GENETIC STRUCTURE OF HARTEBEEST POPULATIONS STRADDLING A TRANSITION ZONE BETWEEN MORPHOTYPES

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Abstract

Variation in mitochondrial and microsatellite DNA was used to define the genetic structure of hartebeest populations that straddle a transition zone between two morphotypes (*Alcelaphus buselaphus cokei* and *A. b. lelwel*) in Kenya. Previous analyses of mtDNA from hartebeest populations across Africa supported the refugium hypothesis, which holds that present day hartebeest morphotypes diverged in allopatry, as a result of climate change. In this analysis of morphologically distinct populations in close geographical proximity, the majority of genetic variation was found within populations, with relatively little distinction, and varying levels of subdivision, among populations. Patterns of shared alleles, and a reduced tendency for mtDNA haplotypes to cluster phylogenetically according to morphotype, were suggestive of gene flow between populations. Thus, there was sharp disparity between the apparently seamless genetic transition between populations and the marked disjunction in gross morphology over distances as short as 100 km. Hartebeest in the transitional zone between *A. b. lelwel* and *A. b. cokei*, including populations in Laikipia District, Ruma, and Meru National Parks, are the only remaining examples, each genetically and morphologically different from the other, of what appears to be resumed contact between two lineages that diverged in allopatry. Our results underscore the importance of using both genetic and morphological information to explicitly define evolutionary processes as targets for conservation. Yet conservation protocols and practices are ill-defined for hybrids between species and morphotypes. In many African countries, including Kenya, where there has been little mixing of populations by translocation, opportunities to conserve ongoing evolutionary processes persist, and should be strenuously pursued.

Introduction

Conservation in Africa has necessarily emphasized proximate threats to the persistence of large mammals, such as habitat loss and illegal hunting. Community-based conservation is increasingly promoted to provide essential space for wide-ranging wildlife species in human-occupied areas that cannot be formally protected as national parks or reserves. The importance of 'ultimate' threats that are fundamentally biological, such as genetic integrity in small populations, have been stressed in the literature (e.g. Greig 1979; Amos and Balmford, 2001), but under-attended *in situ*. This is partly because ultimate threats are too often assumed to become relevant only after proximate threats have been overcome, and partly from lack of funds or capacity. Ultimate threats merit attention because the survival of large mammals in relatively small and decreasingly natural areas that have become 'islands in a sea of humanity' will entail more than maintaining species lists, or patterns of relative abundance. To the extent possible, fragmented landscapes must be actively managed as functionally intact ecological communities, aiming to perpetuate natural processes that have been operating over vast ecological space and long evolutionary time (Young and Clarke, 2000). These include relatively complex processes, such as gene flow and natural selection that often do not translate in simple ways into appropriate conservation action. This study was intended to advance the application of these principles to the conservation of hartebeest (*Alcelaphus bucelaphus*) in Kenya.

Modern hartebeest lineages appeared at least 740,000 years ago (Vrba, 1995a), and diverged during periods of radiation that coincided with rapid climate change (Arctander *et al.,* 1999; Flagstad *et al.,* 2001; Pitra *et al.,* 1998; Matthee and Robinson

1999). Seven extant 'subspecies', described according to variation in horn shape and size, coat colour and body size (Sidney, 1965), are considered either as variants within a single species (*Alcelaphus buselaphus*; Kingdon, 1982) or as two species, comprising a southern lineage (*A. lichtensteini,* including *lichtensteini* and *caama*; Cillie, 1987) , and a northern lineage (*A. buselaphus,* including *major*, