	Ghanzi	Botswana	8	Dry Skin
12	Okavango	Botswana	8	Dry Skins
13	Chobe	Botswana	14	Skin biopsy
14	Kafue	Zambia	1	Skin biopsy
15	Luangwa	Zambia	3	Skin biopsy
16	Chitambo	Zambia	4	Dry Skins
17	Bulawayo	Zimbabwe	9	Dry Skins
18	Shangani	Zimbabwe	6	Skin biopsy
20	Ikiri-Rungwa, Kizingo	Tanzania	9	Skin biopsy
21	Ugalla West, Wembere, Ugalla	Tanzania	3	Skin biopsy
22	Arusha, Burko, Maasai, Makau	Tanzania	18	Dry Skins
23	Samburu	Kenya	1	Skin biopsy
24	Chad	Chad	4	Museum skins

2.4.2.2 Estimation of haplotype cladogram

A haplotype cladogram displaying the number of mutational steps between haplotypes was generated using the program TCS (Clement et al. 2000) which incorporates the cladogram estimation algorithm described by Templeton et al. (1992). Using this program, a matrix of absolute pairwise differences was calculated using gaps as a fifth state. The matrix was then used to construct a cladogram with haplotype branch connections above the 95% limit. Haplotypes were nested using the algorithm of Templeton and Sing (1993) into 1-step, 2-step and 3-step clades until the entire cladogram was nested. For each clade and haplotype, the topological position (tip or interior) was noted. According to neutral coalescence theory (Hudson 1990), haplotypes or clades found at the tip are younger than those found in the interior. Nesting at each level is related to divergence, and therefore correlates to evolutionary time.

2.4.2.3 Nested contingency and clade analysis

From the haplotype cladogram obtained, clades that exhibited genetic or geographic variation were tested for geographic association (see Templeton 1998). Chi- square tests were used to evaluate the significance of the association between clades at each nesting hierarchy with geographical locations in the program GEODIS (Posada et al. 2000). Those clades that exhibited significant association with geographical locations were used in the nested clade analysis. Nested clade analysis was performed using the program GEODIS to differentiate between historical and contemporary evolutionary processes. The program incorporates the methods of Templeton et al. (1995) and estimates two distances: the clade distance $D_c(X)$ and the nested clade distance $D_n(X)$. $D_c(X)$ is the average distance of individuals in clade X from the geographical centre of that clade. $D_n(X)$ is the average distance of the clade X from the geographical centre of the higher level clade in which clade X is nested. The average distances between the tip and interior clades within the nested group $(I\text{-}Tip)_c$, and the tip to interior distance for the nesting clade $(I\text{-}Tip)_n$ were estimated.

To determine whether these distances were significantly small or large at the 5% level, the permutation procedure of Roff & Bentzen (1989) was used with 1000 replicates as implemented in the program GEODIS. Interpretation of the results followed the guidelines in the inference key given in Templeton et al. (1995).

2.4.3 Microsatellite DNA analysis

2.4.3.1 Genetic variation

Genetic variability in the 13 greater kudu populations was determined by examining the mean number of alleles per locus, allele frequencies per locus, observed heterozygosity (H_O), and Nei's unbiased expected heterozygosity (H_E) (Nei 1987). The average number of alleles per locus per population was obtained using the program MICROSAT (Minch et al. 1996).

Correlation between the number of samples, the number of alleles and heterozygosity per population was determined using Pearson product moment correlation (Sokal & Rohlf 1995). This analysis examines whether a particular population has experienced recent bottlenecks, since rare alleles are generally lost faster than heterozygosity (Hedrick et al. 1986).

2.4.3.2 Genetic distance

Genetic distance was estimated using the proportion of shared alleles distance measure, which has been shown to be appropriate for closely related populations (Bowcock et al. 1994). The option 1-p was used as implemented in the program MICROSAT. The resulting genetic distance was used to reconstruct phylogenetic trees using the program NEIGHBOUR [included in the package PHYLIP ver 3.5 (Felsenstein 1993)]. Confidence estimates for tree topologies were obtained by performing 1000 bootstraps in PHYLIP.

2.4.3.3 Analysis of heterozygosity

Observed heterozygosity (H_O) describes the proportion of heterozygotes observed in a population, and was obtained for each of the 104 locus/population combinations by counting the number of heterozygous genotypes.

An unbiased estimate of gene diversity or expected heterozygosity (H_E) was derived for each locus/population combination using the following equation:

$$H_E = 2n(1-\Sigma p_i^2)/(2n-1)$$

where *n* is the number of individuals sampled and pi is the frequency of each of the alleles at a particular locus (Nei 1987).

2.4.3.4 Hardy-Weinberg Equilibrium (HWE)

The Hardy-Weinberg equilibrium principle describes the prediction of expected proportions of genotypes from observed allele frequencies in a population (Hartl and Clark 1988).

Possible causes of deviations from Hardy-Weinberg equilibrium include subdivision within a population, natural selection acting on loci under consideration, bias towards particular genotypes and null alleles segregating in the population (indicated by excess of homozygotes). Substructure within a population leads to deviations from Hardy-Weinberg equilibrium at all loci, whereas other causes of deviation from Hardy-Weinberg equilibrium are mostly locus-specific. Significant deviation from HWE at a number of independent loci in a population may indicate migration or non-random mating (Hartl and Clark 1988).

The test for deviations from HWE was performed using the program GENEPOP ver 3.3 (Raymond & Rousset 1995). This program performs an exact test and additionally uses the Markov chain algorithm for all loci with more than four alleles, which allows an unbiased estimate of the exact probabilities of being wrong in rejecting HWE. For all comparisons in GENEPOP, the Markov chain was set to 100 batches, 1000 iterations and 2000 dememorizations. Critical significance levels for locus/population combinations were computed using sequential Bonferroni tests, which evaluate all the p values and corrects all simultaneous statistical tests (Rice 1989).

2.4.3.5 Genotypic linkage disequilibrium

Genotypic linkage disequilibrium is the non-random association of alleles at different loci. Linkage disequilibrium arise from a variety of factors, including physical linkage of loci, epistatic selection, genetic hitchhiking, random drift in finite populations and demographic factors such as coancestry, migration and population admixture (Hartl & Clark 1988).

Genotypic linkage disequilibrium was examined for all pairwise combinations of loci in each population. The tests were carried out using the program GENEPOP ver 3.3.

The null hypothesis (Ho) tested was: genotypes at one locus are independent from genotypes at a second locus. The algorithm used is based on analysis of contingency tables, and each contingency table is analysed using the Markov chain method in a similar manner to the test for HWE expectations described above (Raymond & Rousset 1995).

2.4.3.6 Analysis of population genetic substructure

The extent of genetic differentiation among populations was investigated using an analysis of molecular variance (AMOVA) to derive Wright's F statistic. Estimates of Φ_{ST} (an analogue of F_{ST}) and F_{IS} (inbreeding coefficient) were calculated for all pairs of populations as implemented in the program ARLEQUIN. Additionally, R_{ST} was calculated using the program RSTCALC (Goodman 1997). R_{ST} is an F_{ST} analogue that assumes a stepwise mutation model (SMM), measures the variance in allele size and takes into account sample size differences (Slatkin 1995). Although there is debate whether microsatellite loci follow strict SMM (Direnzo et al. 1994), R_{ST} is likely to give less biased estimates of differentiation than with the standard measure using F_{ST} which assumes the infinite alleles model (IAM) (Weir & Cockerham 1984, Weir 1990, Marshall et al. 1999). Permutation tests were used to evaluate the significance of F_{ST} estimates in ARLEQUIN and R_{ST} estimates in RSTCALC.

The significance of the differences in distribution of alleles and genotypes at each population, using all microsatellite loci was performed using Fisher's exact test in the program GENEPOP. Probability values were corrected for multiple comparisons by using sequential Bonferroni tests (Rice 1989).

2.4.3.7 Assignment test

The assignment test provides a powerful approach for inferring how distinct populations are from each other. The test was performed using the approach suggested by Paetkau et al. (1995). The power of the test depends, however, on the number of loci used and assumes linkage disequilibrium and random mating within each population (Waser & Strobeck 1998). To avoid instances where the expected genotype frequencies are zero, an allele frequency of 0.01 was used for alleles not observed in a particular distribution (Paetkau & Strobeck 1994). The expected frequency of each individual's genotype was calculated based on likelihood score. Each individual was assigned to a population where its genotype has the highest likelihood of occurrence.

Significance of the test was determined using 1000 replicates. To assess the likelihood of an individual's genotype belonging to a particular population, logarithms of likelihood scores were plotted to produce a scatter diagram. The degree of overlap of genotypes between populations, in the scatter plot is a measure of genetic differentiation between two populations. Assignment tests were performed using a calculator from the website <http://www.biology.ualberta.ca/jbrzusto>.

CHAPTER 3

Results

3.1 Mitochondrial DNA data

3.1.1 Sequence variation

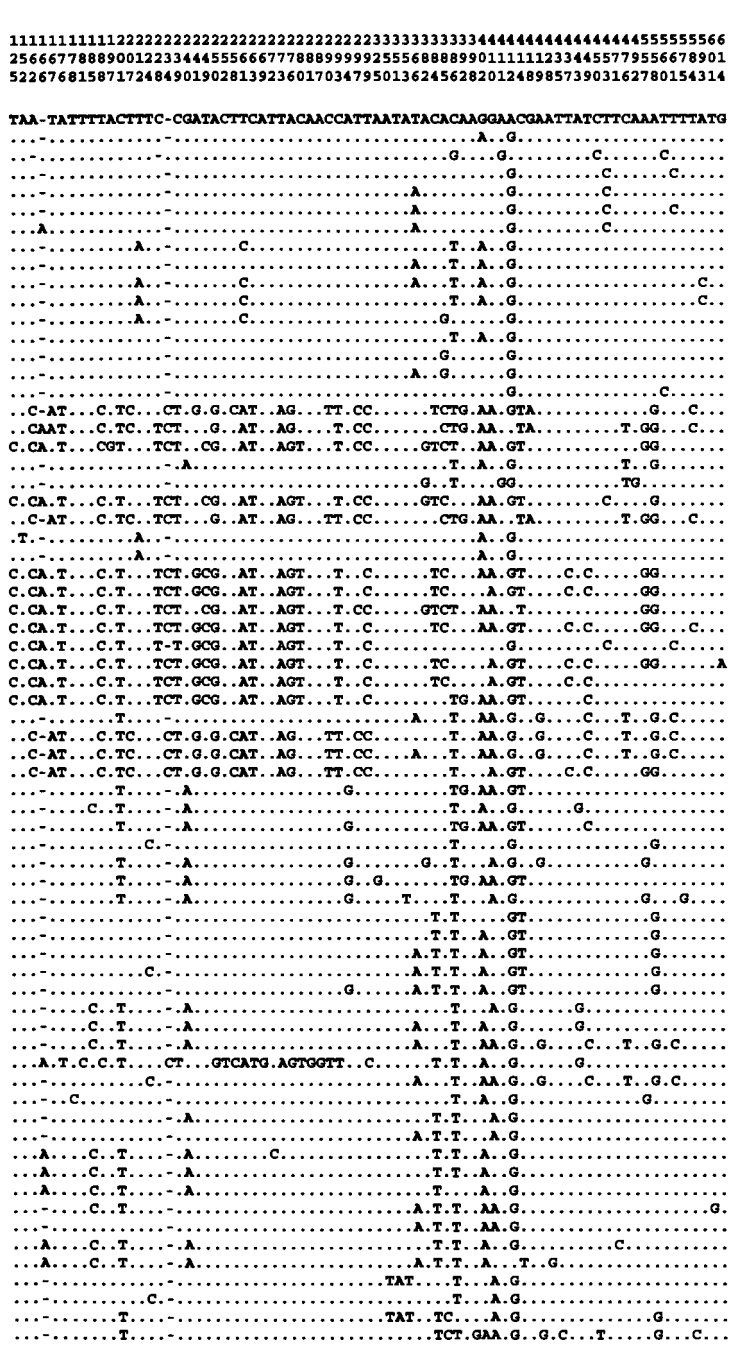
A 622 bp segment of the 5' end of the control region was sequenced in 94 samples obtained from 12 localities (Fig. 1, Table 2). Seventy-five nucleotide positions (12.06%) were polymorphic of which 61 were transitions and 14 were transversions. Two indels were observed at position 166 and 234. Insertions at these two positions were observed in all haplotypes from the Eastern Cape, except haplotype number 30 (Fig. 2). The estimated transition / transversion ratio was 4.36 and the among site rate variation was moderate at α = 0.533. The 75 variable sites defined 68 haplotypes of which only two were shared. The shared haplotypes were numbers 26 (between Otjiwarongo in Namibia and Eastern Cape in South Africa) and 41 (between Mokolodi in Botswana and Mpumalanga in South Africa). The most frequent haplotype (number 26) was found in the Eastern Cape population and was scored in seven individuals.

The average sequence divergence between populations was 2.3% and ranged from 0% to 5.7% (Appendix I). The highest divergence was found between haplotype number 21 (from Otjiwarongo in Namibia) and 29 (from Eastern Cape in South Africa).

3.1.2 Phylogenetic relationships among greater kudu haplotypes

Phylogenetic relationships among the 68 greater kudu haplotypes were reconstructed using the HKY85 distances with gamma correction and the neighbour-joining algorithm. A mid-point rooted tree (Fig. 3) shows the presence of two discrete groups. The first comprises haplotypes exclusively from the Eastern Cape and from Kimberley and Otjiwarongo. The second consists of all the remaining haplotypes, including one haplotype from Kimberley (34) and four haplotypes (20, 21, 24 and 25) from Otjiwarongo.

The phylogenetic tree was then rooted using eland as outgroup and within the first group (Eastern Cape, Kimberley and Otjiwarongo), there were two groups supported by a bootstrap value of 72% (Fig. 4). Haplotypes in the second group were unresolved with bootstrap support of less than 50%. Fifty-two nucleotide positions were parsimony informative. Because of the large number of haplotypes compared to number of informative sites, the maximum parsimony method



	Populations											
	BOK	BOG	BOM	NTJ	SEC	SKM	SMP	SLM	SKZ	ZAM	ZIM	CHD
1	1	-	-	-	-	-	-	-	-	-	-	-
2	1	-	-	-	-	-	-	-	-	-	-	-
3	1	-	-	-	-	-	-	-	-	-	-	-
4	2	-	-	-	-	-	-	-	-	-	-	-
5	1	-	-	-	-	-	-	-	-	-	-	-
6	1	-	-	-	-	-	-	-	-	-	-	-
7	1	-	-	-	-	-	-	-	-	-	-	-
8	-	1	-	-	-	-	-	-	-	-	-	-
9	-	1	-	-	-	-	-	-	-	-	-	-
10	-	1	-	-	-	-	-	-	-	-	-	-
11	-	1	-	-	-	-	-	-	-	-	-	-
12	-	1	-	-	-	-	-	-	-	-	-	-
13	-	1	-	-	-	-	-	-	-	-	-	-
14	-	1	-	-	-	-	-	-	-	-	-	-
15	-	1	-	-	-	-	-	-	-	-	-	-
16	-	-	2	-	-	-	-	-	-	-	-	-
17	-	-	-	1	-	-	-	-	-	-	-	-
18	-	-	-	2	-	-	-	-	-	-	-	-
19	-	-	-	1	-	-	-	-	-	-	-	-
20	-	-	-	1	-	-	-	-	-	-	-	-
21	-	-	-	1	-	-	-	-	-	-	-	-
22	-	-	-	2	-	-	-	-	-	-	-	-
23	-	-	-	2	-	-	-	-	-	-	-	-
24	-	-	-	1	-	-	-	-	-	-	-	-
25	-	-	-	1	-	-	-	-	-	-	-	-
26	-	-	-	1	3	-	-	-	-	-	-	-
27	-	-	-	-	7	-	-	-	-	-	-	-
28	-	-	-	-	1	-	-	-	-	-	-	-
29	-	-	-	-	2	-	-	-	-	-	-	-
30	-	-	-	-	1	-	-	-	-	-	-	-
31	-	-		-	1	-	-	-	-	-	-	-
32	-	-	-	-	1	-	-	-	-	-	-	-
33	-	-	-	-	1	-	-	-	-	-	-	-
34	-	-	-	-	-	1	-	-	-	-	-	
35	-	-	-	-	-	1	-	-	-	-	-	
36	-	-	-	-	-	1	-	-	-	-	-	-
37	-	-	-	-	-	1	-	-	-	-	-	-
38	-	-	-	-	-	-	1	-	-	-	-	-
39	-	-	-	-	-	-	1	-	-	-	-	-
40	-	-	-	-	-	-	2	-	-	-	-	-
41	-	-	5	-	-	-	1	-	-	-	-	-
42	-	-	-	-	-	-	1	-	-	-	-	-
43	-	-	-	-	-	-	1	-	-	-	-	-
44	-	-	-	-	-	-	1	-	-	-	-	-
45	-	-	-	-	-	-	-	1	-	-	-	-
46	-	-	-	-	-	-	-	2	-	-	-	-
47	-	-	-	-	-	-	-	1	-	-	-	-
48	-	-	-	-	-	-	-	1	-	-	-	-
49	-	-	-	-	-	-	-	1	-	-	-	-
50	-	-	-	-	-	-	-	-	1	-	-	-
51	-	-	-	-	-	-	-	-	1	-	-	-
52	-	-	-	-	-	-	-	-	1	-	-	-
53	-	-	-	-	-	-	-	-	1	-	-	-
54	-	-	-	-	-	-	-	-	1	-	-	-
55	-	-	-	-	-	-	-	-	-	1	-	-
56	-	-	-	-	-	-	-	-	-	2	-	-
57	-	-	-	-	-	-	-	-	-	1	-	-
58	-	-	-	-	-	-	-	-	-	-	2	-
59	-	-	-		-	-	-	-	-	-	2	-
60	-	-	-	-	-	-	-	-	-	-	1	-
61	-	-	-	-	-	-	-	-	-	-	1	-
62	-	-	-	-	-	-	-	-	-	-	1	-
63	-	-	-	-	-	-	-	-	-	-	1	-
64	-	-	-	-	-	-	-	-	-	-	1	-
65	-	-	-	-	-	-	-	-	-	-	-	1
66	-	-	-	-	-	-	-	-	-	-	-	1
67	-	-	-	-	-	-	-	-	-	-	-	1
68	-	-	-	-	-	-	-	-	-	-	-	1

Fig. 2. Genetic variation observed in a 622 bp fragment of the mtDNA control region from 94 samples of greater Kudu (*Tragelaphus strepsiceros*) obtained from 12 sampling locations (see Table 1). Haplotype numbers are given on the left and the number of individuals observed for each haplotype are shown on the right. A dot indicates similarity with haplotype 1. For abbreviations see Table 2.

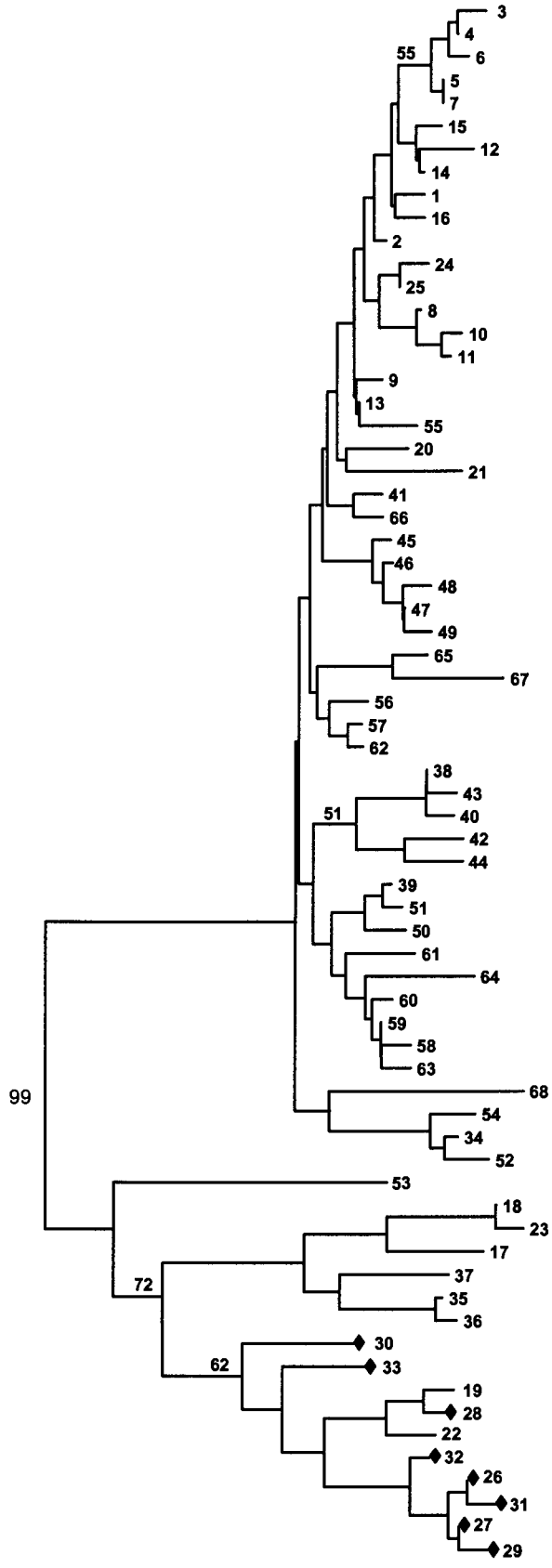


Fig. 3. A mid-point rooted neighbour-joining phylogram showing the phylogenetic relationship among 68 greater Kudu haplotypes. Phylogenetic reconstruction was done using NJ algorithm and HKY85 model with gamma corrected genetic distances. Haplotype numbers are given in Fig. 2 and (◆) refers to haplotypes from the Eastern Cape population. Only bootstrap values > 50% are shown.

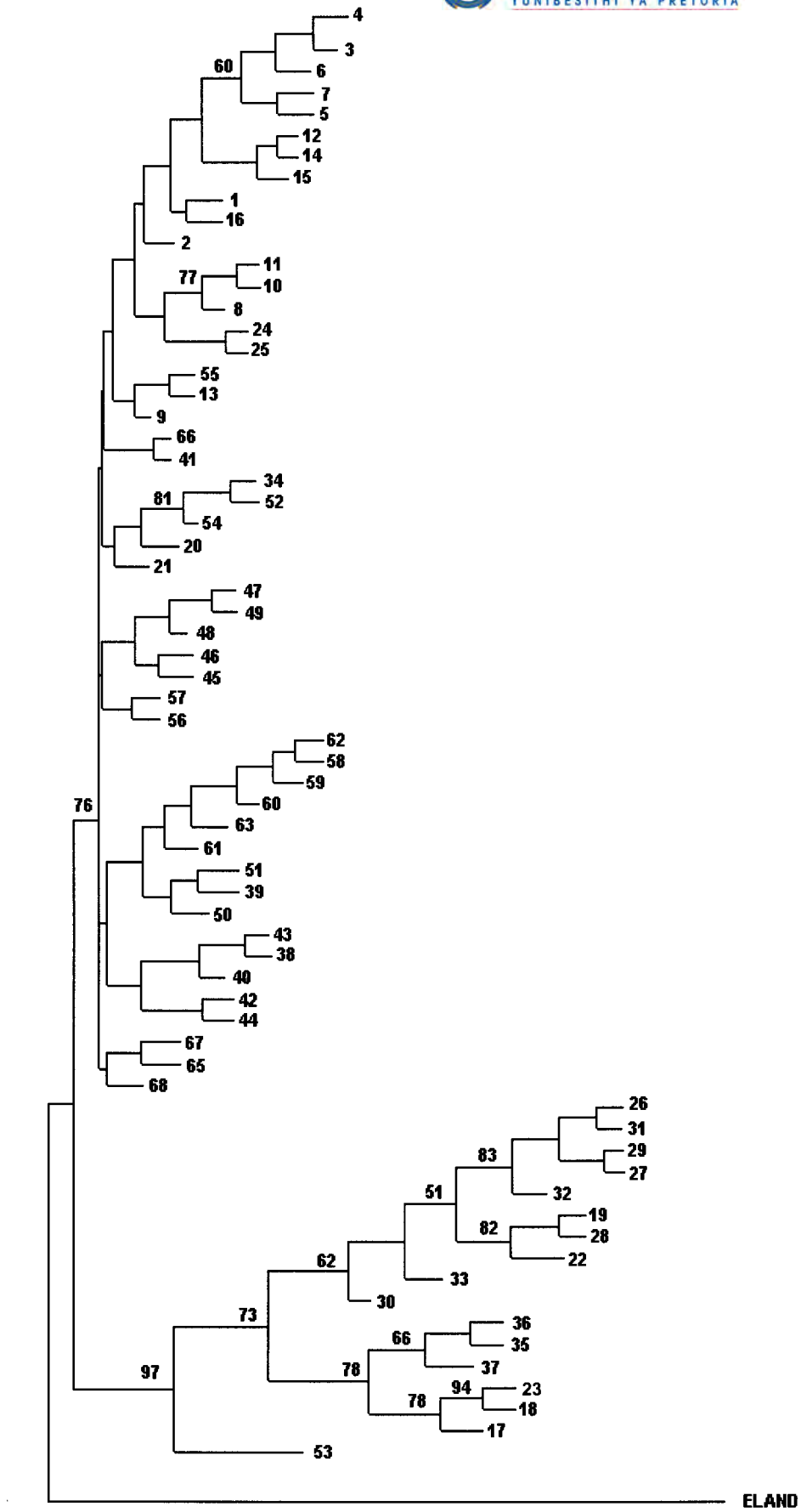


Fig. 4. Phylogenetic relationships among 68 greater Kudu haplotypes obtained from 12 locations. Phylogram reconstruction was done by the neighbour-joining algorithm using HKY85 with gamma corrected genetic distances with the eland as outgroup. Bootstrap values > 50% are shown. Haplotype numbers correspond to Fig. 2.

(Swofford & Olsen 1990) was not used to reconstruct phylogenetic relationships in the greater kudu.

3.1.3 Population genetic differentiation

To investigate the extent of genetic partitioning, three scenarios were explored. Populations were categorised into groups based on subspecies as described by Ansell (1971), geographic isolation, as well as the grouping suggested by the neighbour-joining tree. Significant p values for population subdivision were obtained when populations were grouped into two groups as derived from the neighbour-joining phylogram (data not shown). The first group consisted of haplotypes from the Eastern Cape, Kimberley and Namibia (Table 9), while the second comprised of the remaining haplotypes. The percentage of total variance attributed to Φ_{CT} and Φ_{ST} were higher than Φ_{SC} (15%) suggesting that populations in one group were more closely related to each other than they were to the other group. The presence of haplotypes from Kimberley and Otjiwarongo in the first and second group resulted in a considerably lower proportion of total variance attributed to differentiation at population level (Φ_{ST} = 30%).

Pairwise comparisons of Φ_{ST} among the 12 populations are given in Table 10. The pairwise Φ_{ST} values ranged from 0.127 to 0.885 and the largest Φ_{ST} value was found between the Eastern Cape population and Mokolodi from southern Botswana. Out of the 66 comparisons, six were non-significant. All the non-significant comparisons were found in populations with small sample sizes.

Apart from populations from the Eastern Cape, Kimberley and Chad, all greater kudu populations used in this study have, to a large extent, historically contiguous geographical distributions. Estimates of the number of migrants per generation between populations could not be used to measure gene flow because of the influence of historical events on greater kudu populations as shown by nested clade analysis (see later). Several studies of African bovid species (buffalo, wildebeest and impala) (Templeton & Georgiadis 1996) have shown that non-zero estimates of the number of migrants can arise due to retention of ancestral haplotypes between populations. Inference of gene flow in such circumstances would therefore be erroneous.

Table 9. Hierarchical analysis of molecular variance (AMOVA) of mtDNA control region sequences among 12 greater kudu populations.

Hierarchy	d.f.	% Total variance	Φ Statistic		*p* value
Among groups	1	55.62	Φ_{CT}	0.556	<0.001
Among populations / within groups	10	14.76	Φ_{SC}	0.332	<0.001
Within populations	82	29.63	Φ_{ST}	0.704	<0.001

Table 10. Pairwise Φ_{ST} values (below diagonal) and the associated *p* values (above diagonal) calculated for mtDNA control region sequences in AMOVA for 12 greater kudu populations.

		1	2	3	4	5	6	7	8	9	10	11	12
1	Okavango		**	**	**	***	**	**	**	**	**	**	**
2	Eastern Cape	0.882		***	***	**	**	**	***	**	**	**	**
3	Ghanzi	0.367	0.870		**	***	**	*	**	**	**	**	**
4	Mpumalanga	0.536	0.842	0.435		*	*	*	*	*	*	**	*
5	Limpopo	0.705	0.879	0.556	0.520		*	*	*	**	*	*	**
6	Zambia	0.498	0.864	0.328	0.269	0.555		*	*	*	NS	**	NS
7	Zimbabwe	0.641	0.864	0.539	0.414	0.599	0.420		*	**	**	**	*
8	Otjiwarongo	0.469	0.404	0.429	0.398	0.401	0.358	0.455		*	**	NS	NS
9	Mokolodi	0.509	0.885	0.405	0.493	0.647	0.482	0.659	0.444		*	*	*
10	Chad	0.470	0.833	0.363	0.282	0.476	0.135	0.467	0.319	0.366		*	NS
11	Kimberley	0.742	0.720	0.700	0.622	0.721	0.626	0.723	0.173	0.732	0.535		NS
12	Kwa-Zulu Natal	0.381	0.766	0.290	0.167	0.345	0.127	0.223	0.257	0.341	0.129	0.305	

NS represents not significant, * represents $p < 0.01$, ** indicates $p < 0.001$, *** indicates $p < 0.0001$.

3.1.4 Mismatch frequency distribution

Mismatch frequency distributions of pairwise nucleotide differences were examined in five populations (Eastern Cape, Otjiwarongo, Okavango, Ghanzi and Zimbabwe) as shown in Fig. 5. The remaining populations were not considered due to small sample size. A chi-square test in all populations indicated a non-significant departure of observed from expected frequencies. Populations from the Eastern Cape and Otjiwarongo exhibited multimodal distributions suggesting stable populations in the past. The shape of the expected frequency curve in the Eastern Cape population is compatible with a population that is a remnant of a once larger population (see Fig. 10 from Rogers and Harpending 1992). The remaining three populations (Ghanzi, Okavango and Zimbabwe) revealed signatures characteristic of expanding populations.

Summary statistics for measures of genetic diversity observed in the 12 populations are shown in Table 11. High levels of haplotype diversity were observed within greater kudu populations and the overall value was 0.99. The lowest haplotype diversity value (H = 0.48) was found in the population from Mokolodi (southern Botswana).

Nucleotide diversity (π) values were estimated according to Nei (1987) and ranged from 0.003 ± 0.001 in Mokolodi to 0.029 ± 0.003 in Otjiwarongo. The overall nucleotide diversity was 0.027 ± 0.001. Comparison of the results with those obtained in studies on other large African antelopes with similar distribution patterns such as buffalo (π = 0.050, Simonsen et al. 1998), hartebeest (π = 0.032, Arctander et al. 1999) and wildebeest (π = 0.025, Arctander et al. 1999) indicate that the greater kudu have moderate mtDNA diversity.

3.2 Nested clade data

Studies have shown that the non-random association of lineages or haplotypes with geographical location can arise from restricted gene flow, historical events (fragmentation, range expansion or colonisation) or a combination of these factors (Templeton et al. 1992). For the greater kudu, discriminating among these factors for the probable cause was performed using a nested clade analysis.

3.2.1 Haplotype networks

Estimation of the relationship between haplotypes followed the method of Templeton et al. (1992). The method begins by estimating the minimum number of mutational steps

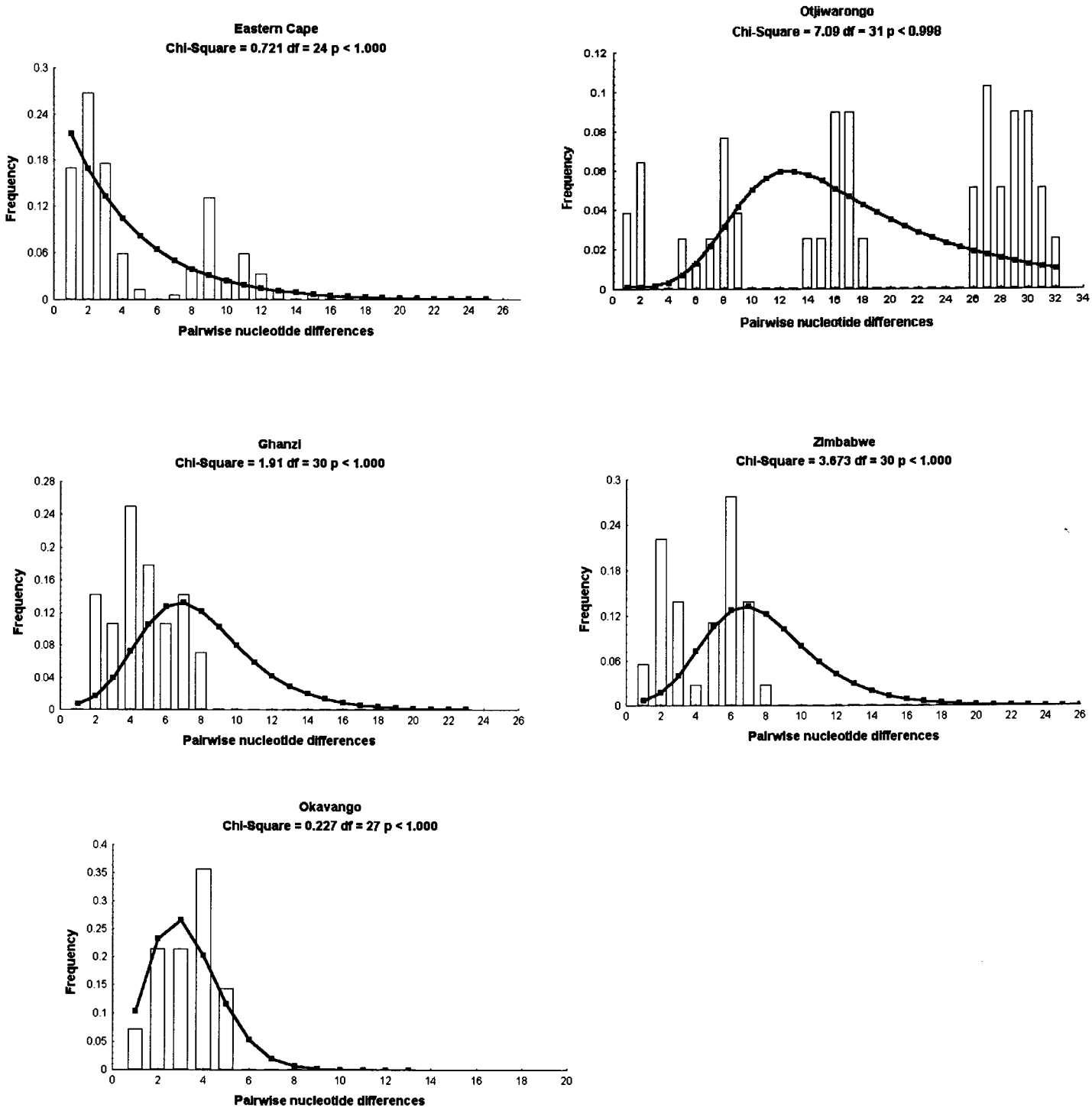


Fig.5. Mismatch frequency distribution of the pairwise nucleotide differences in five populations of greater kudu (*Tragelaphus strepsiceros*). The observed values are shown as bars and expected values are shown as curves. The observed and expected frequencies were tested using the x^2 test of goodness of fit.

Table 11. Measures of genetic diversity observed in mtDNA control region sequences of 12 greater kudu populations.

Population	No. of samples	No. of mtDNA haplotypes	No. of bp differences	% Pairwise divergence*	Haplotype diversity (H)	Nucleotide diversity (π)
Eastern Cape	18	8	0 - 12	0.00 - 2.35	0.8301 ± 0.0734	0.00586 ± 0.00179
Mpumalanga	8	7	0 - 10	0.00 - 1.67	0.9640 ± 0.0770	0.00991 ± 0.00176
Limpopo	6	5	0 - 3	0.00 - 0.49	0.9339 ± 0.1226	0.00258 ± 0.00056
Kimberley	4	4	1 - 18	0.16 - 4.31	1.0000 ± 0.1772	0.02043 ± 0.00743
KwaZulu-Natal	5	5	3 - 29	0.49 - 5.10	1.0000 ± 0.1260	0.02387 ± 0.00769
Okavango	8	6	0 - 4	0.00 - 0.65	0.9295 ± 0.0840	0.00369 ± 0.00065
Ghanzi	8	8	1 - 7	0.16 - 1.15	1.0000 ± 0.0637	0.00599 ± 0.00073
Mokolodi	7	2	0 - 4	0.00 - 0.65	0.4764 ± 0.1715	0.00307 ± 0.00111
Otjiwarongo	13	10	0 - 30	0.00 - 5.26	0.9623 ± 0.0419	0.02891 ± 0.00307
Zambia	4	3	0 - 6	0.00 - 0.98	0.8338 ± 0.2226	0.00591 ± 0.00220
Zimbabwe	9	7	0 - 7	0.00 - 1.16	0.9446 ± 0.0701	0.00556 ± 0.00124
Chad	4	4	4 - 13	0.65 - 2.17	1.0000 ± 0.1773	0.01398 ± 0.00344
Total	94	68	0 - 32	0.00 - 5.64	0.9901 ± 0.0046	0.02692 ± 0.00138

*Pairwise sequence divergences estimated using the HKY85 (Hasegawa et al. 1985) model with gamma correction.

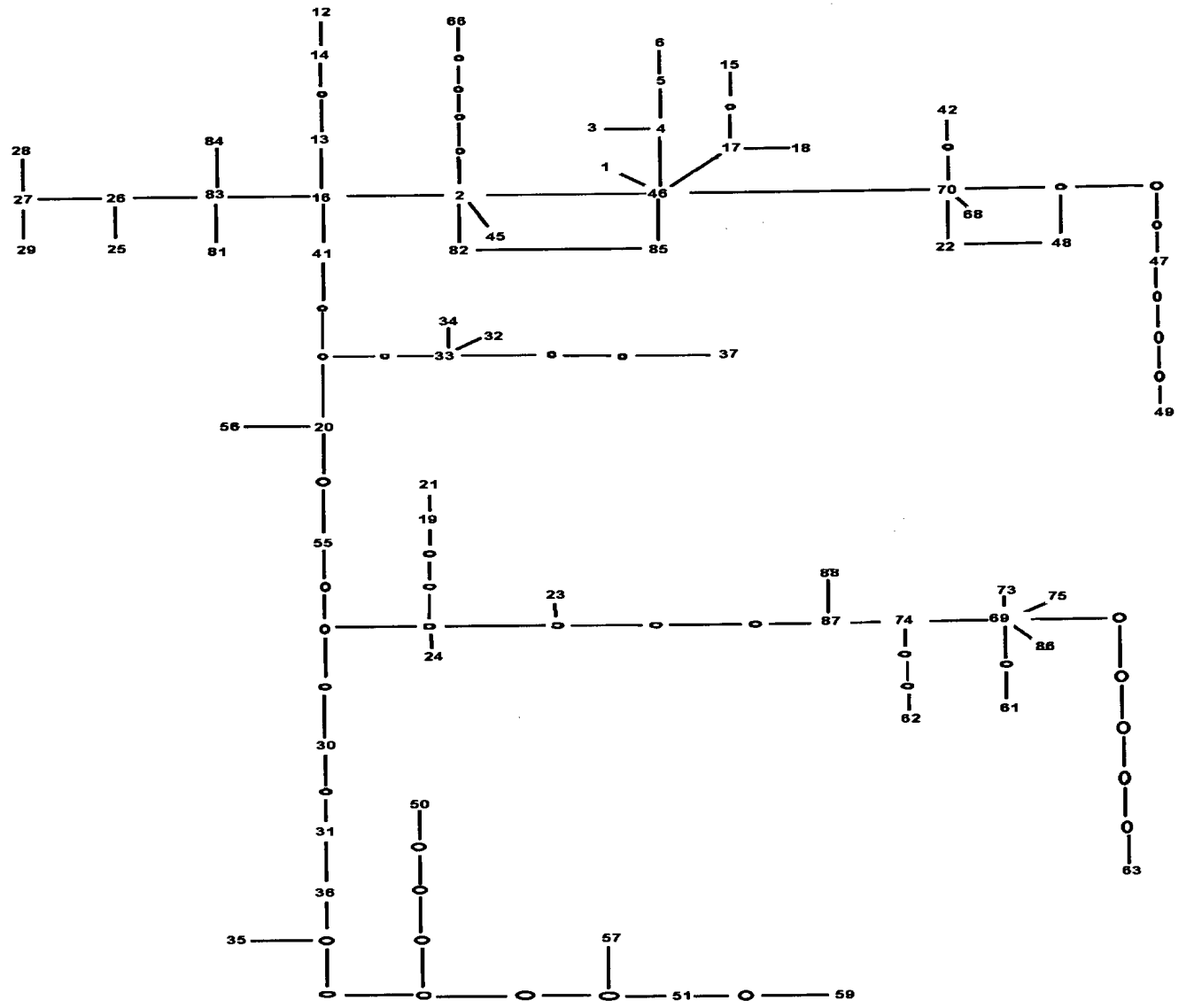
Table 12. Haplotype number, subspecies, sample origin, geographic co-ordinates and number of individuals per haplotype used for nested clade analysis. Haplotype numbers corresponds to those used in Fig. 6.

Haplotype number	Subspecies	Country	Locality	Geographical* co-ordinates	No. of samples
1	*T. s. strepsiceros*	Botswana	Okavango	-20.40, 23.10	1
2	*T. s. strepsiceros*	Botswana	Okavango	-20.40, 23.20	1
3	*T. s. strepsiceros*	Botswana	Okavango	-20.40, 23.30	1
4	*T. s. strepsiceros*	Botswana	Chobe	-19.40, 25.20	2
	T. s. strepsiceros	Botswana	Okavango	-20.40, 23.40	2
5	*T. s. strepsiceros*	Botswana	Okavango	-20.40, 23.50	2
6	*T. s. strepsiceros*	Botswana	Okavango	-20.40, 23.60	1
7	*T. s. strepsiceros*	South Africa	Eastern Cape	-33.57, 26.14	10
8	*T. s. strepsiceros*	South Africa	Eastern Cape	-33.57, 26.15	5
	T. s. strepsiceros	Namibia	Otjiwarongo	-20.57, 16.60	1
9	*T. s. strepsiceros*	Namibia	Mt. View	-20.40, 19.90	2
	T. s. strepsiceros	Namibia	Otjiwarongo	-20.57, 16.70	1
10	*T. s. strepsiceros*	South Africa	Eastern Cape	-33.57, 26.16	2
11	*T. s. strepsiceros*	South Africa	Eastern Cape	-33.57, 26.17	1
12	*T. s. strepsiceros*	Botswana	Ghanzi	-21.70, 21.70	1
13	*T. s. strepsiceros*	Botswana	Ghanzi	-21.70, 21.80	1
14	*T. s. strepsiceros*	Botswana	Ghanzi	-21.70, 21.90	1
15	*T. s. strepsiceros*	Botswana	Ghanzi	-21.70, 21.10	1
16	*T. s. strepsiceros*	Zambia	Chitambo	-15.20, 24.80	1
	T. s. strepsiceros	Botswana	Ghanzi	-21.70, 21.11	1
	T. s. strepsiceros	Zambia	Kafue	-15.20, 24.80	1
17	*T. s. strepsiceros*	Botswana	Ghanzi	-21.70, 21.12	1
18	*T. s. strepsiceros*	Botswana	Ghanzi	-21.70, 21.13	1
19	*T. s. strepsiceros*	South Africa	Mpumalanga	-25.50, 31.07	2
20	*T. s. strepsiceros*	South Africa	Mpumalanga	-25.50, 31.08	1
21	*T. s. strepsiceros*	South Africa	Mpumalanga	-25.50, 31.09	1
22	*T. s. strepsiceros*	Botswana	Mokolodi	-24.20, 27.20	5
	T. s. strepsiceros	South Africa	Mpumalanga	-25.50, 31.10	2
23	*T. s. strepsiceros*	South Africa	Mpumalanga	-25.50, 31.11	1
24	*T. s. strepsiceros*	South Africa	Mpumalanga	-25.50, 31.12	1
25	*T. s. strepsiceros*	South Africa	Limpopo	-21.50, 27.30	1
26	*T. s. strepsiceros*	South Africa	Limpopo	-21.50, 27.40	2
27	*T. s. strepsiceros*	South Africa	Limpopo	-21.50, 27.50	1
28	*T. s. strepsiceros*	South Africa	Limpopo	-21.50, 27.60	1
29	*T. s. strepsiceros*	South Africa	Limpopo	-21.50, 27.70	1
30	*T. s. strepsiceros*	Zambia	Chitambo	-15.20, 24.90	2
	T. s. strepsiceros	Zambia	Luangwa	-15.70, 26.70	2
31	*T. s. strepsiceros*	Zambia	Chitambo	-15.20, 24.10	1
32	*T. s. strepsiceros*	Zimbabwe	Bulawayo	-18.70, 27.90	2
	T. s. strepsiceros	Zimbabwe	Shangani	-18.70, 28.90	2
33	*T. s. strepsiceros*	Zimbabwe	Bulawayo	-18.70, 27.10	2
	T. s. strepsiceros	Zimbabwe	Shangani	-18.70, 28.10	3
34	*T. s. strepsiceros*	Zimbabwe	Bulawayo	-18.70, 27.90	1
	T. s. strepsiceros	Zimbabwe	Shangani	-18.70, 28.11	1
35	*T. s. strepsiceros*	Zimbabwe	Bulawayo	-18.70, 27.10	1
36	*T. s. strepsiceros*	Zimbabwe	Bulawayo	-18.70, 27.11	1
37	*T. s. strepsiceros*	Zimbabwe	Bulawayo	-18.70, 27.12	1
38	*T. s. strepsiceros*	Namibia	Otjiwarongo	-20.57, 16.80	1
39	*T. s. strepsiceros*	Namibia	Otjiwarongo	-20.57, 16.90	1
40	*T. s. strepsiceros*	Namibia	Otjiwarongo	-20.57, 16.10	1
41	*T. s. strepsiceros*	Namibia	Corona	-23.50, 17.10	2
	T. s. strepsiceros	Namibia	Otjiwarongo	-20.57, 16.11	1
42	*T. s. strepsiceros*	Namibia	Otjiwarongo	-20.57, 16.12	1

Table 12 (continued).

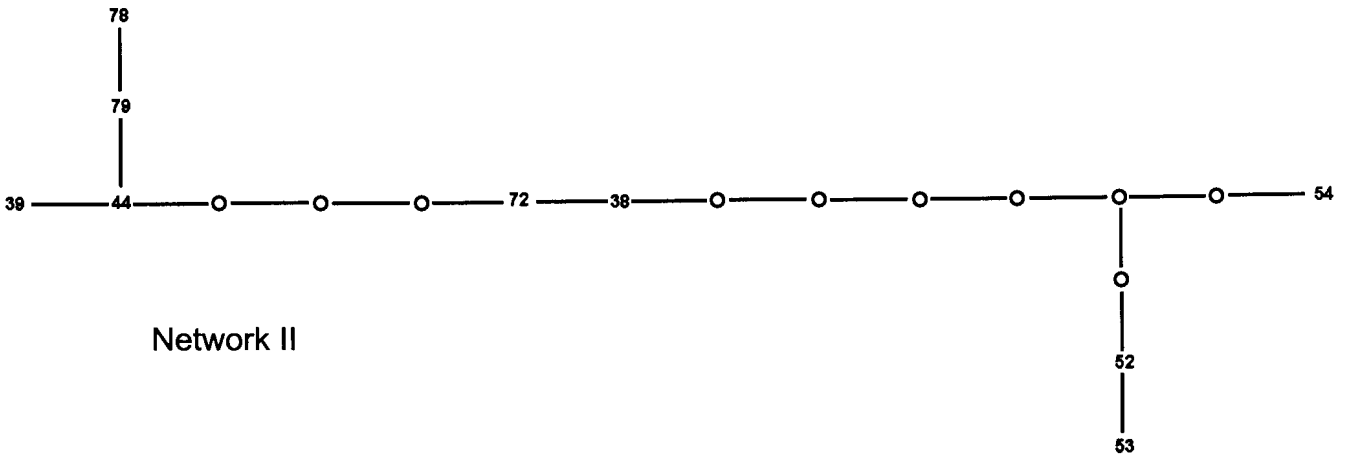
Haplotype number	Subspecies	Country	Locality	Geographical* co-ordinates	No. of samples
43	*T. s. strepsiceros*	Namibia	Mt View	-20.40, 19.50	1
	T. s. strepsiceros	Namibia	Otjiwarongo	-20.57, 16.13	2
44	*T. s. strepsiceros*	Namibia	Otjiwarongo	-20.57, 16.14	2
45	*T. s. strepsiceros*	Namibia	Otjiwarongo	-20.57, 16.15	2
46	*T. s. strepsiceros*	Botswana	Chobe	-19.40, 25.30	1
	T. s. strepsiceros	Botswana	Ghanzi	-21.70, 21.14	1
	T. s. strepsiceros	Botswana	Mokolodi	-24.20, 27.30	2
47	*T. s. burlacei*	Chad	Chad	13.60, 22.54	1
48	*T. s. burlacei*	Chad	Chad	13.60, 22.55	1
49	*T. s. burlacei*	Chad	Chad	13.60, 22.56	1
50	*T. s. burlacei*	Chad	Chad	13.60, 22.57	1
51	*T. s. strepsiceros*	South Africa	Kimberley	-28.20, 24.90	1
52	*T. s. strepsiceros*	South Africa	Kimberley	-28.20, 24.10	1
53	*T. s. strepsiceros*	South Africa	Kimberley	-28.20, 24.11	1
54	*T. s. strepsiceros*	South Africa	Kimberley	-28.20, 24.12	1
55	*T. s. strepsiceros*	South Africa	KwaZulu-Natal	-27.20, 30.10	1
56	*T. s. strepsiceros*	South Africa	KwaZulu-Natal	-27.20, 30.20	1
57	*T. s. strepsiceros*	South Africa	KwaZulu-Natal	-27.20, 30.30	1
58	*T. s. strepsiceros*	South Africa	KwaZulu-Natal	-27.20, 30.40	1
59	*T. s. strepsiceros*	South Africa	KwaZulu-Natal	-27.20, 30.50	1
60	*T. s. chora*	Kenya	Samburu	1.70, 38.40	1
61	*T. s. bea*	Tanzania	Ugalla	-4.50, 31.50	1
62	*T. s. bea*	Tanzania	Ugalla	-4.50, 31.60	1
63	*T. s. bea*	Tanzania	Ugalla	-4.50, 31.70	1
64	*T. s. strepsiceros*	South Africa	Kimberley	-28.20, 24.13	1
65	*T. s. strepsiceros*	Namibia	Mt View	-20.40, 19.60	1
66	*T. s. strepsiceros*	Botwana	Chobe	-19.40, 25.40	1
67	*T. s. strepsiceros*	Namibia	Corona	-23.50, 17.20	3
67	*T. s. strepsiceros*	Namibia	Mt View	-20.40, 19.70	4
68	*T. s. strepsiceros*	Namibia	Mt View	-20.40, 19.80	1
69	*T. s. bea*	Tanzania	Arusha	-2.50, 34.20	10
	T. s. bea	Tanzania	Rungwa	-5.40, 32.70	1
70	*T. s. strepsiceros*	Zambia	Luangwa	-15.70, 26.80	1
71	*T. s. strepsiceros*	Namibia	Etosha	-19.20, 16.60	2
72	*T. s. strepsiceros*	Namibia	Etosha	-19.20, 16.70	4
73	*T. s. bea*	Tanzania	Rungwa	-5.40, 32.80	6
74	*T. s. bea*	Tanzania	Rungwa	-5.40, 32.90	1
75	*T. s. bea*	Tanzania	Rungwa	-5.40, 32.10	1
76	*T. s. strepsiceros*	Namibia	Etosha	-19.20, 16.80	1
77	*T. s. strepsiceros*	Namibia	Etosha	-19.20, 16.90	3
78	*T. s. strepsiceros*	Namibia	Corona	-23.50, 17.30	3
79	*T. s. strepsiceros*	Namibia	Corona	-23.50, 17.40	2
80	*T. s. strepsiceros*	Namibia	Corona	-23.50, 17.50	2
81	*T. s. strepsiceros*	Botwana	Chobe	-19.40, 25.50	5
82	*T. s. strepsiceros*	Botwana	Chobe	-19.40, 25.60	1
83	*T. s. strepsiceros*	Botwana	Chobe	-19.40, 25.70	2
84	*T. s. strepsiceros*	Botwana	Chobe	-19.40, 25.80	1
85	*T. s. strepsiceros*	Botwana	Chobe	-19.40, 25.90	1
86	*T. s. bea*	Tanzania	Arusha	-2.50, 34.30	3
87	*T. s. bea*	Tanzania	Arusha	-2.50, 34.40	1
88	*T. s. bea*	Tanzania	Arusha	-2.5,0 34.50	4
Total					180

* Geographical co-ordinates are given in decimal degrees.

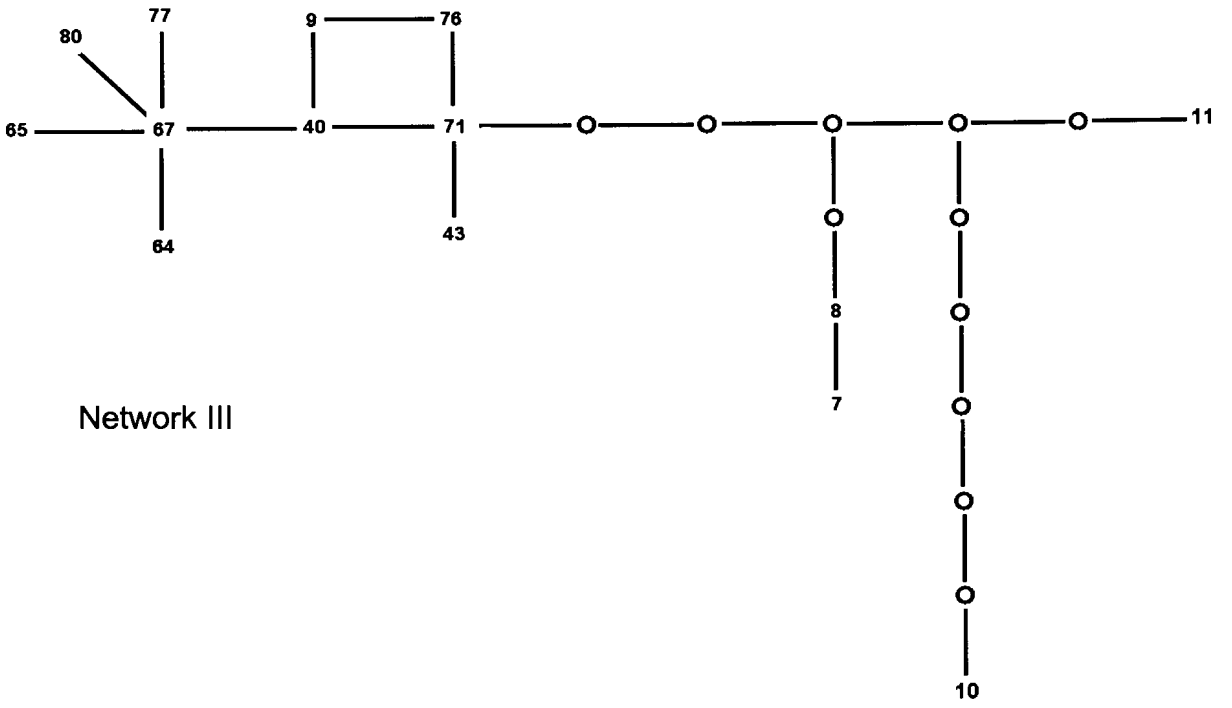


Network I

Fig. 6. Haplotype networks for mtDNA control region in the greater Kudu *(Tragelaphus strepsiceros)* resolved by 8 steps at 95% plausible connections with the algorithm of Templeton et al. (1992). These networks represent the most parsimonious connections for the set of haplotypes. Each connection represents a single mutational step and 'O' represents an intermediate haplotype not observed in the population. Haplotype designations are given in Table 12.



Network II



Network III

Fig. 6 (continued).

between haplotypes under criteria of parsimony with a probability equal to or higher than 95%. An unrooted cladogram was constructed using 88 haplotypes derived from 180 greater kudu samples (Table 12). The unrooted cladogram yielded three highly divergent networks after eight mutational steps (P_8 = 95%) (Fig. 6). Network I consists of 63 haplotypes (Fig. 7) and is connected to network III by a minimum of 16 mutational steps. The 16 steps are well beyond the confidence limits of parsimony, and therefore it is difficult to determine which haplotype or clade connects the two networks. Haplotypes 58 (from KwaZulu-Natal in South Africa) and 60 (from Samburu in Kenya) were omitted from the analysis due to the large number of mutational steps (31) that connected them to network I.

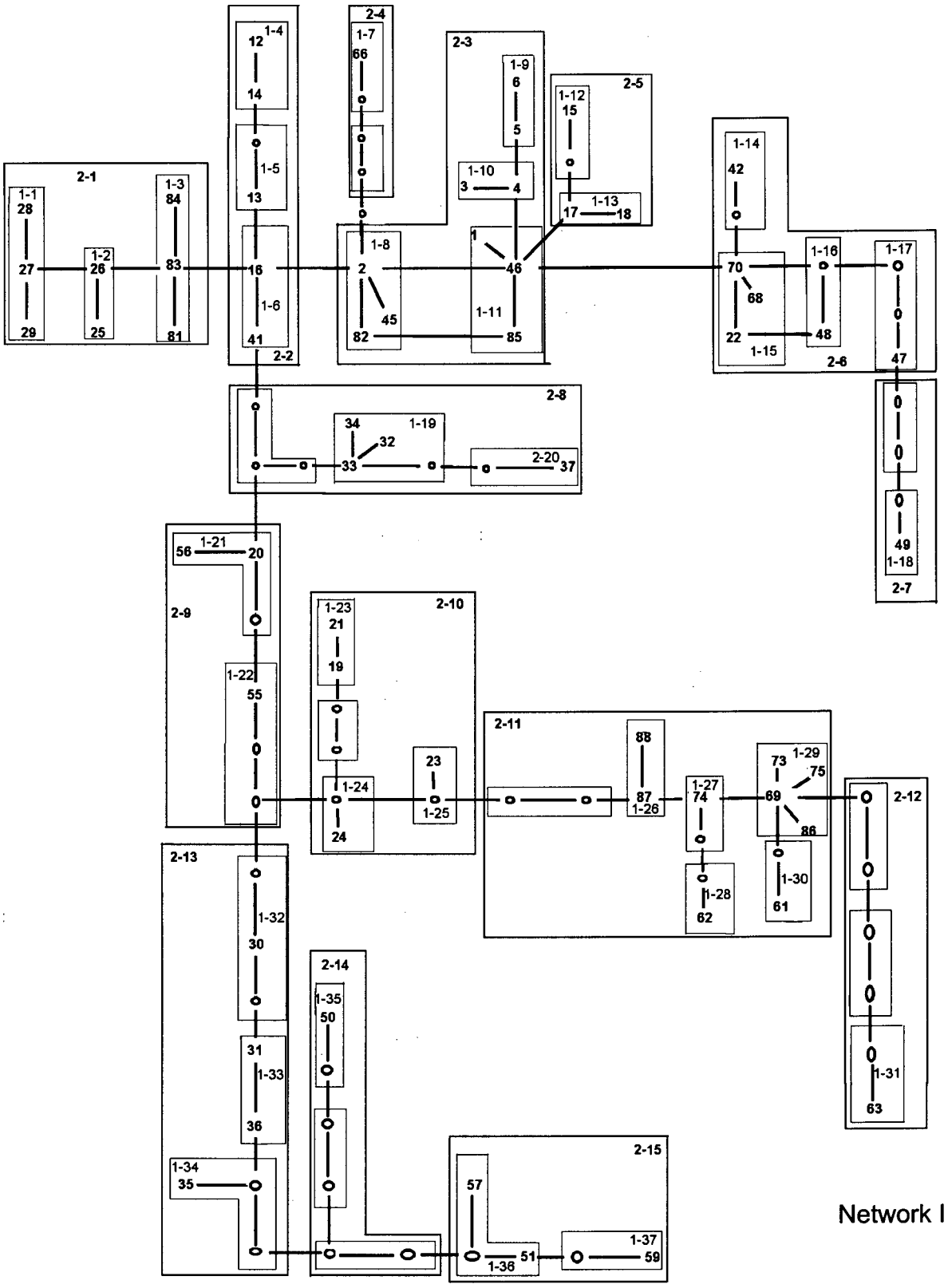
Network II and III consists of nine and 14 haplotypes respectively (Fig. 8) and are connected by nine mutational steps at clade 2-16 and 2-19 (Fig. 9). Network II and III consists of haplotypes exclusively from the Eastern Cape and Kimberley (South Africa) and Otjiwarongo and Corona (Namibia). The remaining haplotypes were grouped into network I.

3.2.3 *Nested contingency analysis*

Results of the nested contingency analysis for geographic subdivision are given in Table 13. The analysis was performed for all nested clades with genetic and geographic variation by permuting the lower clades within a nested clade with the sampling localities included in the clade. The *p* value for each analysis was estimated using the chi-square statistic. The null hypotheses of no geographic association was rejected ($p < 0.05$) in 15 of the nested categories. Contingency analysis of the whole cladogram rejected the null hypothesis of no association with geographic location ($p < 0.001$) (Table 13) indicating that the distribution of lineages was not random with respect to geographic location.

3.2.4 Nested clade analysis for geographical subdivision

Results of the nested clade analysis are given in Fig. 10a for clade 5-1 and Fig. 10b for clade 5-2. Nineteen nested categories resulted in significant values for D_c and D_n distances (see materials and methods for description of D_c and D_n), however only 15 nested clades had significant *p* values from the nested contingency test (Table 13). These nested categories were used to infer patterns based on predictions about



Network I

Fig. 7. The unrooted mtDNA cladogram for *Tragelaphus strepsiceros* showing network I resolved by 8-step. The network is estimated from 95% plausible haplotype connections using the algorithm of Templeton et al. (1992). Zeros indicate haplotypes that are intermediate between existing haplotypes but were not found in the population. The 1-n clades represents the 1 step clades where *n* is the specific number of the clade. The 2-n step clades indicate the nesting of 1-n step clades. Two intersecting loops in clade 2-3 and 2-6 did not affect nesting categories.

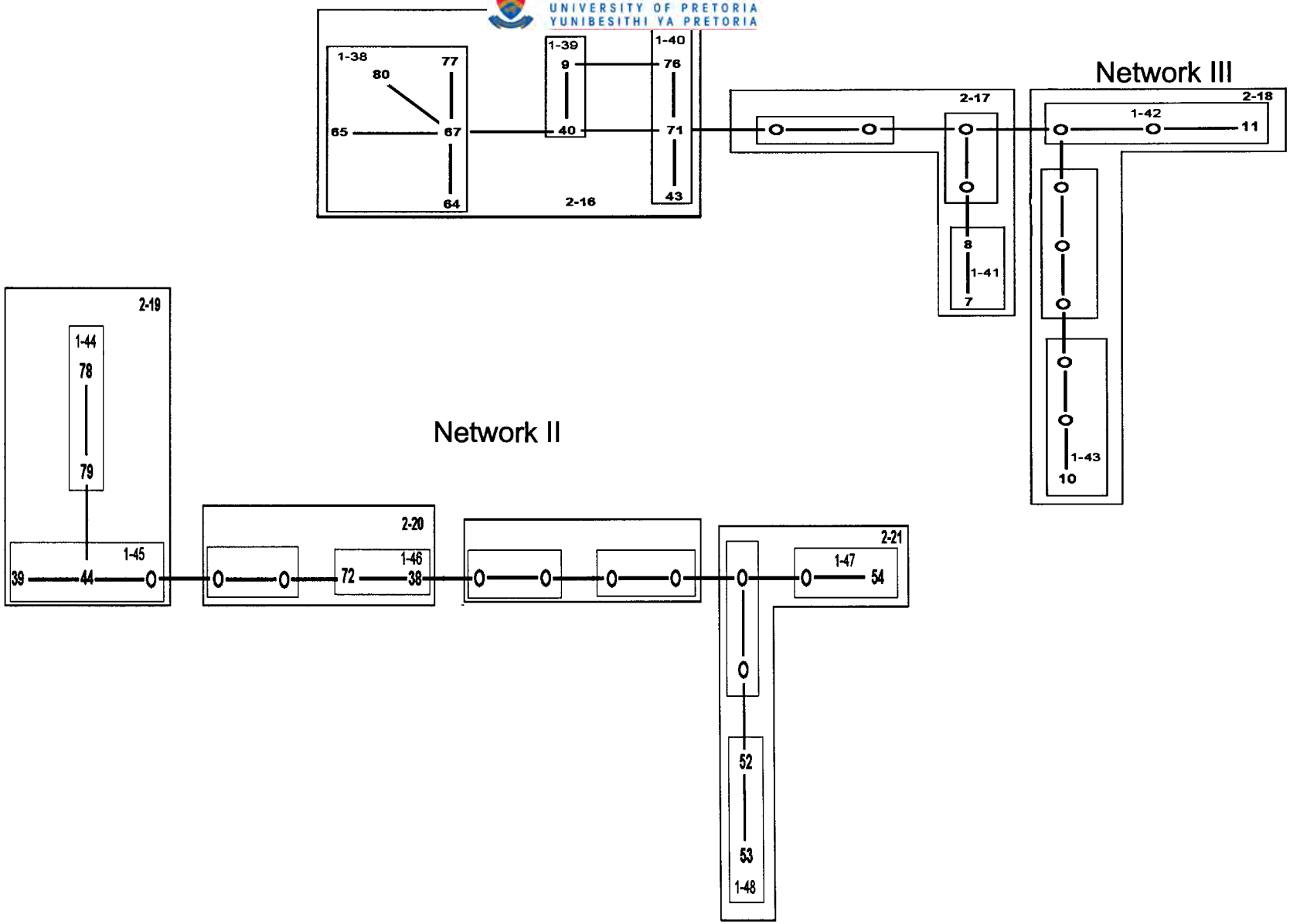


Fig. 8. The unrooted mtDNA cladogram for *Tragelaphus strepsiceros* showing network II and III resolved by 8-step. The network is estimated from 95% plausible haplotype connections using the algorithm of Templeton et al. 1992. A solid branch indicates a single mutation between haplotypes. Zeros indicate haploytpes that are intermediate between existing haplotypes but were not found in the population. The 1-n clades represents the 1 step clades where *n* is the specific number of the clade. The 2-n step clades indicate the nesting of 1-n step clades.

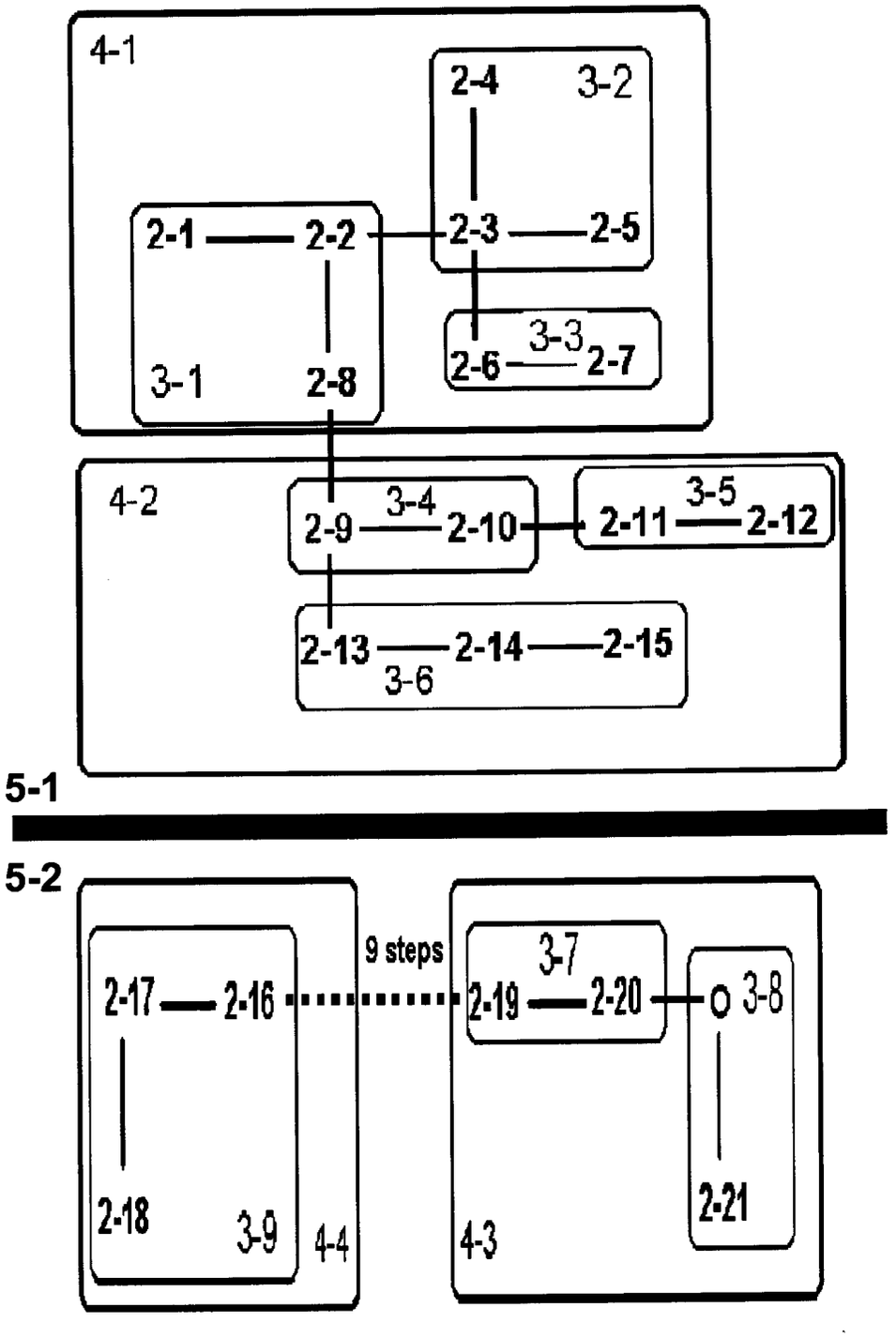


Fig. 9. Higher nested clades from the three networks in Fig. 7 and Fig. 8 were connected into a single cladogram. Heavy line indicate a large number of mutational steps (clades 5-1 and 5-2 are connected by16 mutational steps, well beyond the confidence limits for parsimony while clade 4-3 and 4-4 are connected by 9 steps).

Table 13. Analysis of associations between geographic locations and nested clades, using an exact permutational contingency test. Nested clades with a probability value ≤ 0.05 indicate significant geographical structure. Clades with no genetic or geographic variation were excluded.

Nested Clade	Permutational Chi-square statistic	Probability
1-6	6.000	0.210
1-8	8.000	0.151
1-10	0.750	1.000
1-11	9.800	0.292
1-15	18.000	0.012*
1-21	2.000	1.000
1-29	17.145	0.000*
1-36	2.000	1.000
1-38	32.400	0.000*
1-39	4.000	0.503
1-40	2.100	0.619
2-1	14.000	0.000*
2-2	6.666	0.512
2-3	18.803	0.068
2-6	24.000	0.375
2-9	0.750	1.000
2-11	7.964	0.011*
2-13	4.550	0.146
2-15	0.750	1.000
2-16	12.500	0.108
2-19	9.000	0.008*
3-1	72.000	0.000*
3-2	5.155	0.073
3-3	3.611	0.615
3-4	3.750	0.146
3-5	1.551	0.415
3-6	22.000	0.005*
3-7	4.321	0.079
3-9	36.377	0.000*
4-1	74.113	0.000*
4-2	92.263	0.000*
4-3	17.000	0.000*
5-1	103.126	0.000*
5-2	Very large	0.000*
Entire cladogram	151.932	0.000*

* significance at the 0.05 level

Fig. 10a

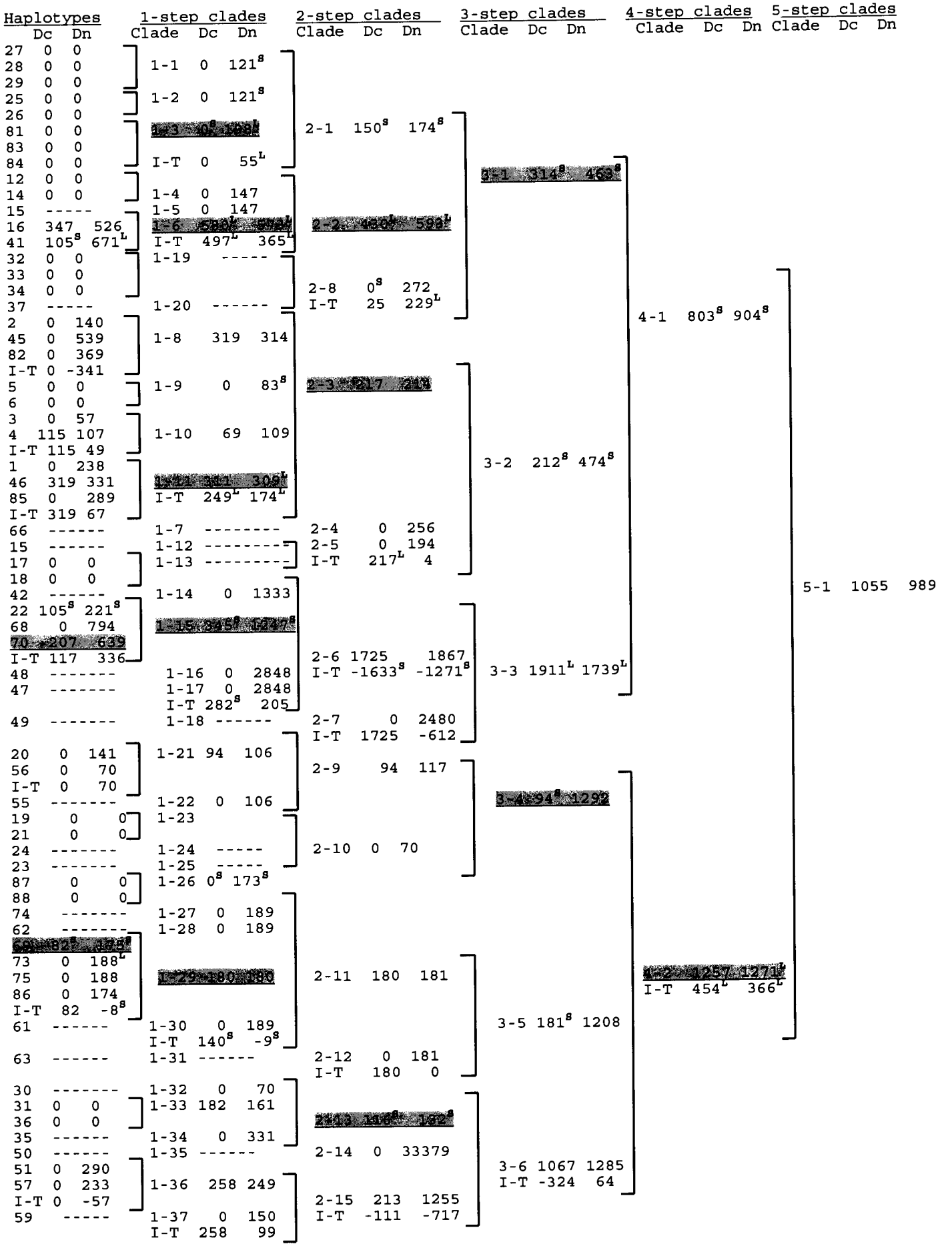

Haplotypes
Dc Dn
1-step clades
Clade Dc Dn
2-step clades
Clade Dc Dn
3-step clades
Clade Dc Dn
4-step clades
Clade Dc Dn
5-step clades
Clade Dc Dn
27 0 0
28 0 0
29 0 0
1-1 0 121S
25 0 0
26 0 0
1-2 0 121S
81 0 0
83 0 0
84 0 0
1-3 0S 198L
I-T 0 55L
2-1 150S 174S
12 0 0
14 0 0
1-4 0 147
15 -----
1-5 0 147
16 347 526
41 105S 671L
1-6 580L 579L
I-T 497L 365L
2-2 480L 599L
4-1 314S 468S
32 0 0
33 0 0
34 0 0
37 -----
1-19 -----
1-20 ------
2-8 0S 272
I-T 25 229L
2 0 140
45 0 539
82 0 369
I-T 0 -341
1-8 319 314
4-1 803S 904S
5 0 0
6 0 0
1-9 0 83S
2-3 217 214
3 0 57
4 115 107
I-T 115 49
1-10 69 109
1 0 238
46 319 331
85 0 289
I-T 319 67
1-21 311 309L
I-T 249L 174L
3-2 212S 474S
66 ------
15 ------
17 0 0
18 0 0
1-7 --------
1-12 --------
1-13 --------
2-4 0 256
2-5 0 194
I-T 217L 4
42 ------
22 105S 221S
68 0 794
70 207 639
I-T 117 336
1-14 0 1333
1-15 345S 1247S
2-6 1725 1867
I-T -1633S -1271S
5-1 1055 989
48 -------
47 -------
1-16 0 2848
1-17 0 2848
I-T 282S 205
3-3 1911L 1739L
49 -------
1-18 ------
2-7 0 2480
I-T 1725 -612
20 0 141
56 0 70
I-T 0 70
1-21 94 106
2-9 94 117
55 -------
1-22 0 106
3-4 94S 1292
19 0 0
21 0 0
1-23
24 -------
23 -------
1-24 -----
1-25 -----
2-10 0 70
87 0 0
88 0 0
1-26 0S 173S
74 -------
62 -------
1-27 0 189
1-28 0 189
69 82S 176S
73 0 188L
75 0 188
86 0 174
I-T 82 -8S
1-29 180 180
2-11 180 181
4-2 1257 1271L
I-T 454L 366L
61 ------
1-30 0 189
I-T 140S -9S
3-5 181S 1208
63 ------
1-31 ------
2-12 0 181
I-T 180 0
30 -------
31 0 0
36 0 0
1-32 0 70
1-33 182 161
35 ------
1-34 0 331
2-13 198S 182S
50 ------
1-35 ------
2-14 0 33379
51 0 290
57 0 233
I-T 0 -57
1-36 258 249
3-6 1067 1285
I-T -324 64
59 -----
1-37 0 150
I-T 258 99
2-15 213 1255
I-T -111 -717

Fig. 10b

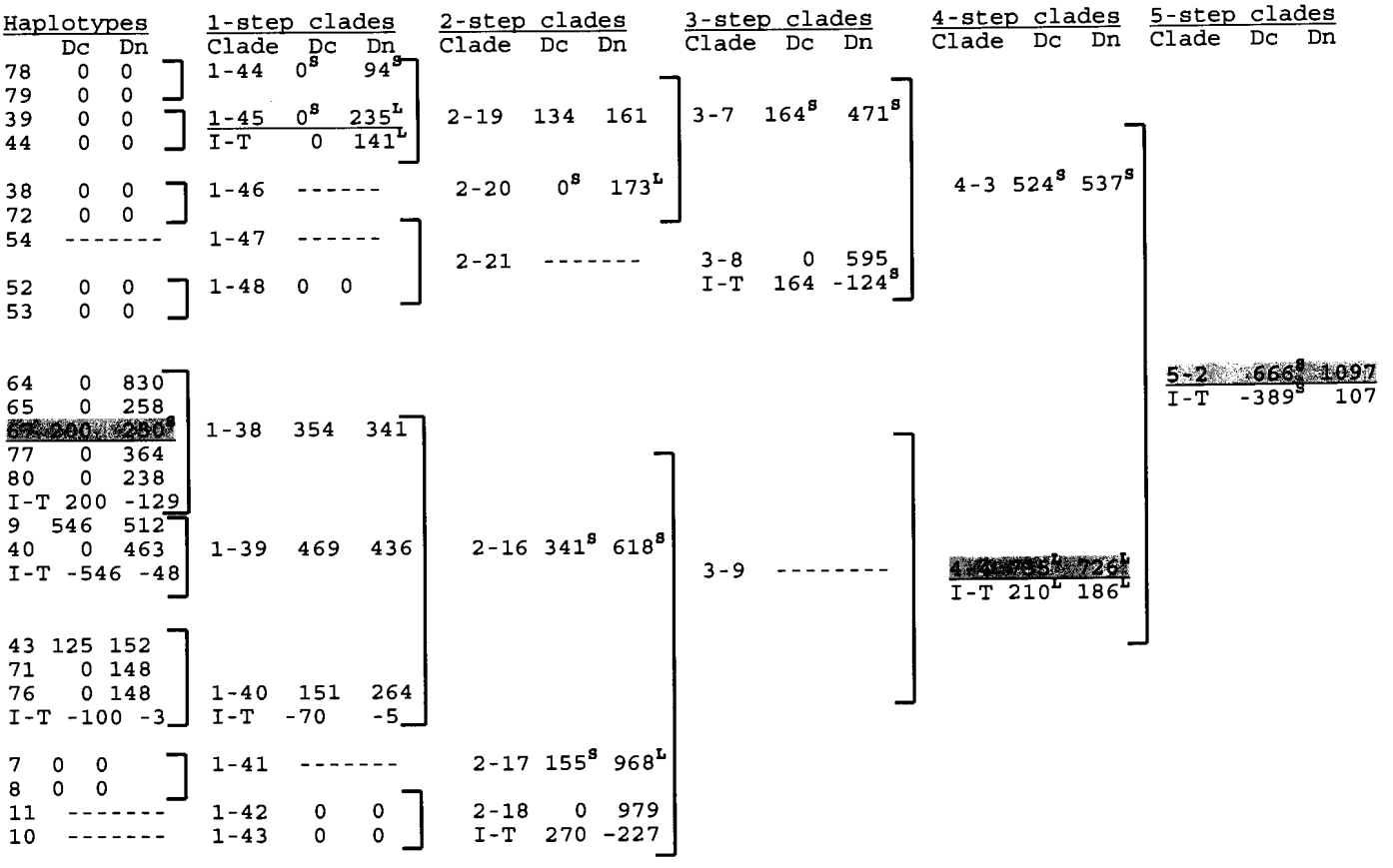


Fig.10. The results of nested clade and geographic distance analyses of mtDNA control region haplotypes in the greater Kudu. The hierarchical design and clade designations used are given in Fig. 7 and Fig. 8. Fig 10 (a) represents analysis from clade 5-1 and Fig. 10 (b) represents analysis from clade 5-2. Haplotypes are given on the far left and nesting level increases from left to right, across columns. Brackets indicate clade-nesting structure. Each clade consists of lower level nested categories. D_c and D_n indicate clade and nested clade distances, I-T indicates the interior verses tip clade test. A superscript 'S' or ' L' means that the distance measure was significantly small or large than expected at $p < 0.05$ level. Tip/interior clades were determined using predictions from the coalescent theory (Castelloe & Templeton 1994) and interior clades are underlined and shaded.

population structure and history using an inference key given in Templeton et al. (1995).

Nested clade analysis uses genealogical information to infer the basis of the observed geographic associations by statistically evaluating the expected patterns, a population exhibits under different models of population structure and historical events. The expected patterns are restricted range expansion, allopatric fragmentation and restricted gene flow via isolation by distance (Templeton et al. 1995). The inferred patterns are shown in Table 14.

Contiguous range expansion and restricted gene flow via isolation by distance was the best explanation for the distribution of mtDNA haplotypes in 1-step clades. For 2-and 3-step clades, the most frequently inferred pattern was restricted gene flow. All 4-step clades were due to past fragmentation.

At 5-step clades, the 86 haplotypes were divided into two clades, 5-1 and 5-2 (Fig. 9). The inferred pattern for clade 5-1 was restricted gene flow via isolation by distance. The restricted gene flow under isolation by distance prediction is characterised by significantly small D_C values for tip clades and significantly large D_n values for interior clades. Clade 5-1 comprises of two clades, clade 4-1 (tip) and clade 4-2 (interior). Clade 4-1 has a significantly small value for D_c, whereas clade 4-2 has a significantly large value for D_n. Additionally the D_c and D_n values for I-T were significantly large (Fig. 10a).

The inferred pattern for clade 5-2 was allopatric fragmentation. Allopatric fragmentation is characterised by significantly small D_c values at the higher clade level. The D_n value at the higher level may suddenly increase rapidly while the D_c value remains restricted. Additionally, 3-step or 4-step clades tend to be connected to the rest of the cladogram by a larger than the average number of mutational steps (Crandall & Templeton 1996). From Fig. 9, clades 4-3 and 4-4 are connected by nine mutational steps which are marginally higher than the maximum number of steps needed to resolve the cladogram (P_8 = 95%). From Fig. 10, 4-step categories within clade 5-2 show a rapid increase in D_n values while D_c values remain relatively constrained.

Table 14. Nested clades containing significant distance measures (Fig. 10) and a chain of inferred patterns for mtDNA control region haplotype data in *Tragelaphus strepsiceros*. Nested clades are given in Fig. 7 and Fig. 8. Inference key obtained from Templeton et al. (1995).

Nested clades	Chain of inference	Inferred pattern
1-15	1-2-3-4-NO	Restricted gene flow via isolation by distance
1-29	1-2-11-12-NO	Contiguous range expansion
1-38	1-2-11-12-NO	Contiguous range expansion
2-1	1-2-3-4-NO	Restricted gene flow via isolation by distance
2-11	1-2-11-17-4-NO	Restricted gene flow via isolation by distance
2-19	1-2-3-4-NO	Restricted gene flow via isolation by distance
3-1	1-2-3-4-NO	Restricted gene flow via isolation by distance
3-6	1-2-11-12-NO	Contiguous range expansion
3-9	1-2-3-5-15-16-YES	Allopatric fragmentation
4-1	1-2-3-5-15-NO	Past fragmentation
4-2	1-2-3-4-9-NO	Past fragmentation
4-3	1-2-3-5-15-NO	Past fragmentation
5-1	1-2-3-4-NO	Restricted gene flow via isolation by distance
5-2	1-2-11-17-4-9-10-YES	Allopatric fragmentation
Total	1-2-3-11-NO	Contiguous range expansion

3.3.Microsatellite DNA data

3.3.1 Test for Hardy Weinberg equilibrium and genotypic linkage disequilibrium

The test for heterozygote excess / deficiency resulted in seven of the 13 populations showing deficiency at locus CSSM18 (data not shown). Heterozygote deficit is indicative of population structure (Wahlund effect), assortative mating, presence of null alleles or selection on microsatellite loci or (Callen et al. 1993). In the case of locus CSSM18, the most probable reason for the deficiency was presence of null alleles caused by a mutation in the flanking region of the microsatellite. Additionally, an examination of the distribution of alleles at this locus in a pairwise comparison for all populations revealed that approximately 41% of all comparisons did not show significant differences in allele distribution (data not shown). This locus was therefore omitted from subsequent analyses. The test for HWE in the remaining loci using locus / population combination revealed no significance at $p < 0.01$ (Table 15). The three populations with small sample sizes (Mpumalanga, Zambia and Lukwati) did not show deviation from HWE. They were included in subsequent analyses.

Exact tests for genotypic linkage disequilibrium resulted in significant values for 12 of 364 comparisons. This proportion is lower than what would be expected by chance alone (18.2 expected from type I error at $p < 0.05$) (data not shown). A pairwise comparison of loci across all populations revealed two pairs of microsatellite loci with significant values at $p < 0.05$ (Table 16). These results indicate no physical linkage of the loci. They also indicate that there was no substructure within populations.

3.3.2 Allelic variation

Allelic variation at eight microsatellite loci was recorded from 203 greater kudu samples (Appendix II). A total of 95 alleles were scored across the eight loci in 13 populations. Of the eight loci, the most variable locus was OARFC304 with 17 alleles scored across all populations. The least variable locus was RBP3 with seven alleles scored (Appendix III). The number of alleles detected in each population varied and the highest was found in the Limpopo population (63) while the lowest was observed in the population from Mpumalanga (38) and Zambia (39). The low number of alleles scored in the populations from Zambia and Mpumalanga may be due to small sample sizes. Private alleles i.e. alleles found in only one population constituted 13.6% of the total number and were observed in six populations. The population from Zimbabwe had the highest number of private alleles (Appendix III).

Table 15. The observed (H_O) and expected (H_E) heterozygosity, inbreeding coefficient (F_{IS}) and exact probabilities of Hardy-Weinberg proportions are listed for each locus and population. For abbreviations see Table 4.

Locus		SEC	SMP	SLM	BOK	BOG	NTJ	NCO	TRU	TAB	TAR	TLK	ZAM	ZIM
RPB3														
	Ho	0.70	0.14	0.52	0.35	0.40	0.57	0.50	0.50	0.80	0.21	0.89	0.80	0.69
	He	0.57	0.14	0.45	0.37	0.42	0.49	0.45	0.55	0.62	0.44	0.63	0.64	0.71
	Fis	-0.24	-0.08	-0.17	0.005	0.03	-0.20	-0.14	0.06	-0.34	0.51	-0.50	-0.38	0.03
	P(HW)	0.22	0.97	0.75	0.28	0.37	0.53	0.47	0.05	0.01	0.02	0.29	0.36	0.55
BMC3224														
	Ho	0.27	0.57	0.48	0.41	0.50	0.50	0.28	0.33	0.40	0.11	0.44	0.20	0.13
	He	0.56	0.49	0.42	0.63	0.69	0.51	0.35	0.49	0.43	0.45	0.39	0.51	0.12
	Fis	0.36	-0.24	-0.18	0.33	0.26	-0.01	0.18	0.28	0.04	0.21	-0.22	0.43	-0.05
	P(HW)	0.06	0.26	0.97	0.15	0.87	0.78	0.33	0.14	0.02	0.04	0.83	0.03	1.00
OARFC304														
	Ho	0.41	0.86	0.84	0.94	0.78	0.93	0.94	0.42	0.67	0.58	1.00	1.00	0.75
	He	0.66	0.90	0.88	0.84	0.86	0.88	0.85	0.73	0.68	0.73	0.92	0.89	0.88
	Fis	0.37	-0.02	0.03	-0.16	0.07	-0.10	-0.15	0.40	-0.01	0.19	-0.15	-0.25	0.12
	P(HW)	0.07	0.16	0.03	0.63	0.07	0.18	0.10	0.04	0.06	0.19	0.05	0.60	0.42
OARHH64														
	Ho	0.68	0.71	0.80	0.71	0.89	0.86	0.67	0.75	0.53	0.58	0.78	0.80	0.81
	He	0.78	0.66	0.82	0.77	0.79	0.76	0.80	0.74	0.53	0.72	0.77	0.89	0.80
	Fis	0.11	-0.17	0.00	0.05	-0.16	-0.17	0.14	-0.05	-0.05	0.17	-0.07	0.00	-0.04
	P(HW)	0.42	0.16	0.34	0.09	0.33	0.82	0.30	0.07	0.71	0.57	0.55	0.66	0.16

Table 15 (continued).

Locus	SEC	SMP	SLM	BOK	BOG	NTJ	NCO	TRU	TAB	TAR	TLK	ZAM	ZIM
ETH225													
Ho	0.70	0.71	0.72	0.65	0.45	0.57	0.72	0.67	0.53	0.74	0.56	0.80	0.56
He	0.74	0.76	0.70	0.60	0.79	0.74	0.83	0.88	0.81	0.79	0.86	0.64	0.62
Fis	0.03	-0.01	-0.05	-0.11	0.42	0.19	0.11	0.21	0.32	0.04	0.32	-0.38	0.07
P(HW)	0.99	0.65	0.92	0.70	0.08	0.10	0.07	0.24	0.43	0.51	0.40	0.90	0.43
OARCP26													
Ho	0.74	0.43	0.68	0.88	0.85	0.71	0.56	0.92	0.87	0.79	0.89	1.00	0.88
He	0.76	0.73	0.82	0.78	0.75	0.79	0.78	0.83	0.79	0.85	0.90	0.91	0.69
Fis	0.01	0.36	0.15	-0.17	-0.16	0.06	0.27	-0.15	-0.14	0.04	-0.04	-0.22	-0.31
P(HW)	0.17	0.20	0.69	0.64	0.38	0.02	0.47	0.92	0.74	0.11	0.47	0.40	0.01
MAF46													
Ho	0.64	0.57	0.92	0.82	0.60	0.86	0.56	0.58	0.73	0.37	0.56	0.40	0.69
He	0.79	0.77	0.80	0.68	0.75	0.74	0.62	0.71	0.69	0.56	0.80	0.51	0.71
Fis	0.18	0.20	-0.18	-0.26	0.18	-0.20	0.08	0.14	-0.10	0.33	0.26	0.13	-0.03
P(HW)	0.05	0.85	0.86	1.00	0.61	0.99	0.43	0.09	0.27	0.19	0.14	0.26	0.55
BMS 1237													
Ho	0.57	0.29	0.60	0.76	0.78	0.71	0.39	0.55	0.80	0.63	0.67	0.40	0.31
He	0.88	0.84	0.88	0.85	0.90	0.69	0.83	0.83	0.80	0.83	0.86	0.87	0.82
Fis	0.03	0.26	0.14	0.08	0.11	-0.07	0.15	0.03	-0.04	0.22	0.18	0.18	0.21
P(HW)	0.08	0.03	0.36	0.09	0.31	0.48	0.03	0.03	0.18	0.39	0.44	0.02	0.03

Table 16. Summary of genotypic linkage disequilibrium observed from pairwise comparison of eight microsatellite loci in 13 greater kudu populations. The probability test was performed using the Markov chain algorithm (see material and methods).

Locus pair			Chi-square	d.f.	p-value
BMC3224	&	BMS 123	16.38	24	0.874
BMC3224	&	ETH225	29.94	26	0.270
BMC3224	&	MAF46	26.10	26	0.458
BMC3224	&	OARCP26	21.40	26	0.721
BMC3224	&	OARFC30	27.62	24	0.277
BMC3224	&	OARHH64	20.70	24	0.657
ETH225	&	BMS 123	18.39	24	0.784
ETH225	&	MAF46	12.64	26	0.987
ETH225	&	OARCP26	21.92	26	0.693
MAF46	&	BMS 123	14.77	24	0.927
OARCP26	&	BMS 123	17.88	24	0.809
OARCP26	&	MAF46	46.01	26	0.019*
OARFC30	&	BMS 123	13.92	22	0.904
OARFC30	&	ETH225	33.95	24	0.086
OARFC30	&	MAF46	10.70	24	0.991
OARFC30	&	OARCP26	18.99	24	0.753
OARFC30	&	OARHH64	24.67	22	0.099
OARHH64	&	BMS 123	22.25	24	0.564
OARHH64	&	ETH225	43.89	24	0.021*
OARHH64	&	MAF46	22.88	24	0.527
OARHH64	&	OARCP26	33.25	24	0.099
RPB3	&	BMC3224	26.39	26	0.442
RPB3	&	BMS 123	20.55	24	0.665
RPB3	&	ETH225	15.67	26	0.944
RPB3	&	MAF46	31.97	26	0.194
RPB3	&	OARCP26	30.25	26	0.258
RPB3	&	OARFC30	26.50	24	0.329
RPB3	&	OARHH64	19.07	24	0.748

* indicates significance at the 0.05 level

Significant differences were observed in allele frequency distribution at each of the eight microsatellite loci using Fisher's exact test ($p < 0.001$) (data not shown). However, an examination of pairwise comparison of populations using all loci revealed non-significant differences ($p < 0.05$) in seven out of 78 comparisons. The seven comparisons are Limpopo and Mpumalanga, Limpopo and Okavango, Limpopo and Lukwati, Okavango and Corona, Otjiwarongo and Corona, Ruaha and Tabora and Lukwati and Zambia. Except for Limpopo and Lukwati, and Limpopo and Okavango, the above result shows that there were no significant differences in allele frequency distribution in adjacent greater kudu populations. The Eastern Cape population consistently exhibited highly significant differences in the distribution of allele frequencies in all pairwise comparisons.

The overall levels of genetic diversity across the 13 greater kudu populations were moderate to high (Table 17) with an average expected heterozygosity of 0.7038 ± 0.0802. The mean observed heterozygosity ranged from 0.500 ± 0.0806 for the population from Arusha in Tanzania to 0.7143 ± 0.075 for the population from Otjiwarongo in Namibia. The mean estimated gene diversity ranged from 0.6607 ± 0.1314 for the population in Mpumalanga in South Africa to 0.7655 ± 0.0998 for the population from Lukwati in Tanzania. There was no significant difference between the observed and the expected heterozygosity values within the 13 greater kudu populations ($r^2 = 0.273$, $p < 0.05$), which suggests that, for the most part, the populations are in Hardy-Weinberg equilibrium (HWE). The average gene diversity estimate obtained in this study (0.704 ± 0.080) was similar to previously reported estimates in the African buffalo using 14 microsatellite loci (0.759) (Van Hooft et al. 2000) and in a global genetic survey of cattle using 20 microsatellite loci (0.709) (Loftus et al. 1999).

A positive correlation was found between the number of samples per population and the allelic diversity (average number of alleles per locus) ($r^2 = 0.699$, $p < 0.05$). However, there was no correlation between the number of samples and expected heterozygosity ($r^2 = 0.008$, $p < 0.05$). There was also no significant correlation between the average number of alleles per locus and the expected heterozygosity ($r^2 = 0.030$, $p < 0.05$).

Table 17. The mean observed (H_O) and expected (H_E) heterozygosity values obtained from the eight microsatellite loci for each of the 13 greater kudu populations. H_O and H_E calculated according to Nei (1987), n refers to sample size and A refers to the average number of alleles per locus.

Population	Code	n	H_O	H_E	A (full sample)[a]	A (uniform sample)[b]
Eastern Cape	SEC	23	0.58 ± 0.06	0.71 ± 0.05	6.25	5.13
Mpumalanga	SMP	7	0.53 ± 0.13	0.66 ± 0.13	4.75	-
Limpopo	SLM	25	0.69 ± 0.05	0.72 ± 0.06	7.75	6.75
Okavango	BOK	18	0.69 ± 0.07	0.68 ± 0.06	6.50	6.38
Ghanzi	BOG	20	0.65 ± 0.07	0.74 ± 0.06	7.00	6.50
Otjiwarongo	NTJ	15	0.71 ± 0.07	0.70 ± 0.06	6.50	6.50
Corona	NCO	18	0.57 ± 0.07	0.68 ± 0.07	6.75	6.50
Ruaha	TRU	12	0.58 ± 0.08	0.72 ± 0.07	5.38	-
Tabora	TAB	15	0.66 ± 0.07	0.66 ± 0.06	5.63	5.63
Arusha	TAR	20	0.50 ± 0.08	0.67 ± 0.06	7.00	6.13
Lukwati	TLK	9	0.72 ± 0.10	0.76 ± 0.09	6.38	-
Zambia	ZAM	5	0.67 ± 0.17	0.73 ± 0.13	4.63	-
Zimbabwe	ZIM	16	0.60 ± 0.08	0.66 ± 0.08	5.88	5.75

Average H_E =0.704 ± 0.080

[a]The average number of alleles per locus was calculated using all the samples in each population.

[b]A uniform sample size (15 randomly chosen) was used to calculate the average number of alleles per locus in each population.

The average number of alleles observed per locus for each population is considered a good measure of genetic variability provided that the sample sizes are more or less the same for each population and the populations are at mutation-drift equilibrium (Nei 1987). In order to remove bias due to unequal sample size, the average number of alleles per locus was calculated using 15 samples, randomly chosen from each population. Assuming mutation-drift equilibrium in each population, analysis was performed for nine populations excluding four (Mpumalanga, Zambia, Lukwati and Ruaha) with small sample sizes (Table 17). The results indicate that the Eastern Cape population had the lowest number of alleles detected per locus with an average of 5.13. This reduction in allelic diversity may be due to genetic isolation, historical population bottlenecks or founder effects.

3.3.3 *Phylogenetic relationships*

The phylogenetic analysis of microsatellite variation across the 13 populations did not show evidence of geographical structure; however, there was some (albeit weak) evidence of grouping of populations from adjacent regions (Fig. 11). At the continental level, there were two weakly supported groups (55% bootstrap support). Phylogenetic relationships generated using other microsatellite distance measures revealed similar results (data not shown).

3.3.4 Population genetic subdivision

Pairwise analysis of population differentiation revealed generally low Φ_{ST} and R_{ST} estimates derived from the eight microsatellite loci (Table 18). Φ_{ST} estimates ranged from 0.001 to 0.133 with an average of 0.046. With the exception of six, all pairwise comparisons were significantly different (Table 18). The population from the Eastern Cape had the highest Φ_{ST} values in all pairwise comparisons (average = 0.108). In the case of R_{ST} estimates, approximately 55% (43 out of 78) of the comparisons were not significant at $p < 0.05$. The reasons for these findings are two fold; first, the microsatellite loci used may not strictly adhere to stepwise mutation model (SMM) assumptions. Secondly, R_{ST} measures the variance in allele size and takes into account differences in sample size (Slatkin 1995). In this case, the variance within some populations may be greater than between populations resulting in non-significance.

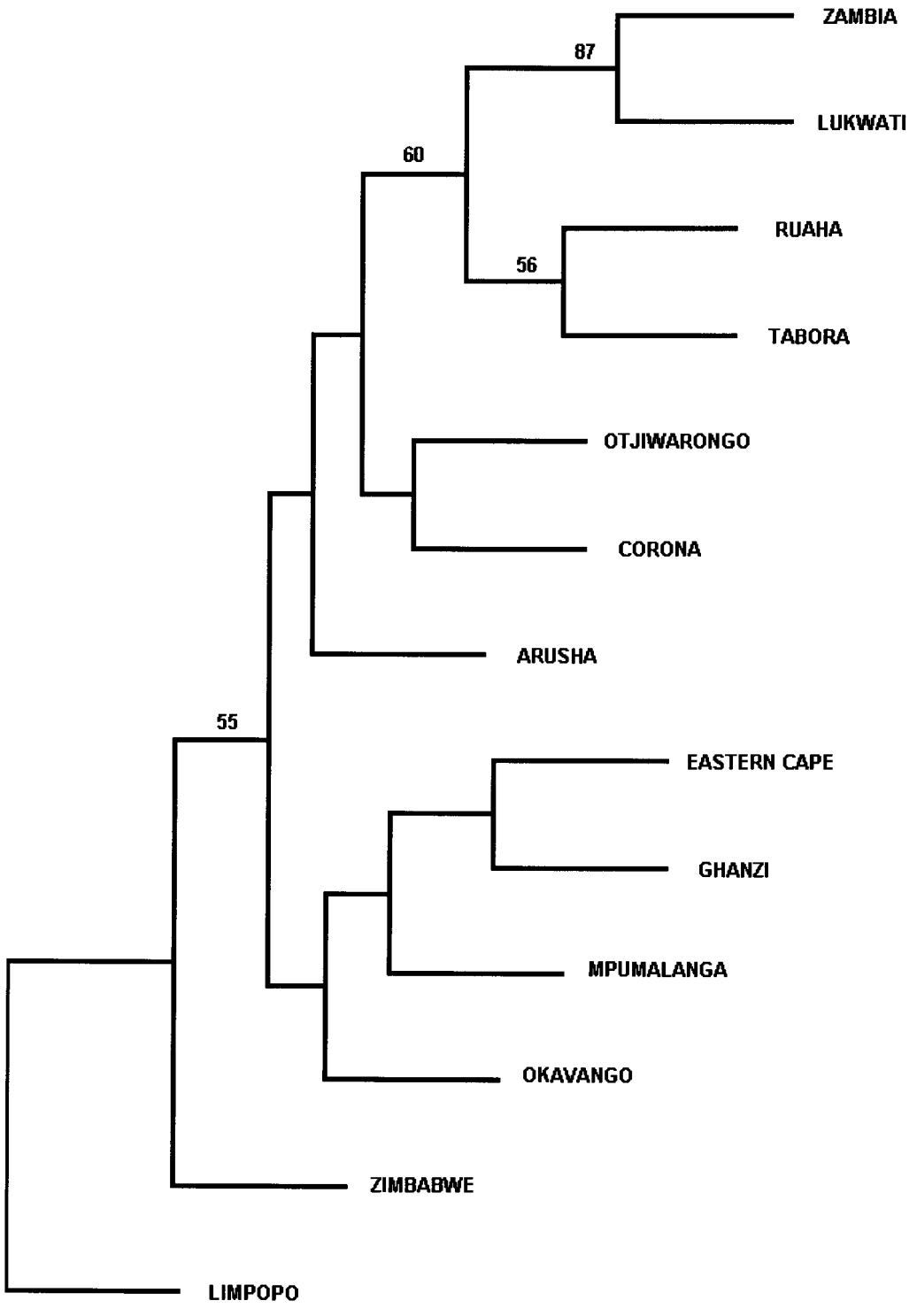


Fig. 11. Amid-point rooted neighbour-joining phylogram showing phylogenetic relationships among 13 greater Kudu populations based on genetic variation at eight microsatellite loci. The tree was reconstructed using the proportion of shared alleles (1-p) distance measure. Values above branches represent bootstrap support > 50 %.

Table 18. Pairwise comparison of Φ_{ST} and R_{ST} values in 13 greater kudu populations based on eight microsatellite loci. Values below the diagonal represent Φ_{ST} estimates while values above represent R_{ST} estimates.

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	Eastern Cape		0.270*	0.258*	0.241*	0.208*	0.254*	0.306*	0.389*	0.443*	0.298*	0.343*	0.342*	0.337*
2	Mpumalanga	0.111*		0.055	0.055	0.077*	0.039*	0.067*	0.196*	0.295*	0.131*	0.063	0.014*	0.215
3	Limpopo	0.093*	0.025*		0.004	0.014	0.002	0.007	0.026	0.071*	0.012	0.013	0.018	0.052*
4	Okavango	0.119*	0.042*	0.006		0.012	0.017	0.002	0.024	0.051*	0.033*	0.046	0.018	0.098*
5	Ghanzi	0.070*	0.028*	0.013*	0.008		0.005	0.014	0.060*	0.106*	0.050*	0.030	0.047	0.067*
6	Otjiwarongo	0.106*	0.023*	0.031*	0.041*	0.039*		0.021	0.033	0.070*	0.033*	0.021	0.009	0.089*
7	Corona	0.100*	0.035*	0.021*	0.019*	0.023*	0.001		0.002	0.047	0.012	0.001	0.002	0.089*
8	Ruaha	0.102*	0.070*	0.041*	0.051*	0.043*	0.043*	0.016*		0.007	0.005	0.007	0.024	0.113*
9	Tabora	0.127*	0.112*	0.068*	0.076*	0.077*	0.055*	0.033*	0.002		0.053*	0.089	0.108	0.146*
10	Arusha	0.126*	0.084*	0.032*	0.040*	0.048*	0.060*	0.024*	0.037*	0.034*		0.009	0.009	0.061*
11	Lukwati	0.097*	0.048*	0.013*	0.044*	0.031*	0.036*	0.028*	0.024*	0.054*	0.054*		0.069	0.039
12	Zambia	0.133*	0.072*	0.019*	0.027*	0.030*	0.049*	0.019*	0.035*	0.051*	0.0219	0.005		0.036
13	Zimbabwe	0.109*	0.079*	0.026*	0.059*	0.055*	0.071*	0.059*	0.062*	0.073*	0.075*	0.044*	0.053*	

* indicates significance ($p<0.05$).

Out of 78 pairwise comparisons, 31 had lower estimates of R_{ST} than Φ_{ST}. Estimates for R_{ST} are expected to be higher than those for Φ_{ST} when populations have evolved independently and when divergence time is such that drift and mutation contribute significantly to genetic differentiation (Slatkin 1995). The size of the bias towards higher estimates of R_{ST} is expected to increase with time of separation. Genetic drift and mutation become important causes of genetic differentiation when estimates of Φ_{ST} and R_{ST} are ≥ 0.2 indicating a migration rate of less than one migrant per generation (Goodman 1998). The Eastern Cape population consistently exhibited R_{ST} estimates of > 0.2 in all pairwise comparisons and an average of 0.307. This indicates that genetic differentiation in this population is primarily due to drift and mutation.

In an attempt to identify the most probable geographical partitioning in the greater kudu, populations were categorised into several hypothetical groups (data not shown). The highest estimates of Φ_{ST} (0.1037) and R_{ST} (0.276) were obtained when the 13 populations were divided into two groups with the population from the Eastern Cape in one group and the rest of the populations in the other. These findings are not indicative of geographic partitioning in the greater kudu given the low Φ_{ST} and R_{ST} values, but rather suggest a pattern that may be interpreted as isolation by distance over most of the species' range.

3.3.5 Assignment test results

Ninety-two of the 203 individuals (45%) were assigned to their correct populations (Table 19). Most of the mis-assigned individuals were generally distributed in neighbouring source populations. The Eastern Cape population had the highest proportion (0.78) of individuals correctly assigned while the population from Corona and Lukwati had the least (0.22). The likelihood that individuals from two populations would assign to either population was plotted on a scatter diagram. The scatter plot generated shows the relative amount of relatedness among populations. A tight cluster would represent individuals from closely related populations with a lack of overlap reflecting significant differences among populations. A scatter plot of log likelihood scores from the populations from Eastern Cape and Otjiwarongo (Fig. 12), and from Arusha and Zimbabwe (Fig. 13) shows overlap of genotypes. Pairwise comparison of the remaining populations resulted in similar associations. It is worth noting that the power of the test depends on the number of loci used (Waser & Strobeck 1998). This

suggests that the eight loci may not be sufficient to provide higher resolution or separate the genotypes.

Table 19. The proportion of individuals assigned to each of the 13 greater kudu population using the assignment test. The proportion of Individuals correctly assigned to their source population is shown in bold while the proportion of individuals assigned to a population other than the source population is given below and above the diagonal.

			Population to which individuals are assigned												
Source population		2n	1	2	3	4	5	6	7	8	9	10	11	12	13
1	Eastern Cape	46	**0.783**	0.000	0.043	0.000	0.043	0.043	0.043	0.000	0.000	0.000	0.000	0.000	0.043
2	Mpumalanga	14	0.000	**0.714**	0.000	0.000	0.000	0.143	0.000	0.000	0.000	0.000	0.000	0.143	0.000
3	Limpopo	50	0.000	0.080	**0.360**	0.320	0.040	0.000	0.000	0.000	0.040	0.080	0.040	0.000	0.040
4	Okavango	36	0.000	0.056	0.167	**0.389**	0.056	0.111	0.167	0.000	0.056	0.000	0.000	0.000	0.000
5	Ghanzi	40	0.050	0.100	0.000	0.150	**0.400**	0.000	0.150	0.000	0.000	0.100	0.050	0.000	0.000
6	Otjiwarongo	30	0.000	0.067	0.067	0.067	0.000	**0.533**	0.200	0.000	0.067	0.000	0.000	0.000	0.000
7	Corona	36	0.000	0.000	0.056	0.167	0.056	0.167	**0.222**	0.056	0.111	0.111	0.056	0.000	0.000
8	Ruaha	24	0.000	0.000	0.000	0.000	0.000	0.000	0.083	**0.417**	0.167	0.333	0.000	0.000	0.000
9	Tabora	30	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.267	**0.467**	0.067	0.067	0.000	0.067
10	Arusha	40	0.000	0.050	0.150	0.000	0.050	0.000	0.100	0.150	0.150	**0.350**	0.000	0.000	0.000
11	Lukwati	18	0.000	0.000	0.222	0.000	0.111	0.000	0.000	0.000	0.222	0.000	**0.222**	0.222	0.000
12	Zambia	10	0.000	0.000	0.000	0.000	0.200	0.000	0.000	0.000	0.000	0.200	0.200	**0.400**	0.000
13	Zimbabwe	32	0.000	0.000	0.000	0.000	0.063	0.000	0.063	0.000	0.063	0.188	0.000	0.000	**0.625**

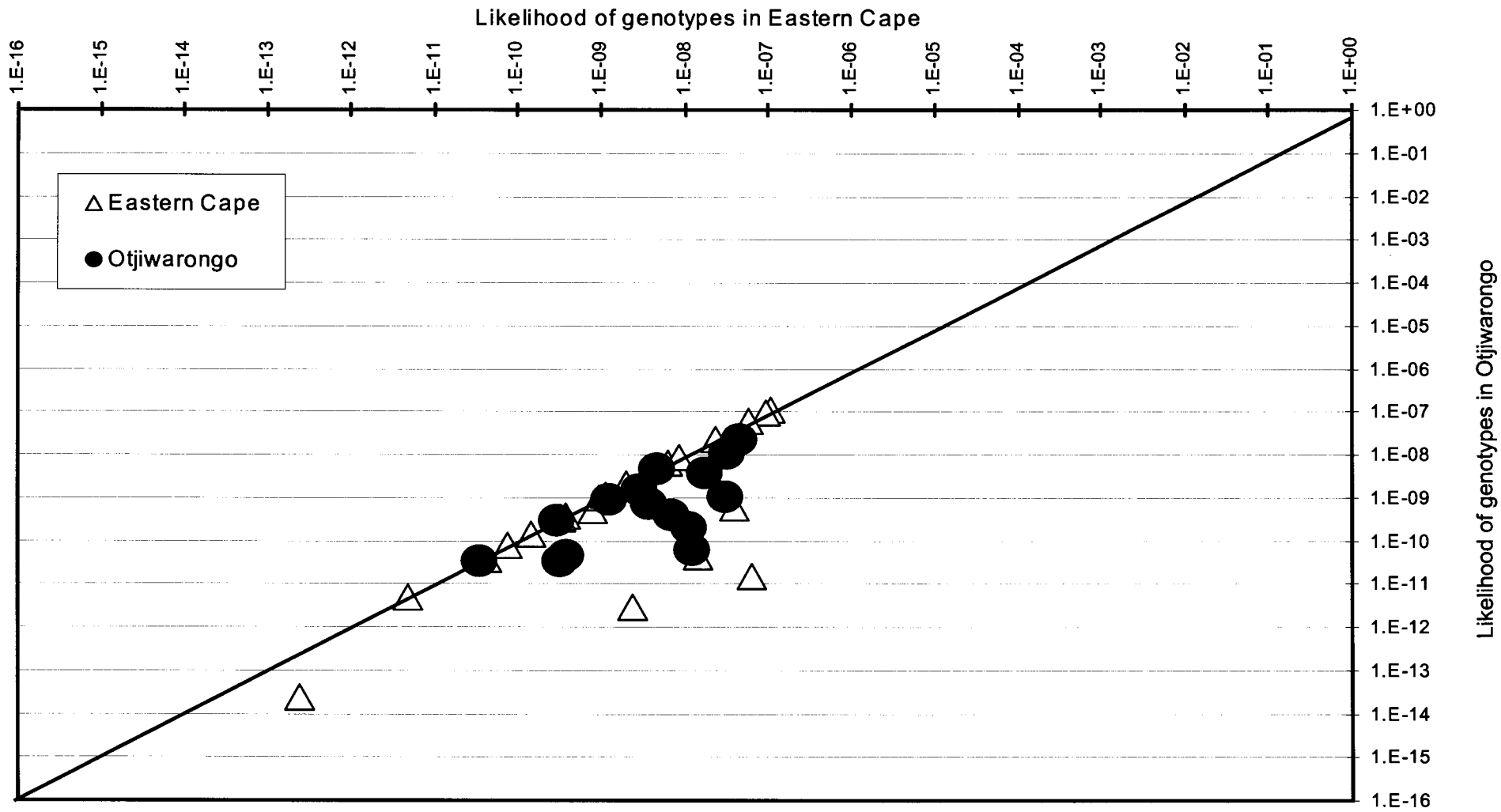


Fig. 12. The assignment of individuals to the population from the Eastern Cape (23) and Otjiwarongo (15) using the logarithm of likelihood scores calculated from allele frequencies.

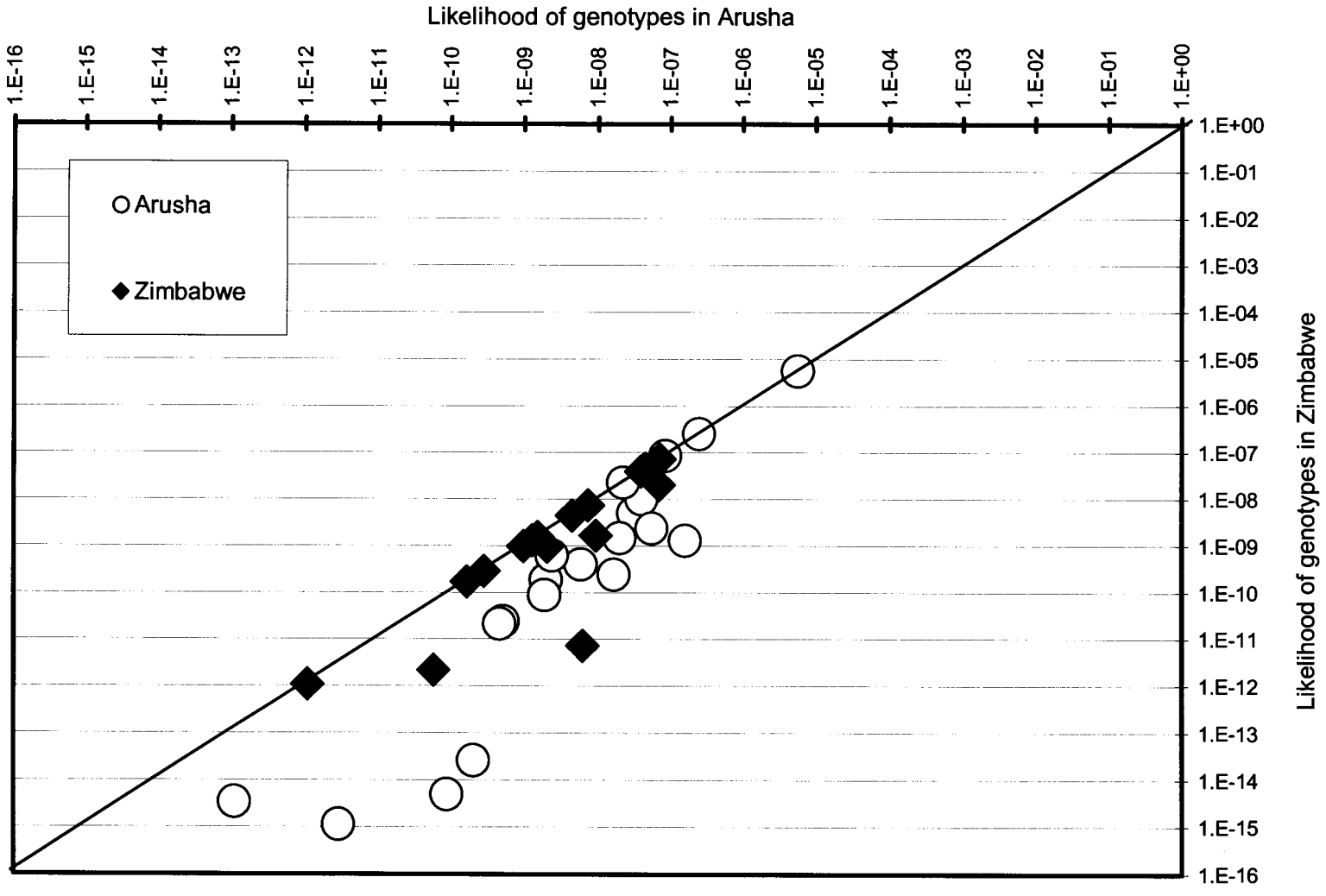


Fig. 13. The assignment of individuals to the population from Arusha (20) and Zimbabwe (16) using the logarithm of likelihood scores calculated from allele frequencies.

CHAPTER 4

Discussion

Previous analyses of phylogeographic data have depended on estimation of haplotype trees and their geographic distribution to make biological inference by visual inspection of how haplotypic networks overlay upon geography (Avise 1998). These analyses do not make full use of historical genealogical information in the data and are limited in several ways. First, they do not include estimation and comparison of competing evolutionary hypotheses that best explain genetic patterns observed in extant populations. In this regard, the recent development of the nested clade analysis of phylogeographic data offers a useful framework in which to test various hypotheses (Templeton et al. 1995, Templeton 1998). Second, these approaches are limited in inferring the dynamics of historical demographic processes. The analysis of mismatch distribution provides a framework in which to estimate the magnitude of demographic changes in historical populations, under the hypotheses of equilibrium or population expansion (Rogers & Harpending 1992, Schneider & Excoffier 1999). The combined use of size variation in eight microsatellite loci, and analyses of phylogenetic relationships, mismatch frequency distribution and nested clades of mtDNA control region sequences revealed complex patterns in the evolutionary history, demography and distribution of genetic variation in the greater kudu.

4.1 Phylogeography and population genetic structure

The levels of genetic variation in the greater kudu were low to moderate for mtDNA control region data and of the 68 haplotypes only two were shared between locations. At the local level, the data revealed shallow geographical structure, while at the continental level there were two significantly supported groups (Fig. 4). Group I was paraphyletic relative to group II and comprised of haplotypes from the Eastern Cape and from Kimberley and Namibia while group II consist of haplotypes from the rest of the range of the species together with four haplotypes from Namibia and Kimberley. The general trend of haplotypes found in group I was that they exhibited marginally longer branches and populations were more differentiated as evidenced by the grouping together of haplotypes from the Eastern Cape. This suggests that haplotypes from this group are the oldest in the greater kudu

The level of heterozygosity observed from the eight microsatellite loci was medium to high suggesting an outbred population and was comparable to results from similar studies in other African bovid species e.g. buffalo (Simonsen et al. 1998, Van Hooft et al. 2000). From the assignment test, the proportion of correctly assigned individuals

was 45%. An examination of individual populations revealed that two populations (Eastern Cape and Mpumalanga) were genetically distinct. The rest of the populations exhibited lower proportions, which indicate close relationships of genotypes due to either the sharing of recent founders, or protracted gene flow. The proportion of correctly assigned individuals, in this study was similar to that found in buffalo, but considerably lower than that found in the North American wapiti (*Cervus elephus*). The wapiti is a species that has low genetic variability due to severe bottlenecks in the past (Polziehn et al. 2001).

Examination of allele frequency distribution in a pairwise comparison of all greater kudu populations revealed that the distribution in the Eastern Cape population was significantly different from other populations in all comparisons. This implies that the population has been separated for sufficient time for genetic drift, in the absence of gene flow, to result in appreciable difference in allele frequencies. This result is supported by Φ_{ST} estimates from microsatellite loci as well as mtDNA haplotype data.

Comparisons of the level of genetic partitioning show that generally Φ_{ST} was higher for mtDNA than for microsatellite data. This supports the observation that females of the greater kudu are philopatric and males disperse longer distances, therefore males contribute more to gene flow. However, the result may also be due to the differences in the effective population size of nuclear and mtDNA markers. Mitochondrial DNA markers have a four-fold decrease in the effective population size compared to microsatellite markers, and are therefore more sensitive to the effects of bottlenecks or founder events (Avise 1994).

According to Slatkin & Barton (1989) estimates of Φ_{ST} and R_{ST} from microsatellite size variation data are expected to vary widely among populations for several reasons. Estimates for R_{ST} are expected to be higher than those for Φ_{ST} when populations have evolved independently and when divergence time is such that drift and mutation contribute to genetic differentiation (Slatkin 1995). The size of the bias towards higher estimates of R_{ST} is expected to increase with time of separation. Genetic drift and mutation become important contributors to genetic differentiation when estimates of Φ_{ST} and R_{ST} are equal or more than 0.2 indicating a migration rate of less than one (Goodman 1998). In this study, the R_{ST} estimates were higher than Φ_{ST} estimates in two populations (Eastern Cape and Mpumalanga) but lower for all pairwise

comparisons in the remaining populations. This result suggests that the two populations have evolved independently for a long time. This explanation is plausible for the Eastern Cape population given the disjunct geographical distribution, however, due to small sample size it is difficult to draw similar conclusions for the population from Mpumalanga. Moreover, the inference of an independent evolutionary history for the Eastern Cape population is supported by analysis for allele frequency distribution. For the remaining populations, in all pairwise comparisons, R_{ST} estimates were lower than Φ_{ST}, suggesting that these populations share recent founders (Slatkin 1995).

Although the Otjiwarongo population from Namibia is in close proximity to the Ghanzi and Okavango populations in Botswana, there were no close genetic relationships evident from the mtDNA haplotype data. The absence of obvious geographic barriers to gene flow suggests that the evolutionary history of these populations may have been influenced by climatic changes during the Pleistocene period rather than by vicariance. This is supported by evidence which indicate shifting patterns of vegetation types in the Kalahari after the last glacial maximum (approximately 18,000 years BP) (Lancaster 1979). During this period, vegetation in the Kalahari area (central southern Africa) included woodland and savanna grassland (Lancaster 1979). In contrast, the microsatellite data suggest demographic connections among populations in Namibia and Botswana as shown by the low R_{ST} and Φ_{ST} estimates. There are two possible explanations for this apparent discordance; first, microsatellite DNA evolves faster than mtDNA (Avise 1994) and therefore historical events are easily obscured. Second, if gene flow in the greater kudu is mainly male mediated, then genetic differentiation will not be registered at biparental loci.

4.2 Historical population demography

The distribution of pairwise nucleotide differences within a population provides a powerful way of examining demographic history of a population (Harpending et al. 1993). The shift in peaks to the right along a scale of increased pairwise difference results from the gradual accumulation in the number of differences between descendant sequences. Multimodal distribution indicates a stable population while unimodal distribution represents an expanding population. A unimodal peak at a low level of pairwise difference indicates a recently established population. Similar peaks at a higher level of pairwise difference suggest that those sets of sequences belong to a much older population (Harpending et al. 1993).

Populations from the Eastern Cape and Otjiwarongo showed multimodal distribution indicating stability in the past, however an examination of the observed distributions shows that the Eastern Cape population has the highest peak at 2 mutational steps while the population from Otjiwarongo has the highest peak at 27 mutational steps. This pattern is consistent with the interpretation that the Eastern Cape population experienced a genetic bottleneck. In accordance with this explanation, this population had significantly reduced allelic diversity. Examination of the shape of the curve for the Eastern Cape population shows that it originated from a population with a small effective size (see Fig. 10 of Rogers & Harpending 1992). Indeed, this interpretation is also supported by high haplotype and moderate nucleotide diversity indices, which suggest that the population experienced transient bottlenecks (see Grant & Bowen 1998).

Populations from the rest of the range in Ghanzi, Zimbabwe and Okavango exhibited unimodal frequency distributions and a star like phylogeny which is characteristic of expanding populations (Nee et al. 1996). Additionally, the highest peak for these populations was between 4 and 6 mutational steps indicating that these populations are of relatively recent origin. However, inference of population expansion should be viewed with caution since unimodal distributions have been shown to be influenced by factors other than sudden expansion. These factors include selective fixation of mtDNA haplotypes (Rogers & Harpending 1992, Rogers 1995), sample size (Arctander et al. 1999), and non-random mating within populations (Rogers et al. 1996).

4.3 Evolutionary history of the greater kudu

Previous studies have shown that nested clade analyses of haplotype cladograms with geographical data are more robust in detecting genetic and geographical partitioning than analyses based on analogues of F-statistics (Templeton 1998, references there in). In this study, nested clade analysis revealed significant evidence of geographic structure at several hierarchical levels. The inferred pattern for the total cladogram was one of contiguous range expansion, with the colonising individuals originating from the oldest population in clade 5-2 (Fig. 10). Estimates from root probability indicated that the oldest haplotypes came from the population from northern Namibia.

Two clades were retrieved at the highest nesting level (Fig. 9, Fig. 10). The first clade 5-1 comprises of lineages from Chad, Tanzania, Zambia, Zimbabwe, Botswana, Mpumalanga, Limpopo and KwaZulu-Natal (Fig. 10, also see group II in Fig. 4). The inferred patterns for this clade are explained by gene flow restricted by the isolation by distance model of population structure (Table 14). Under this model, expansion of populations is due to short-distance dispersal of individuals and younger haplotypes are scattered throughout the range (Templeton et al. 1995). From coalescence theory and outgroup root probability, clade 5-1 (Fig. 10) occupies a tip position indicating that the majority of haplotypes are of recent origin (Crandall & Templeton 1993).

The second clade (5-2) comprises of haplotypes from the Eastern Cape population, Kimberley and Namibia. Patterns observed in this clade are explained by allopatric fragmentation. Allopatric fragmentation is a historical occurrence that describes events in which an ancestral population is subdivided into two or more sub-populations that are currently non-overlapping (Hudson 1990). The clade that identifies the fragmentation event separates clade 5-2 into northern (clade 2-16) and southern (clades 2-17 and 2-18) populations (Fig. 10b). The northern population comprises nine haplotypes from Namibia and one from Kimberley (Fig. 10b, Table 12). The southern population comprises three haplotypes from the Eastern Cape population and one (haplotype number 8, see Table 12) shared between Eastern Cape and Otjiwarongo in Namibia. This implies that although the population from Kimberley is geographically isolated and in close proximity to the Eastern Cape, this population originated from northern Namibia (Otjiwarongo and Etosha). This suggests that the isolation of the Kimberley population from Namibia (Fig. 1) is a recent event.

During the 1950s and 1960s, several large antelopes, including the greater kudu began invading the Karoo (MacDonald 1992). The reason for the invasion was overgrazing by domestic livestock, which resulted in encroachment of the Karoo by woodland plant species such as *Acacia karoo* and *Lycium sp.* along drainage lines (Acocks 1964). It is conceivable that migrations between populations in the Eastern Cape and Kimberley may have resulted in mating between greater kudu from the two locations. However, this inference is not evident due to insufficient time for haplotypes from immigrants to be fixed or reach detectable levels.

One haplotype (number 8, see Table 12) was shared between populations from the Eastern Cape and Otjiwarongo in northern Namibia. There are two possible explanations for this observation; first, there was secondary contact between the two populations after fragmentation following vicariance. Second, due to insufficient time, ancestral haplotypes in the two populations have not been sorted. The first explanation of secondary contact would require range expansion to bring the two populations together. This explanation, however, is not supported by the nested clade analysis.

From the nested clade analysis, there is strong evidence to suggest that the greater kudu originated from Namibia. This interpretation is supported by studies of other arid adapted species (Arctander et al. 1999). The narrow distribution of the oldest clade 5-2 (Namibia, Kimberley and Eastern Cape) suggests that vast areas of sub-Saharan Africa were covered by unsuitable habitat for the greater kudu. The widespread distribution of haplotypes in clade 5-1 suggests that mtDNA lineages in this clade may be of recent origin, an interpretation that is supported by analyses from mismatch frequency distribution.

4.4 Influence of Pleistocene climatic changes on population distribution

From the fossil record, the greater kudu appeared approximately two million years ago, however, from nested clade, mismatch frequency distribution and phylogenetic analyses, greater kudu sequences suggest more recent coalescence than would be expected from the current population size. Assuming equilibrium between genetic drift and mutation, the expected coalescence time in generations is $2N_{E(F)}$ where $N_{E(F)}$ is the effective number of females in the population (Hartl & Clark 1989). For the greater kudu, the current census size throughout the range stands at hundreds of thousands, which when calibrated for $N_{E(F)}$ suggests a much older coalescence time. A plausible explanation for the recent coalescence is that the greater kudu experienced wide fluctuations in the mean effective population size during the Pleistocene glacial-interglacial cycles that resulted in expansion and contraction of the geographical range of the greater kudu. Wide fluctuations in the mean effective population size have been shown to result in more recent coalescence times than predicted from census population size (Avise et al. 1984). During glacial periods (cold and dry conditions), the species range would have contracted leaving several geographically isolated populations. It is possible that some of these populations became extinct, while those that prevailed, went through severe bottlenecks. The repeated expansion and

contraction to refugia that greater kudu populations experienced may have drastically reduced the genetic variability (due to founder effects), leading to shallow genetic structure and lack of geographic partitioning. Another explanation for the lack of phylogeographic structure is that the greater kudu are large antelope that exhibit moderate maternal philopatry (Kingdon 1982) and males are capable of moving over large distances. Consequently, during interglacial periods movement of individuals between previously geographically isolated populations would obscure past phylogeographic structures in many populations.

CHAPTER 5

Conclusion

The survey of microsatellite size variation and the combined use of phylogeographic, nested clade and mismatch analyses of mtDNA sequence data presented in this thesis has illuminated many aspects of evolutionary history, phylogeography and historical demography in the greater kudu. These aspects have significant implications for the conservation and management of the species throughout the range. The results indicate a generally outbred species, which lacks deep geographical divisions throughout the distribution. The results also show evidence of recent origin for all populations, with the exception of populations from Namibia, Kimberley and the Eastern Cape of South Africa.

Four subspecies have previously been described in the greater kudu based on morphological features such as colour, number of stripes and horn length (Ansell 1971). From this study, there is no evidence to support the existence of populations, which could be viewed as subspecies. These results therefore call for a re-examination of the traditionally recognised subspecies within the greater kudu.

5.1 Implications for conservation and management of greater kudu populations

Over the last one hundred years, many greater kudu populations decreased in numbers due to hunting for trophy and loss of natural habitats leading to fragmentation and isolation. Nevertheless, sufficient numbers remain in the wild and the overall status throughout the range is thought to be satisfactory. According to the IUCN (1996), the greater kudu is classified as a species in the lower risk category whose continued survival depends upon active conservation measures. The conservation actions taken should aim to preserve adaptive diversity and evolutionary processes across the geographical range of the species (Crandall et al 2000), rather than on preserving distinct intraspecific phenotypes (Moritz 1995, Moritz 1999).

Results from this study show that populations from Namibia, Kimberley and the Eastern Cape form a genetically distinct group. Although this group does not exhibit reciprocal monophyly of the mtDNA control region, efforts should be made towards preserving what appears to be a distinct evolutionary pathway. This group should certainly be regarded as a management unit (MU). Within this group there is evidence that the population from the Eastern Cape exhibits significant differentiation at both mtDNA control region sequences and microsatellite loci. The genetic distinctiveness of the Eastern Cape population is supported by the fact that individuals in this population are

considerably smaller in size, have shorter horns, have fewer stripes and are pale coloured compared to greater kudu found in other populations in southern and eastern Africa (SCI 1997). From a conservation and management perspective, movement of individuals from neighbouring areas, for instance Mpumalanga or KwaZulu-Natal, to this population should be discouraged as this would lead to mixing of individuals from populations with different evolutionary histories. Additionally, this will lead to potential loss of genes that are unique and possibly adaptive in the Eastern Cape population given the population's historical isolation.

The remaining populations constitute the second MU, on the grounds that they form a distinct group, which exhibits weak geographic partitioning. The degree of differentiation in this group suggests demographic connection that may have been caused by shared ancestry or protracted gene flow. Lack of geographical structure may also be interpreted as an outcome of past episodes of isolation followed by admixture. From an evolutionary perspective, admixture was probably a common feature of the historical demography of the greater kudu, which has recently been interrupted by human disturbance. Translocation or establishment of dispersal corridors to facilitate movement of individuals between adjacent populations within this management unit, is therefore a management option that would approximate natural historical processes. This option should be explored for areas where the greater kudu have been wiped out, are reduced in numbers or where human activities prohibit natural migration. However, before identifying source populations for translocation, it is imperative to establish the impact of fitness-related phenotypic differences (Hedrick 1999). For instance, adult greater kudu found in Chad have short horns and small body size compared to those found in parts of eastern and southern Africa. If access to females is dependent upon body size and horn length, then translocating males from central Africa to southern Africa will result in those males having no contribution to the gene pool.

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Appendices

Appendix I

Appendix I. Estimates of sequence divergence between pairs of *Acacia karroo* haplotypes obtained using the HKY85 model with gamma correction. Refer to Fig. 2 for haplotype numbers.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1															
2	0.0033														
3	0.0065	0.0065													
4	0.0049	0.0049	0.0016												
5	0.0049	0.0049	0.0049	0.0032											
6	0.0065	0.0065	0.0032	0.0016	0.0016										
7	0.0049	0.0049	0.0049	0.0032	0.0016	0.0016									
8	0.0082	0.0049	0.0115	0.0098	0.0098	0.0115	0.0098								
9	0.0065	0.0032	0.0098	0.0082	0.0049	0.0065	0.0049	0.0049							
10	0.0115	0.0082	0.0148	0.0132	0.0098	0.0115	0.0098	0.0032	0.0049						
11	0.0098	0.0065	0.0132	0.0115	0.0115	0.0132	0.0115	0.0016	0.0065	0.0016					
12	0.0065	0.0065	0.0098	0.0082	0.0082	0.0098	0.0082	0.0049	0.0098	0.0082	0.0065				
13	0.0049	0.0016	0.0082	0.0065	0.0065	0.0082	0.0065	0.0032	0.0016	0.0065	0.0049	0.0082			
14	0.0033	0.0033	0.0065	0.0049	0.0049	0.0065	0.0049	0.0082	0.0065	0.0115	0.0098	0.0032	0.0049		
15	0.0049	0.0049	0.0082	0.0065	0.0033	0.0049	0.0033	0.0098	0.0049	0.0098	0.0115	0.0049	0.0065	0.0016	
16	0.0033	0.0033	0.0065	0.0049	0.0049	0.0065	0.0049	0.0082	0.0065	0.0115	0.0098	0.0065	0.0049	0.0033	0.0049
17	0.0507	0.0469	0.0507	0.0526	0.0525	0.0545	0.0525	0.0468	0.0468	0.0504	0.0486	0.0506	0.0450	0.0488	0.0506
18	0.0469	0.0469	0.0507	0.0526	0.0525	0.0545	0.0524	0.0468	0.0468	0.0504	0.0486	0.0506	0.0450	0.0488	0.0506
19	0.0489	0.0451	0.0528	0.0508	0.0507	0.0526	0.0506	0.0450	0.0450	0.0486	0.0468	0.0488	0.0432	0.0470	0.0488
20	0.0099	0.0065	0.0133	0.0116	0.0115	0.0132	0.0115	0.0082	0.0065	0.0115	0.0098	0.0132	0.0049	0.0099	0.0115
21	0.0099	0.0099	0.0133	0.0116	0.0115	0.0132	0.0115	0.0115	0.0098	0.0148	0.0132	0.0132	0.0082	0.0099	0.0115
22	0.0451	0.0414	0.0451	0.0432	0.0431	0.0450	0.0431	0.0450	0.0450	0.0486	0.0468	0.0450	0.0432	0.0432	0.0450
23	0.0488	0.0488	0.0526	0.0546	0.0545	0.0564	0.0545	0.0487	0.0487	0.0523	0.0505	0.0525	0.0469	0.0507	0.0525
24	0.0065	0.0032	0.0098	0.0082	0.0082	0.0098	0.0082	0.0049	0.0065	0.0082	0.0065	0.0065	0.0049	0.0065	0.0082
25	0.0049	0.0016	0.0082	0.0065	0.0065	0.0082	0.0065	0.0033	0.0049	0.0065	0.0049	0.0049	0.0032	0.0049	0.0065
26	0.0451	0.0451	0.0489	0.0470	0.0469	0.0488	0.0468	0.0488	0.0488	0.0524	0.0506	0.0450	0.0470	0.0432	0.0450
27	0.0470	0.0432	0.0508	0.0489	0.0488	0.0507	0.0487	0.0469	0.0469	0.0505	0.0487	0.0469	0.0451	0.0451	0.0469
28	0.0451	0.0451	0.0528	0.0508	0.0507	0.0526	0.0506	0.0450	0.0450	0.0486	0.0468	0.0488	0.0432	0.0470	0.0488
29	0.0489	0.0451	0.0528	0.0508	0.0507	0.0526	0.0506	0.0488	0.0488	0.0524	0.0506	0.0488	0.0470	0.0470	0.0488
30	0.0341	0.0341	0.0305	0.0287	0.0322	0.0304	0.0322	0.0377	0.0377	0.0412	0.0394	0.0358	0.0359	0.0341	0.0358
31	0.0470	0.0470	0.0508	0.0489	0.0488	0.0507	0.0487	0.0507	0.0507	0.0543	0.0525	0.0469	0.0489	0.0451	0.0469
32	0.0414	0.0414	0.0451	0.0432	0.0431	0.0450	0.0431	0.0450	0.0450	0.0486	0.0468	0.0413	0.0432	0.0395	0.0413
33	0.0415	0.0378	0.0415	0.0433	0.0432	0.0451	0.0432	0.0377	0.0377	0.0412	0.0394	0.0432	0.0359	0.0415	0.0432
34	0.0183	0.0149	0.0183	0.0166	0.0167	0.0150	0.0167	0.0166	0.0116	0.0166	0.0183	0.0218	0.0132	0.0183	0.0167
35	0.0470	0.0432	0.0470	0.0451	0.0488	0.0469	0.0488	0.0431	0.0431	0.0467	0.0449	0.0488	0.0414	0.0470	0.0488
36	0.0488	0.0450	0.0488	0.0469	0.0470	0.0451	0.0470	0.0449	0.0414	0.0449	0.0467	0.0506	0.0431	0.0488	0.0470
37	0.0451	0.0451	0.0489	0.0470	0.0469	0.0488	0.0469	0.0450	0.0450	0.0486	0.0468	0.0469	0.0432	0.0451	0.0469
38	0.0150	0.0116	0.0150	0.0167	0.0166	0.0183	0.0166	0.0132	0.0115	0.0165	0.0149	0.0183	0.0099	0.0150	0.0166
39	0.0116	0.0082	0.0150	0.0133	0.0132	0.0149	0.0132	0.0098	0.0082	0.0132	0.0115	0.0149	0.0065	0.0116	0.0132
40	0.0167	0.0133	0.0167	0.0184	0.0183	0.0201	0.0183	0.0149	0.0132	0.0183	0.0166	0.0201	0.0116	0.0167	0.0183
41	0.0065	0.0065	0.0099	0.0082	0.0082	0.0098	0.0082	0.0082	0.0065	0.0115	0.0098	0.0098	0.0049	0.0065	0.0082
42	0.0150	0.0150	0.0184	0.0167	0.0166	0.0183	0.0166	0.0166	0.0149	0.0200	0.0183	0.0183	0.0133	0.0150	0.0166
43	0.0167	0.0133	0.0167	0.0184	0.0183	0.0201	0.0183	0.0149	0.0132	0.0183	0.0166	0.0201	0.0116	0.0167	0.0183
44	0.0149	0.0149	0.0183	0.0166	0.0165	0.0183	0.0165	0.0165	0.0148	0.0199	0.0182	0.0183	0.0132	0.0149	0.0165
45	0.0082	0.0082	0.0116	0.0099	0.0098	0.0115	0.0098	0.0098	0.0082	0.0132	0.0115	0.0115	0.0065	0.0082	0.0098
46	0.0099	0.0065	0.0133	0.0116	0.0115	0.0132	0.0115	0.0082	0.0065	0.0115	0.0098	0.0132	0.0049	0.0099	0.0115
47	0.0115	0.0082	0.0149	0.0132	0.0099	0.0116	0.0099	0.0098	0.0049	0.0098	0.0115	0.0149	0.0065	0.0115	0.0099
48	0.0132	0.0098	0.0166	0.0149	0.0116	0.0133	0.0116	0.0115	0.0065	0.0115	0.0132	0.0166	0.0082	0.0132	0.0116
49	0.0132	0.0098	0.0166	0.0149	0.0116	0.0133	0.0116	0.0115	0.0065	0.0115	0.0132	0.0166	0.0082	0.0132	0.0116
50	0.0116	0.0116	0.0150	0.0133	0.0132	0.0149	0.0132	0.0132	0.0115	0.0165	0.0149	0.0149	0.0099	0.0116	0.0132
51	0.0132	0.0098	0.0166	0.0149	0.0116	0.0133	0.0116	0.0115	0.0065	0.0115	0.0132	0.0166	0.0082	0.0132	0.0116
52	0.0218	0.0183	0.0218	0.0201	0.0201	0.0184	0.0201	0.0200	0.0150	0.0200	0.0217	0.0253	0.0166	0.0218	0.0201
53	0.0416	0.0379	0.0454	0.0435	0.0433	0.0452	0.0433	0.0378	0.0378	0.0413	0.0395	0.0433	0.0360	0.0416	0.0433
54	0.0183	0.0149	0.0183	0.0166	0.0167	0.0150	0.0167	0.0166	0.0116	0.0166	0.0183	0.0218	0.0132	0.0183	0.0167
55	0.0082	0.0049	0.0116	0.0099	0.0098	0.0115	0.0098	0.0065	0.0049	0.0098	0.0082	0.0115	0.0033	0.0082	0.0098
56	0.0082	0.0082	0.0116	0.0099	0.0098	0.0115	0.0098	0.0098	0.0082	0.0132	0.0115	0.0115	0.0065	0.0082	0.0098
57	0.0082	0.0082	0.0115	0.0098	0.0065	0.0082	0.0065	0.0098	0.0049	0.0098	0.0115	0.0115	0.0065	0.0082	0.0065
58	0.0133	0.0099	0.0167	0.0150	0.0149	0.0166	0.0149	0.0115	0.0098	0.0148	0.0132	0.0166	0.0082	0.0133	0.0149
59	0.0116	0.0082	0.0150	0.0133	0.0132	0.0149	0.0132	0.0098	0.0082	0.0132	0.0115	0.0149	0.0065	0.0116	0.0132
60	0.0099	0.0065	0.0133	0.0116	0.0115	0.0132	0.0115	0.0115	0.0098	0.0148	0.0132	0.0132	0.0082	0.0099	0.0115
61	0.0149	0.0115	0.0183	0.0166	0.0132	0.0149	0.0132	0.0132	0.0082	0.0132	0.0148	0.0183	0.0098	0.0149	0.0132
62	0.0098	0.0065	0.0132	0.0115	0.0082	0.0099	0.0082	0.0082	0.0033	0.0082	0.0098	0.0132	0.0049	0.0098	0.0082
63	0.0133	0.0099	0.0167	0.0150	0.0149	0.0166	0.0149	0.0115	0.0098	0.0148	0.0132	0.0166	0.0082	0.0133	0.0149
64	0.0149	0.0149	0.0218	0.0201	0.0167	0.0184	0.0166	0.0166	0.0116	0.0166	0.0183	0.0218	0.0132	0.0183	0.0167
65	0.0098	0.0098	0.0132	0.0115	0.0115	0.0131	0.0115	0.0115	0.0098	0.0148	0.0131	0.0131	0.0082	0.0098	0.0115
66	0.0065	0.0065	0.0099	0.0082	0.0082	0.0098	0.0082	0.0082	0.0065	0.0115	0.0098	0.0098	0.0049	0.0065	0.0082
67	0.0148	0.0148	0.0182	0.0165	0.0165	0.0182	0.0165	0.0199	0.0182	0.0233	0.0216	0.0165	0.0165	0.0131	0.0148
68	0.0218	0.0183	0.0253	0.0236	0.0235	0.0253	0.0235	0.0200	0.0183	0.0234	0.0217	0.0235	0.0166	0.0201	0.0218

Appendix I (continued).

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1															
2															
3															
4															
5															
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7															
8															
9															
10															
11															
12															
13															
14															
15															
16															
17	0.0507														
18	0.0507	0.0132													
19	0.0489	0.0253	0.0217												
20	0.0099	0.0469	0.0431	0.0451											
21	0.0099	0.0546	0.0507	0.0489	0.0099										
22	0.0451	0.0253	0.0253	0.0065	0.0451	0.0489									
23	0.0526	0.0115	0.0016	0.0235	0.0450	0.0526	0.0271								
24	0.0065	0.0505	0.0505	0.0487	0.0098	0.0132	0.0449	0.0524							
25	0.0049	0.0487	0.0487	0.0469	0.0082	0.0115	0.0431	0.0506	0.0016						
26	0.0451	0.0289	0.0288	0.0132	0.0489	0.0528	0.0132	0.0307	0.0487	0.0469					
27	0.0470	0.0271	0.0270	0.0115	0.0470	0.0547	0.0115	0.0289	0.0468	0.0450	0.0016				
28	0.0489	0.0253	0.0183	0.0032	0.0451	0.0489	0.0065	0.0200	0.0487	0.0469	0.0132	0.0115			
29	0.0489	0.0253	0.0253	0.0132	0.0489	0.0567	0.0132	0.0271	0.0487	0.0469	0.0032	0.0016	0.0132		
30	0.0341	0.0361	0.0360	0.0235	0.0415	0.0415	0.0166	0.0380	0.0376	0.0358	0.0166	0.0183	0.0235	0.0200	
31	0.0470	0.0307	0.0306	0.0149	0.0508	0.0547	0.0149	0.0325	0.0506	0.0488	0.0016	0.0032	0.0149	0.0049	0.0183
32	0.0414	0.0289	0.0324	0.0166	0.0489	0.0489	0.0132	0.0343	0.0449	0.0431	0.0032	0.0049	0.0166	0.0065	0.0132
33	0.0415	0.0252	0.0252	0.0166	0.0415	0.0452	0.0166	0.0270	0.0413	0.0395	0.0132	0.0115	0.0166	0.0132	0.0132
34	0.0183	0.0487	0.0449	0.0469	0.0115	0.0183	0.0469	0.0468	0.0183	0.0166	0.0469	0.0450	0.0469	0.0469	0.0432
35	0.0470	0.0166	0.0235	0.0324	0.0395	0.0470	0.0324	0.0218	0.0468	0.0450	0.0324	0.0306	0.0324	0.0324	0.0289
36	0.0488	0.0183	0.0252	0.0341	0.0413	0.0488	0.0341	0.0235	0.0486	0.0468	0.0341	0.0323	0.0341	0.0341	0.0306
37	0.0451	0.0149	0.0218	0.0270	0.0451	0.0489	0.0306	0.0200	0.0487	0.0469	0.0200	0.0217	0.0270	0.0235	0.0307
38	0.0150	0.0413	0.0413	0.0432	0.0116	0.0184	0.0432	0.0431	0.0149	0.0132	0.0470	0.0451	0.0432	0.0470	0.0433
39	0.0116	0.0488	0.0488	0.0470	0.0082	0.0150	0.0470	0.0507	0.0115	0.0098	0.0508	0.0489	0.0470	0.0508	0.0396
40	0.0167	0.0431	0.0431	0.0451	0.0133	0.0201	0.0451	0.0450	0.0166	0.0149	0.0451	0.0432	0.0451	0.0451	0.0452
41	0.0065	0.0469	0.0469	0.0451	0.0065	0.0099	0.0451	0.0488	0.0098	0.0082	0.0451	0.0470	0.0451	0.0489	0.0378
42	0.0150	0.0526	0.0488	0.0432	0.0150	0.0150	0.0470	0.0507	0.0183	0.0166	0.0470	0.0489	0.0432	0.0508	0.0433
43	0.0167	0.0431	0.0431	0.0451	0.0133	0.0201	0.0451	0.0450	0.0166	0.0149	0.0489	0.0470	0.0451	0.0489	0.0452
44	0.0149	0.0524	0.0486	0.0468	0.0149	0.0183	0.0506	0.0505	0.0182	0.0165	0.0468	0.0487	0.0468	0.0506	0.0431
45	0.0082	0.0413	0.0450	0.0395	0.0082	0.0116	0.0395	0.0469	0.0115	0.0098	0.0395	0.0414	0.0395	0.0432	0.0396
46	0.0099	0.0394	0.0431	0.0377	0.0065	0.0133	0.0377	0.0450	0.0098	0.0082	0.0414	0.0395	0.0377	0.0414	0.0415
47	0.0115	0.0412	0.0449	0.0394	0.0082	0.0149	0.0394	0.0468	0.0115	0.0098	0.0431	0.0413	0.0394	0.0431	0.0432
48	0.0132	0.0430	0.0468	0.0413	0.0098	0.0166	0.0413	0.0487	0.0132	0.0115	0.0450	0.0431	0.0413	0.0450	0.0451
49	0.0132	0.0430	0.0468	0.0413	0.0098	0.0166	0.0413	0.0487	0.0132	0.0115	0.0450	0.0431	0.0413	0.0450	0.0451
50	0.0116	0.0488	0.0488	0.0470	0.0116	0.0150	0.0470	0.0507	0.0149	0.0132	0.0470	0.0489	0.0470	0.0508	0.0396
51	0.0132	0.0506	0.0506	0.0488	0.0098	0.0166	0.0488	0.0525	0.0132	0.0115	0.0526	0.0507	0.0488	0.0526	0.0414
52	0.0218	0.0525	0.0487	0.0507	0.0115	0.0218	0.0507	0.0506	0.0217	0.0200	0.0507	0.0488	0.0507	0.0507	0.0470
53	0.0416	0.0323	0.0433	0.0342	0.0416	0.0454	0.0342	0.0415	0.0414	0.0396	0.0378	0.0360	0.0342	0.0378	0.0307
54	0.0183	0.0525	0.0487	0.0507	0.0115	0.0183	0.0507	0.0506	0.0183	0.0166	0.0507	0.0488	0.0507	0.0507	0.0470
55	0.0082	0.0488	0.0450	0.0432	0.0082	0.0116	0.0470	0.0469	0.0082	0.0065	0.0470	0.0451	0.0432	0.0470	0.0396
56	0.0082	0.0450	0.0488	0.0432	0.0082	0.0116	0.0432	0.0507	0.0115	0.0098	0.0432	0.0451	0.0432	0.0470	0.0396
57	0.0082	0.0449	0.0487	0.0431	0.0115	0.0115	0.0431	0.0506	0.0115	0.0098	0.0431	0.0450	0.0431	0.0469	0.0395
58	0.0133	0.0469	0.0506	0.0450	0.0099	0.0167	0.0450	0.0526	0.0132	0.0115	0.0488	0.0469	0.0450	0.0488	0.0414
59	0.0116	0.0450	0.0487	0.0432	0.0082	0.0150	0.0432	0.0507	0.0115	0.0098	0.0469	0.0450	0.0432	0.0469	0.0395
60	0.0099	0.0469	0.0506	0.0450	0.0099	0.0167	0.0413	0.0526	0.0098	0.0082	0.0450	0.0432	0.0450	0.0450	0.0377
61	0.0149	0.0448	0.0486	0.0430	0.0149	0.0183	0.0430	0.0505	0.0148	0.0132	0.0468	0.0449	0.0430	0.0468	0.0431
62	0.0098	0.0430	0.0468	0.0413	0.0098	0.0132	0.0413	0.0487	0.0098	0.0082	0.0450	0.0431	0.0413	0.0450	0.0414
63	0.0133	0.0469	0.0506	0.0450	0.0099	0.0167	0.0450	0.0526	0.0132	0.0115	0.0488	0.0469	0.0450	0.0488	0.0414
64	0.0183	0.0487	0.0486	0.0468	0.0149	0.0218	0.0468	0.0506	0.0183	0.0166	0.0506	0.0487	0.0431	0.0506	0.0469
65	0.0098	0.0504	0.0504	0.0486	0.0132	0.0132	0.0486	0.0523	0.0131	0.0115	0.0486	0.0505	0.0486	0.0524	0.0412
66	0.0065	0.0469	0.0469	0.0451	0.0099	0.0099	0.0451	0.0488	0.0098	0.0082	0.0451	0.0470	0.0451	0.0489	0.0378
67	0.0148	0.0448	0.0486	0.0430	0.0182	0.0217	0.0393	0.0505	0.0182	0.0165	0.0393	0.0412	0.0430	0.0430	0.0430
68	0.0218	0.0414	0.0451	0.0433	0.0183	0.0253	0.0433	0.0470	0.0217	0.0200	0.0471	0.0452	0.0433	0.0433	0.0508

Appendix I (continued).

	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
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32	0.0049														
33	0.0149	0.0098													
34	0.0488	0.0469	0.0395												
35	0.0342	0.0324	0.0253	0.0304											
36	0.0359	0.0341	0.0270	0.0286	0.0016										
37	0.0217	0.0235	0.0235	0.0431	0.0115	0.0132									
38	0.0489	0.0432	0.0323	0.0166	0.0451	0.0469	0.0432								
39	0.0528	0.0470	0.0396	0.0166	0.0451	0.0469	0.0470	0.0099							
40	0.0470	0.0414	0.0305	0.0149	0.0432	0.0450	0.0414	0.0016	0.0116						
41	0.0470	0.0451	0.0415	0.0149	0.0432	0.0450	0.0414	0.0150	0.0116	0.0167					
42	0.0489	0.0470	0.0433	0.0166	0.0451	0.0469	0.0432	0.0099	0.0133	0.0116	0.0150				
43	0.0508	0.0451	0.0341	0.0183	0.0470	0.0488	0.0451	0.0016	0.0116	0.0033	0.0167	0.0116			
44	0.0487	0.0468	0.0431	0.0200	0.0487	0.0505	0.0430	0.0098	0.0132	0.0115	0.0149	0.0065	0.0115		
45	0.0414	0.0395	0.0396	0.0166	0.0451	0.0469	0.0395	0.0133	0.0133	0.0150	0.0049	0.0167	0.0150	0.0166	
46	0.0432	0.0414	0.0378	0.0149	0.0432	0.0450	0.0414	0.0116	0.0116	0.0133	0.0065	0.0184	0.0133	0.0183	0.0016
47	0.0450	0.0431	0.0395	0.0133	0.0450	0.0432	0.0431	0.0132	0.0132	0.0149	0.0082	0.0201	0.0149	0.0200	0.0032
48	0.0469	0.0450	0.0414	0.0150	0.0469	0.0451	0.0450	0.0149	0.0149	0.0166	0.0065	0.0218	0.0166	0.0217	0.0049
49	0.0469	0.0450	0.0414	0.0150	0.0469	0.0451	0.0450	0.0115	0.0149	0.0132	0.0098	0.0183	0.0132	0.0183	0.0049
50	0.0489	0.0432	0.0396	0.0166	0.0451	0.0469	0.0432	0.0099	0.0033	0.0116	0.0116	0.0099	0.0116	0.0098	0.0133
51	0.0546	0.0488	0.0414	0.0150	0.0469	0.0451	0.0488	0.0115	0.0016	0.0132	0.0132	0.0149	0.0132	0.0148	0.0149
52	0.0526	0.0507	0.0432	0.0033	0.0339	0.0322	0.0469	0.0166	0.0132	0.0149	0.0183	0.0166	0.0183	0.0200	0.0201
53	0.0397	0.0342	0.0306	0.0471	0.0324	0.0341	0.0342	0.0435	0.0360	0.0454	0.0416	0.0473	0.0454	0.0470	0.0397
54	0.0526	0.0507	0.0432	0.0033	0.0339	0.0322	0.0469	0.0201	0.0201	0.0183	0.0115	0.0201	0.0218	0.0235	0.0166
55	0.0489	0.0470	0.0396	0.0166	0.0451	0.0469	0.0432	0.0133	0.0099	0.0150	0.0082	0.0133	0.0150	0.0132	0.0099
56	0.0451	0.0395	0.0396	0.0166	0.0451	0.0469	0.0432	0.0099	0.0099	0.0116	0.0082	0.0099	0.0116	0.0098	0.0065
57	0.0450	0.0394	0.0395	0.0133	0.0450	0.0432	0.0431	0.0132	0.0132	0.0149	0.0082	0.0132	0.0149	0.0132	0.0065
58	0.0508	0.0450	0.0414	0.0183	0.0470	0.0488	0.0489	0.0116	0.0049	0.0133	0.0133	0.0150	0.0133	0.0149	0.0116
59	0.0488	0.0432	0.0395	0.0166	0.0451	0.0469	0.0470	0.0099	0.0033	0.0116	0.0116	0.0133	0.0116	0.0132	0.0099
60	0.0469	0.0413	0.0414	0.0183	0.0470	0.0488	0.0489	0.0116	0.0049	0.0133	0.0133	0.0150	0.0133	0.0149	0.0116
61	0.0487	0.0430	0.0394	0.0132	0.0449	0.0431	0.0468	0.0132	0.0098	0.0149	0.0149	0.0166	0.0149	0.0165	0.0132
62	0.0469	0.0413	0.0377	0.0116	0.0431	0.0414	0.0450	0.0115	0.0115	0.0132	0.0098	0.0149	0.0132	0.0148	0.0082
63	0.0508	0.0450	0.0414	0.0183	0.0470	0.0488	0.0489	0.0116	0.0049	0.0133	0.0133	0.0150	0.0133	0.0149	0.0116
64	0.0526	0.0468	0.0432	0.0201	0.0526	0.0508	0.0507	0.0132	0.0098	0.0149	0.0183	0.0201	0.0149	0.0200	0.0132
65	0.0505	0.0448	0.0412	0.0182	0.0467	0.0485	0.0448	0.0148	0.0148	0.0165	0.0098	0.0148	0.0165	0.0115	0.0115
66	0.0470	0.0414	0.0378	0.0149	0.0432	0.0450	0.0414	0.0116	0.0116	0.0133	0.0033	0.0116	0.0133	0.0115	0.0082
67	0.0412	0.0393	0.0467	0.0199	0.0485	0.0503	0.0466	0.0199	0.0199	0.0217	0.0148	0.0199	0.0217	0.0165	0.0131
68	0.0490	0.0471	0.0470	0.0166	0.0450	0.0468	0.0507	0.0201	0.0201	0.0218	0.0183	0.0201	0.0218	0.0235	0.0166

Appendix I (continued).

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45															
46															
47	0.0016														
48	0.0032	0.0016													
49	0.0032	0.0016	0.0033												
50	0.0150	0.0166	0.0183	0.0183											
51	0.0132	0.0116	0.0133	0.0133	0.0049										
52	0.0183	0.0167	0.0184	0.0184	0.0132	0.0116									
53	0.0379	0.0396	0.0415	0.0415	0.0397	0.0378	0.0510								
54	0.0149	0.0133	0.0116	0.0150	0.0201	0.0184	0.0065	0.0510							
55	0.0082	0.0098	0.0115	0.0115	0.0133	0.0115	0.0201	0.0397	0.0166						
56	0.0082	0.0098	0.0115	0.0115	0.0065	0.0115	0.0166	0.0397	0.0166	0.0099					
57	0.0082	0.0065	0.0082	0.0082	0.0098	0.0116	0.0167	0.0396	0.0133	0.0098	0.0032				
58	0.0099	0.0115	0.0132	0.0132	0.0082	0.0065	0.0149	0.0378	0.0218	0.0116	0.0082	0.0115			
59	0.0082	0.0098	0.0115	0.0115	0.0065	0.0049	0.0132	0.0359	0.0201	0.0099	0.0065	0.0098	0.0016		
60	0.0099	0.0115	0.0132	0.0132	0.0082	0.0065	0.0149	0.0378	0.0218	0.0116	0.0082	0.0115	0.0032	0.0016	
61	0.0115	0.0098	0.0115	0.0115	0.0098	0.0082	0.0132	0.0395	0.0166	0.0132	0.0098	0.0065	0.0082	0.0065	0.0082
62	0.0065	0.0049	0.0065	0.0065	0.0115	0.0099	0.0150	0.0378	0.0116	0.0082	0.0049	0.0016	0.0098	0.0082	0.0098
63	0.0099	0.0115	0.0132	0.0132	0.0082	0.0065	0.0149	0.0378	0.0218	0.0116	0.0082	0.0115	0.0032	0.0016	0.0032
64	0.0115	0.0099	0.0116	0.0116	0.0132	0.0082	0.0167	0.0433	0.0237	0.0166	0.0132	0.0133	0.0082	0.0065	0.0082
65	0.0132	0.0148	0.0165	0.0165	0.0115	0.0165	0.0217	0.0450	0.0182	0.0115	0.0082	0.0082	0.0165	0.0148	0.0165
66	0.0099	0.0115	0.0098	0.0132	0.0082	0.0132	0.0183	0.0416	0.0115	0.0082	0.0049	0.0049	0.0133	0.0116	0.0133
67	0.0148	0.0165	0.0182	0.0182	0.0165	0.0216	0.0234	0.0468	0.0234	0.0199	0.0131	0.0131	0.0182	0.0165	0.0148
68	0.0149	0.0166	0.0183	0.0183	0.0201	0.0218	0.0200	0.0471	0.0200	0.0201	0.0166	0.0166	0.0183	0.0166	0.0183

Appendix I (continued).

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62	0.0049							
63	0.0082	0.0098						
64	0.0098	0.0116	0.0082					
65	0.0148	0.0098	0.0165	0.0217				
66	0.0115	0.0065	0.0133	0.0183	0.0065			
67	0.0165	0.0148	0.0182	0.0234	0.0082	0.0148		
68	0.0165	0.0149	0.0183	0.0235	0.0217	0.0183	0.0165	

Appendix II

Appendix II. The observed (H_O) and expected (H_E) heterozygosity, inbreeding coefficient (F_{IS}) and exact probabilities of Hardy-Weinberg proportions are listed for each locus and population. For abbreviations see Table 4.

Locus		SEC	SMP	SLM	BOK	BOG	NTJ	NCO	TRU	TAB	TAR	TLK	ZAM	ZIM
RPB3														
	Ho	0.70	0.14	0.52	0.35	0.40	0.57	0.50	0.50	0.80	0.21	0.89	0.80	0.69
	He	0.57	0.14	0.45	0.37	0.42	0.49	0.45	0.55	0.62	0.44	0.63	0.64	0.71
	Fis	-0.24	-0.08	-0.17	0.005	0.03	-0.20	-0.14	0.06	-0.34	0.51	-0.50	-0.38	0.03
	P(HW)	0.22	0.97	0.75	0.28	0.37	0.53	0.47	0.05	0.01	0.02	0.29	0.36	0.55
BMC3224														
	Ho	0.27	0.57	0.48	0.41	0.50	0.50	0.28	0.33	0.40	0.11	0.44	0.20	0.13
	He	0.56	0.49	0.42	0.63	0.69	0.51	0.35	0.49	0.43	0.45	0.39	0.51	0.12
	Fis	0.36	-0.24	-0.18	0.33	0.26	-0.01	0.18	0.28	0.04	0.21	-0.22	0.43	-0.05
	P(HW)	0.06	0.26	0.97	0.15	0.87	0.78	0.33	0.14	0.02	0.04	0.83	0.03	1.00
OARFC304														
	Ho	0.41	0.86	0.84	0.94	0.78	0.93	0.94	0.42	0.67	0.58	1.00	1.00	0.75
	He	0.66	0.90	0.88	0.84	0.86	0.88	0.85	0.73	0.68	0.73	0.92	0.89	0.88
	Fis	0.37	-0.02	0.03	-0.16	0.07	-0.10	-0.15	0.40	-0.01	0.19	-0.15	-0.25	0.12
	P(HW)	0.07	0.16	0.03	0.63	0.07	0.18	0.10	0.04	0.06	0.19	0.05	0.60	0.42
OARHH64														
	Ho	0.68	0.71	0.80	0.71	0.89	0.86	0.67	0.75	0.53	0.58	0.78	0.80	0.81
	He	0.78	0.66	0.82	0.77	0.79	0.76	0.80	0.74	0.53	0.72	0.77	0.89	0.80
	Fis	0.11	-0.17	0.00	0.05	-0.16	-0.17	0.14	-0.05	-0.05	0.17	-0.07	0.00	-0.04
	P(HW)	0.42	0.16	0.34	0.09	0.33	0.82	0.30	0.07	0.71	0.57	0.55	0.66	0.16

Appendix II (continued).

Locus		SEC	SMP	SLM	BOK	BOG	NTJ	NCO	TRU	TAB	TAR	TLK	ZAM	ZIM
ETH225														
	Ho	0.70	0.71	0.72	0.65	0.45	0.57	0.72	0.67	0.53	0.74	0.56	0.80	0.56
	He	0.74	0.76	0.70	0.60	0.79	0.74	0.83	0.88	0.81	0.79	0.86	0.64	0.62
	Fis	0.03	-0.01	-0.05	-0.11	0.42	0.19	0.11	0.21	0.32	0.04	0.32	-0.38	0.07
	P(HW)	0.99	0.65	0.92	0.70	0.08	0.10	0.07	0.24	0.43	0.51	0.40	0.90	0.43
OARCP26														
	Ho	0.74	0.43	0.68	0.88	0.85	0.71	0.56	0.92	0.87	0.79	0.89	1.00	0.88
	He	0.76	0.73	0.82	0.78	0.75	0.79	0.78	0.83	0.79	0.85	0.90	0.91	0.69
	Fis	0.01	0.36	0.15	-0.17	-0.16	0.06	0.27	-0.15	-0.14	0.04	-0.04	-0.22	-0.31
	P(HW)	0.17	0.20	0.69	0.64	0.38	0.02	0.47	0.92	0.74	0.11	0.47	0.40	0.01
MAF46														
	Ho	0.64	0.57	0.92	0.82	0.60	0.86	0.56	0.58	0.73	0.37	0.56	0.40	0.69
	He	0.79	0.77	0.80	0.68	0.75	0.74	0.62	0.71	0.69	0.56	0.80	0.51	0.71
	Fis	0.18	0.20	-0.18	-0.26	0.18	-0.20	0.08	0.14	-0.10	0.33	0.26	0.13	-0.03
	P(HW)	0.05	0.85	0.86	1.00	0.61	0.99	0.43	0.09	0.27	0.19	0.14	0.26	0.55
BMS 1237														
	Ho	0.57	0.29	0.60	0.76	0.78	0.71	0.39	0.55	0.80	0.63	0.67	0.40	0.31
	He	0.88	0.84	0.88	0.85	0.90	0.69	0.83	0.83	0.80	0.83	0.86	0.87	0.82
	Fis	0.03	0.26	0.14	0.08	0.11	-0.07	0.15	0.03	-0.04	0.22	0.18	0.18	0.21
	P(HW)	0.08	0.03	0.36	0.09	0.31	0.48	0.03	0.03	0.18	0.39	0.44	0.02	0.03

Appendix II. Allele size variation observed at seven microsatellite loci in the genotype in 203 greater kudu samples. Zero indicates that no alleles were scored in the sample.

	RPB3		BMC3224		OARFC304		OARHH64		ETH225		OARCP26		MAF46		BMS 1237	
EASTCAPE1	128	130	180	180	135	139	120	122	153	153	172	176	92	102	173	173
EASTCAPE2	130	130	180	180	139	157	116	122	153	161	172	176	92	102	167	171
EASTCAPE3	128	134	180	180	135	135	116	120	147	149	176	176	88	90	167	167
EASTCAPE4	130	134	180	180	139	157	122	122	149	149	172	176	96	98	165	173
EASTCAPE5	130	130	180	180	139	139	120	128	153	153	164	176	102	102	167	173
EASTCAPE6	128	134	180	180	133	133	116	120	151	153	172	176	96	102	167	167
EASTCAPE7	128	134	180	180	139	139	120	122	153	153	172	172	96	102	169	169
EASTCAPE8	128	130	180	180	139	139	116	120	153	155	172	178	102	102	165	175
EASTCAPE9	128	128	180	180	135	135	128	128	149	153	164	172	92	102	161	161
EASTCAPE10	130	130	180	180	135	139	122	124	147	149	172	172	88	90	165	173
EASTCAPE11	128	130	180	180	135	139	122	128	153	155	164	176	102	102	175	175
EASTCAPE12	128	130	180	180	135	135	128	128	153	153	164	178	96	96	161	161
EASTCAPE13	128	130	180	180	135	135	122	122	149	153	178	178	96	96	165	165
EASTCAPE14	128	130	172	178	139	139	120	122	145	153	170	172	96	102	161	169
EASTCAPE15	128	130	172	178	137	139	128	128	147	153	172	176	102	102	169	169
EASTCAPE16	128	130	172	176	137	139	122	122	149	153	172	176	96	96	175	179
EASTCAPE17	128	130	172	180	139	139	120	120	149	153	170	176	98	102	171	175
EASTCAPE18	130	130	0	0	137	137	110	120	145	155	164	176	92	100	0	0
EASTCAPE19	130	130	176	178	135	139	120	122	141	145	174	174	92	100	161	179
EASTCAPE20	128	130	178	178	139	139	120	124	149	153	172	176	98	98	161	171
EASTCAPE21	128	130	176	180	139	139	0	0	149	149	174	176	98	102	167	169
EASTCAPE22	130	130	178	178	0	0	110	128	143	159	176	184	0	0	0	0
EASTCAPE23	128	130	176	176	135	137	116	122	153	153	176	176	98	100	169	171
MPUMA1	130	130	180	180	137	149	116	116	149	149	176	176	90	96	165	165
MPUMA2	130	130	178	180	137	151	116	122	147	149	178	186	100	102	163	171
MPUMA3	130	130	180	180	137	153	116	120	151	153	172	176	100	100	171	171
MPUMA4	130	130	180	180	139	151	110	124	147	151	178	188	90	92	165	173
MPUMA5	130	132	176	180	149	151	116	122	149	151	176	176	90	90	169	169
MPUMA6	130	130	180	182	135	153	116	116	149	151	176	176	94	100	169	169
MPUMA7	130	130	180	182	145	145	116	122	147	147	188	188	100	100	173	173
LIMPOPO1	130	132	180	182	145	147	116	116	149	159	164	164	94	100	165	167
LIMPOPO2	130	130	180	182	137	149	110	126	149	159	168	168	88	90	159	169
LIMPOPO3	130	130	180	182	149	149	110	122	147	149	178	178	90	92	165	177
LIMPOPO4	130	130	180	180	157	159	116	124	147	149	176	178	90	102	169	181
LIMPOPO5	130	130	180	180	139	153	116	120	149	149	178	188	88	90	171	173
LIMPOPO6	130	132	176	180	149	149	122	122	147	149	164	174	96	100	167	167
LIMPOPO7	130	134	180	180	149	159	110	122	149	149	176	178	90	100	175	175
LIMPOPO8	130	132	180	180	123	151	110	124	149	161	178	178	90	102	167	177
LIMPOPO9	130	130	180	180	145	157	120	120	149	149	174	178	92	102	173	177
LIMPOPO10	130	130	180	180	147	149	116	120	145	149	168	178	88	102	173	173
LIMPOPO11	130	132	180	182	147	149	116	124	149	149	164	172	90	92	165	165
LIMPOPO12	130	132	178	180	157	157	116	122	145	147	178	178	90	94	165	177
LIMPOPO13	130	132	178	180	137	137	116	124	151	151	176	186	88	94	171	171
LIMPOPO14	130	132	178	180	137	139	116	116	153	159	172	176	90	100	167	169
LIMPOPO15	130	132	180	180	145	147	120	126	149	153	176	176	90	94	167	167
LIMPOPO16	130	130	180	180	137	157	122	124	149	149	176	178	90	94	159	177
LIMPOPO17	130	134	174	180	137	147	122	124	149	153	172	176	88	90	159	159
LIMPOPO18	130	130	180	180	139	161	120	126	145	149	178	178	92	96	169	173
LIMPOPO19	130	130	174	180	145	149	116	122	149	149	172	176	90	92	165	165
LIMPOPO20	132	136	180	180	157	159	120	122	149	153	172	178	90	100	163	167
LIMPOPO21	130	130	180	180	147	151	116	124	149	151	174	176	90	90	159	165
LIMPOPO22	124	130	176	180	137	149	120	120	147	149	172	184	94	102	173	173
LIMPOPO23	130	130	180	180	147	151	116	120	147	161	176	178	90	90	159	163
LIMPOPO24	130	130	180	180	145	147	116	124	147	151	180	180	90	98	165	173

	RPB3		BMC3224		OARFC304		OARHH64		ETH225		OARCP26		MAF46		BMS 1237	
LIMPOPO25	130	134	174	180	147	149	122	126	149	151	172	176	88	90	173	173
OKAVANGO1	130	130	178	180	149	157	110	120	151	153	172	176	90	102	159	167
OKAVANGO2	130	130	180	182	149	151	120	124	149	159	172	176	90	100	163	167
OKAVANGO3	130	134	180	184	139	149	116	122	145	149	176	186	90	116	159	173
OKAVANGO4	130	130	180	180	135	149	122	124	147	149	164	174	88	94	163	167
OKAVANGO5	130	134	178	180	147	149	116	120	149	149	172	178	90	90	159	167
OKAVANGO6	128	132	180	180	147	149	120	124	149	159	176	178	90	94	159	167
OKAVANGO7	130	130	178	178	133	149	116	116	147	151	176	178	90	116	165	173
OKAVANGO8	130	130	178	180	147	149	124	124	149	149	176	178	90	90	165	167
OKAVANGO9	128	130	180	180	139	145	120	124	149	153	176	178	90	90	159	167
OKAVANGO10	130	130	174	180	147	151	116	116	149	149	176	176	90	100	163	169
OKAVANGO11	130	130	182	182	149	151	120	124	149	159	164	178	90	98	159	177
OKAVANGO12	130	132	180	180	149	149	116	122	149	159	176	186	88	90	159	159
OKAVANGO13	130	134	180	180	151	157	116	122	147	149	164	176	90	116	159	165
OKAVANGO14	130	130	178	180	137	157	116	116	149	149	176	176	90	100	173	177
OKAVANGO15	130	130	180	180	139	159	124	124	149	149	176	180	88	90	173	173
OKAVANGO16	130	130	182	182	147	151	122	124	149	149	174	180	86	90	169	169
OKAVANGO17	130	130	182	182	147	151	120	124	149	153	164	178	90	98	177	177
OKAVANGO18	130	130	180	180	137	147	116	122	147	149	176	176	90	100	159	165
GHANZI1	130	130	174	178	0	0	116	116	149	149	172	176	102	104	145	175
GHANZI2	130	130	178	180	147	149	0	0	151	159	172	180	88	100	155	175
GHANZI3	128	130	164	180	139	139	116	120	147	149	176	180	90	92	0	0
GHANZI4	130	130	178	178	0	0	122	124	149	149	164	178	88	102	0	0
GHANZI5	130	130	172	178	139	139	116	116	151	151	172	178	90	94	161	171
GHANZI7	130	130	178	178	147	149	116	124	143	143	176	178	90	90	161	171
GHANZI8	130	130	176	178	145	151	120	122	147	147	176	176	90	90	171	173
GHANZI9	130	130	176	180	139	139	120	124	149	153	174	178	100	102	145	161
GHANZI10	130	132	176	178	137	139	116	122	143	147	176	178	90	104	167	171
GHANZI11	128	128	178	178	137	139	116	122	147	149	176	176	90	90	155	169
GHANZI12	130	130	180	180	147	149	116	124	143	161	176	178	90	90	165	169
GHANZI13	130	134	180	180	149	151	120	124	149	153	174	178	88	88	169	173
GHANZI14	130	134	178	182	139	149	120	124	149	153	176	178	88	90	169	171
GHANZI15	130	134	180	180	137	145	110	124	153	153	172	176	88	88	173	173
GHANZI16	130	130	180	180	147	149	116	124	145	145	164	180	92	102	165	165
GHANZI17	130	134	180	182	137	157	122	124	149	149	176	176	90	90	159	159
GHANZI18	130	130	180	180	151	161	110	116	149	149	176	180	90	92	169	169
GHANZI19	130	130	180	182	145	145	120	122	149	159	176	180	90	90	165	173
GHANZI20	128	130	180	180	137	145	110	124	153	153	172	176	90	94	159	173
GHANZI21	130	134	180	180	149	151	120	122	149	149	176	178	86	88	173	175
OTJIWARONGO3	128	130	180	182	139	153	110	116	147	149	178	178	86	90	165	165
OTJIWARONGO4	130	130	178	180	135	147	116	124	149	151	176	178	90	90	163	165
OTJIWARONGO5	130	130	178	180	135	153	122	122	167	167	166	178	90	102	165	165
OTJIWARONGO6	130	130	180	182	155	157	120	122	151	151	164	164	90	100	165	173
OTJIWARONGO7	134	130	180	180	155	157	122	122	149	151	176	178	90	94	165	167
OTJIWARONGO8	130	130	180	180	151	153	116	120	147	147	176	178	90	92	167	173
OTJIWARONGO9	130	134	180	180	149	151	116	124	147	147	176	176	90	100	165	167
OTJIWARONGO10	130	134	180	180	135	151	116	122	145	151	176	186	100	100	159	165
OTJIWARONGO11	130	134	182	182	149	149	116	122	151	151	174	182	88	90	165	167
OTJIWARONGO12	130	130	180	180	135	151	116	122	145	151	176	184	90	102	165	173
OTJIWARONGO13	130	134	180	182	135	149	116	120	151	153	174	180	88	90	165	167
OTJIWARONGO14	132	134	180	180	139	149	116	122	149	151	176	186	90	102	165	165
OTJIWARONGO15	130	130	178	180	137	149	110	122	149	151	176	178	100	102	163	169
OTJIWARONGO16	130	134	174	180	135	151	120	124	151	151	176	176	88	90	167	167
OTJIWARONGO17	130	130	180	182	133	149	110	120	147	149	172	176	88	90	165	169
CORONA1	130	134	178	180	135	147	124	124	149	159	176	178	90	100	165	165

	RPB3		BMC3224		OARFC304		OARHH64		ETH225		OARCP26		MAF46		BMS 1237	
CORONA2	130	132	180	180	151	159	116	122	147	149	176	180	90	90	165	167
CORONA3	130	130	182	182	135	151	116	124	151	151	180	180	90	90	169	169
CORONA4	130	134	180	180	139	149	122	122	151	159	176	176	88	90	165	173
CORONA5	130	130	180	180	149	151	116	116	145	149	172	176	86	94	167	167
CORONA6	130	134	180	180	149	151	116	122	145	149	176	176	90	90	165	165
CORONA7	130	130	180	180	149	151	122	124	145	149	176	176	90	90	159	165
CORONA8	130	130	180	180	149	151	120	120	151	153	180	180	90	90	173	173
CORONA9	130	134	180	180	135	135	116	122	149	153	176	176	90	90	159	159
CORONA10	130	130	180	180	149	151	122	124	159	159	172	178	90	92	167	167
CORONA11	130	130	180	180	149	151	110	124	153	153	178	180	90	92	165	167
CORONA12	128	134	180	180	151	153	116	124	153	153	176	186	90	90	159	159
CORONA13	130	134	178	180	139	149	122	122	149	151	172	172	94	104	165	165
CORONA14	130	132	180	180	133	147	110	116	147	149	164	182	90	102	177	177
CORONA15	130	130	174	180	133	147	116	120	151	151	174	176	102	102	169	169
CORONA16	130	130	180	184	149	153	110	120	151	153	176	182	90	104	169	173
CORONA17	130	134	180	182	149	157	120	120	151	153	178	178	86	90	165	169
CORONA18	130	130	180	180	139	153	116	122	145	161	176	188	88	90	163	167
RUAHA1	130	134	180	182	139	149	122	122	149	149	172	176	88	92	0	0
RUAHA2	130	130	174	180	149	149	120	124	145	153	176	182	92	92	159	177
RUAHA3	130	130	180	180	151	151	116	122	153	155	168	176	88	92	159	159
RUAHA4	130	130	178	178	147	147	116	122	151	153	166	172	92	92	159	159
RUAHA6	132	134	180	180	151	151	122	126	153	155	174	184	92	92	169	169
RUAHA7	132	132	180	180	147	151	116	122	151	153	176	176	88	90	165	173
RUAHA8	130	134	180	180	151	151	124	124	145	145	174	176	90	90	165	167
RUAHA9	130	130	180	180	149	149	116	122	145	155	176	180	88	90	165	173
RUAHA10	130	134	174	180	147	147	124	124	149	149	174	188	88	90	165	173
RUAHA11	130	134	174	178	149	151	120	122	157	161	172	176	92	92	165	167
RUAHA12	130	130	180	180	149	151	116	122	155	159	182	188	90	102	177	177
RUAHA13	130	134	180	180	139	149	116	122	159	159	172	176	88	90	165	165
TABORA1	130	134	180	182	147	147	122	122	145	145	172	176	88	90	165	173
TABORA2	130	132	180	182	151	153	116	126	149	153	176	182	90	90	165	167
TABORA3	130	134	180	182	149	149	122	122	159	159	172	176	88	90	159	167
TABORA4	130	134	180	182	149	149	122	122	151	157	168	168	92	92	167	177
TABORA5	130	134	180	182	149	151	116	122	145	145	174	176	88	88	165	167
TABORA6	130	134	180	182	139	149	122	122	149	153	172	176	88	90	165	173
TABORA7	130	134	180	180	149	151	122	126	153	155	172	176	88	90	165	167
TABORA8	130	134	180	180	149	151	122	126	147	161	176	176	88	90	153	159
TABORA9	130	134	180	180	149	151	122	122	149	153	172	182	88	90	165	173
TABORA10	124	124	174	174	149	151	116	122	149	153	168	172	84	102	165	167
TABORA11	130	130	180	180	149	151	122	122	153	153	174	178	88	92	165	173
TABORA12	130	134	180	180	147	147	122	122	149	149	172	176	88	90	165	165
TABORA13	130	132	180	180	151	151	122	124	145	159	176	184	90	98	159	159
TABORA14	130	134	180	180	149	151	116	122	153	153	176	178	88	90	159	177
TABORA15	130	130	180	180	149	151	116	126	153	153	176	180	90	90	159	159
ARUSHA1	130	130	180	180	149	149	116	122	153	159	168	188	90	90	159	159
ARUSHA2	130	132	180	180	149	149	122	126	157	159	168	176	88	90	159	167
ARUSHA3	124	124	174	174	147	149	122	122	149	153	180	180	84	102	165	173
ARUSHA4	130	130	180	180	147	149	122	124	153	155	178	182	88	90	173	173
ARUSHA5	130	130	180	180	137	149	122	122	153	157	168	168	90	90	167	177
ARUSHA6	130	130	180	180	151	151	122	124	145	153	176	176	90	90	167	177
ARUSHA7	130	130	180	180	149	149	116	122	147	161	168	184	90	100	165	173
ARUSHA8	130	130	180	182	137	151	120	124	157	159	180	182	90	90	165	167
ARUSHA9	130	130	180	180	145	149	122	122	157	159	172	176	88	90	167	173
ARUSHA10	132	132	178	178	137	149	122	122	149	149	174	188	92	92	169	177
ARUSHA11	130	134	180	180	137	149	116	120	149	149	168	188	98	98	167	167

	RPB3		BMC3224		OARFC304		OARHH64		ETH225		OARCP26		MAF46		BMS 1237	
ARUSHA12	130	130	180	180	149	149	122	122	149	153	168	176	90	90	167	167
ARUSHA13	134	134	178	178	149	149	120	120	149	153	174	188	90	90	169	169
ARUSHA14	130	130	180	180	137	149	116	120	149	149	168	188	90	90	173	173
ARUSHA15	130	132	176	180	145	151	116	124	153	153	168	176	90	100	165	173
ARUSHA16	130	130	180	180	139	153	110	122	149	149	172	178	94	102	179	181
ARUSHA17	130	130	174	174	151	151	120	124	145	149	168	180	90	90	167	167
ARUSHA18	130	130	180	180	145	149	124	124	153	159	168	168	90	90	165	173
ARUSHA19	130	134	180	180	147	147	122	122	149	153	180	182	90	90	163	167
ARUSHA20	130	134	178	180	137	149	122	122	149	149	172	176	88	90	165	165
LUKWATI1	134	136	174	180	155	159	116	122	143	157	176	180	86	86	177	177
LUKWATI2	130	132	174	180	137	139	116	116	147	149	174	174	96	102	159	173
LUKWATI3	130	134	180	182	137	145	120	126	151	151	172	178	88	92	173	177
LUKWATI4	130	134	180	180	147	149	116	122	149	149	176	178	88	90	165	165
LUKWATI5	130	134	180	180	145	149	116	126	147	161	182	184	92	92	167	177
LUKWATI6	130	130	180	180	151	153	116	116	153	153	184	186	90	90	153	159
LUKWATI7	130	134	180	180	145	149	116	126	147	161	182	184	90	90	167	177
LUKWATI8	130	134	174	180	155	159	120	124	145	149	174	176	88	90	165	177
LUKWATI9	130	134	180	180	137	139	110	120	149	149	172	176	88	90	171	171
ZAMBIA1	134	136	182	182	151	153	120	124	149	149	168	190	90	90	175	177
ZAMBIA2	130	130	180	180	139	149	120	126	145	149	168	188	90	90	165	165
ZAMBIA3	130	134	180	180	145	151	116	116	145	149	168	190	90	90	169	169
ZAMBIA4	130	134	174	180	147	149	110	124	149	153	172	182	88	114	165	177
ZAMBIA5	130	134	180	180	147	149	116	122	149	159	176	180	88	90	171	171
ZIM1	132	136	180	180	149	149	116	126	149	149	178	178	90	90	165	165
ZIM2	130	132	180	180	129	129	122	122	149	149	178	178	88	90	167	179
ZIM3	132	132	180	180	145	145	128	128	149	149	176	178	88	88	175	175
ZIM4	130	134	180	180	147	153	120	124	147	149	172	176	90	92	165	173
ZIM5	130	130	180	180	137	149	116	122	145	149	172	176	88	90	169	169
ZIM6	132	134	180	180	137	149	116	122	147	153	172	176	94	100	175	175
ZIM8	130	138	176	180	139	153	116	118	149	149	172	176	88	90	165	173
ZIM9	130	138	180	180	153	153	116	122	145	149	172	176	88	90	167	167
ZIM10	130	136	180	182	137	139	116	124	149	149	172	176	88	94	167	167
ZIM11	130	130	180	180	147	153	116	124	149	151	172	176	82	82	173	173
ZIM12	130	130	180	180	139	147	120	124	149	151	172	176	88	90	167	171
ZIM13	130	134	180	180	141	147	122	124	151	151	172	176	88	88	167	167
ZIM14	130	132	180	180	139	147	116	122	145	149	174	176	88	98	165	173
ZIM15	130	138	180	180	147	149	116	124	149	149	172	176	88	88	169	169
ZIM16	130	138	180	180	147	149	120	124	145	147	172	176	88	94	173	173

Appendix III

Appendix III. Allele frequencies obtained from eight microsatellite loci genotyped in 13 greater kudu populations. Allele sizes are given in base pairs. For abbreviations see Table 4.

Locus	SEC	SMP	SLM	BOK	BOG	NTJ	NCO	TRU	TAB	TAR	TLK	ZAM	ZIM
RPB3 (bp)													
124			0.02						0.07	0.05			
128	0.37			0.06	0.10	0.03	0.03						
130	0.54	0.93	0.72	0.81	0.75	0.70	0.72	0.63	0.53	0.73	0.50	0.50	0.47
132		0.07	0.18	0.06	0.03	0.03	0.06	0.13	0.07	0.10	0.06		0.25
134	0.09		0.06	0.08	0.13	0.23	0.19	0.25	0.33	0.13	0.39	0.40	0.09
136			0.02								0.06	0.10	0.06
138													0.13
BMC3224													
164					0.03								
172	0.09				0.03								
174			0.06	0.03	0.03	0.03	0.03	0.13	0.07	0.10	0.17	0.10	
176	0.11	0.07	0.04		0.08					0.03			0.03
178	0.16	0.07	0.06	0.17	0.30	0.10	0.06	0.13		0.13			
180	0.64	0.71	0.76	0.58	0.48	0.67	0.81	0.71	0.73	0.73	0.78	0.70	0.94
182		0.14	0.08	0.19	0.08	0.20	0.08	0.04	0.20	0.03	0.06	0.20	0.03
184				0.03			0.03						
OARHH64													
110	0.05	0.07	0.08	0.03	0.08	0.10	0.08			0.03	0.06	0.10	
116	0.11	0.57	0.28	0.31	0.29	0.30	0.28	0.25	0.17	0.13	0.44	0.30	0.28
118													0.03
120	0.27	0.07	0.20	0.19	0.18	0.17	0.17	0.08		0.15	0.17	0.20	0.09
122	0.32	0.21	0.20	0.17	0.18	0.33	0.28	0.42	0.67	0.50	0.11	0.10	0.28
124	0.05	0.07	0.16	0.31	0.26	0.10	0.19	0.21	0.03	0.18	0.06	0.20	0.22
126			0.08					0.04	0.13	0.03	0.17	0.10	0.03
128	0.20		0.00										0.06

Appendix III (continued).

Locus	SEC	SMP	SLM	BOK	BOG	NTJ	NCO	TRU	TAB	TAR	TLK	ZAM	ZIM
OARFC304													
123			0.02										
129													0.06
133	0.05			0.03		0.03	0.06						
135	0.30	0.07		0.03		0.20	0.11						
137	0.11	0.21	0.14	0.06	0.14	0.03				0.15	0.17		0.09
139	0.50	0.07	0.06	0.08	0.25	0.07	0.08	0.08	0.03	0.03	0.11	0.10	0.16
141													0.03
145		0.14	0.10	0.03	0.14					0.08	0.17	0.10	0.06
147			0.18	0.19	0.11	0.03	0.08	0.21	0.13	0.10	0.06	0.20	0.22
149		0.14	0.22	0.31	0.19	0.23	0.28	0.33	0.43	0.48	0.17	0.30	0.19
151		0.21	0.06	0.17	0.11	0.17	0.25	0.38	0.37	0.15	0.06	0.20	
153		0.14	0.02			0.10	0.08		0.03	0.03	0.06	0.10	0.16
155						0.07					0.11		
157	0.05		0.12	0.08	0.03	0.07	0.03						
159			0.06	0.03			0.03				0.11		
161			0.02		0.03								
165													0.03
ETH225 (bp)													
141	0.02												
143	0.02				0.10						0.06		
145	0.07		0.06	0.03	0.05	0.07	0.11	0.17	0.17	0.05	0.06	0.20	0.13
147	0.07	0.29	0.14	0.11	0.13	0.20	0.06		0.03	0.03	0.17		0.09
149	0.24	0.36	0.52	0.61	0.40	0.20	0.22	0.17	0.20	0.38	0.33	0.60	0.59
151	0.02	0.29	0.10	0.06	0.08	0.43	0.25	0.08	0.03		0.11		0.13
153	0.46	0.07	0.08	0.08	0.18	0.03	0.22	0.21	0.37	0.28	0.11	0.10	0.06
155	0.07							0.17	0.03	0.03			
157								0.04	0.03	0.10	0.06		
159	0.02		0.06	0.11	0.05		0.11	0.13	0.10	0.13		0.10	
161	0.02		0.04		0.03		0.03	0.04	0.03	0.03	0.11		
167						0.07							

Appendix III (continued).

Locus	SEC	SMP	SLM	BOK	BOG	NTJ	NCO	TRU	TAB	TAR	TLK	ZAM	ZIM
ARCP26													
164	0.11		0.08	0.11	0.05	0.07	0.03						
166						0.03		0.04					
168			0.06					0.04	0.10	0.30		0.30	
170	0.04												
172	0.30	0.07	0.14	0.08	0.13	0.03	0.11	0.17	0.23	0.08	0.11	0.10	0.38
174	0.07		0.06	0.06	0.05	0.07	0.03	0.13	0.07	0.05	0.17		0.06
176	0.37	0.50	0.24	0.44	0.43	0.40	0.42	0.38	0.40	0.18	0.22	0.10	0.41
178	0.09	0.14	0.32	0.19	0.23	0.23	0.14		0.07	0.05	0.11		0.16
180			0.04	0.06	0.13	0.03	0.17	0.04	0.03	0.13	0.06	0.10	
182						0.03	0.06	0.08	0.07	0.08	0.11	0.10	
184	0.02		0.02			0.03		0.04	0.03	0.03	0.17	1.00	
186		0.07	0.02	0.06		0.07	0.03				0.06	0.91	
188		0.21	0.02				0.03	0.08		0.13		0.10	
190												0.20	
MAF46													
82													0.06
84									0.03	0.03			
86				0.03	0.03	0.03	0.06				0.11		
88	0.05		0.12	0.08	0.20	0.13	0.06	0.25	0.37	0.10	0.22	0.20	0.47
90	0.05	0.29	0.40	0.56	0.45	0.47	0.61	0.29	0.43	0.65	0.39	0.70	0.28
92	0.11	0.07	0.10		0.08	0.03	0.06	0.42	0.10	0.05	0.17		0.03
94		0.07	0.12	0.06	0.05	0.03	0.06			0.03			0.09
96	0.23	0.07	0.04								0.06		
98	0.14		0.02	0.06					0.03	0.05			0.03
100	0.07	0.43	0.10	0.11	0.05	0.17	0.03			0.05			0.03
102	0.36	0.07	0.10	0.03	0.10	0.13	0.08	0.04	0.03	0.05	0.06		
104					0.05		0.06						
114												0.10	
116				0.08									

Appendix III (continued).

Locus	SEC	SMP	SLM	BOK	BOG	NTJ	NCO	TRU	TAB	TAR	TLK	ZAM	ZIM
BMS 1237													
145					0.06								
153									0.03		0.06		
155					0.06								
159			0.12	0.28	0.08	0.03	0.14	0.23	0.23	0.08	0.11		
161	0.17				0.08								
163		0.07	0.04	0.08		0.07	0.03			0.03			
165	0.12	0.21	0.18	0.11	0.11	0.50	0.31	0.32	0.33	0.18	0.17	0.30	0.16
167	0.17		0.16	0.19	0.03	0.23	0.19	0.09	0.20	0.30	0.11		0.31
169	0.17	0.29	0.08	0.08	0.17	0.07	0.17	0.09		0.08		0.20	0.13
171	0.10	0.21	0.06		0.14						0.11	0.20	0.03
173	0.12	0.21	0.20	0.14	0.19	0.10	0.11	0.14	0.13	0.23	0.11		0.22
175	0.12		0.04		0.08							0.10	0.13
177			0.10	0.11			0.06	0.14	0.07	0.08	0.33	0.20	
179	0.05									0.03			0.03
181			0.02							0.03			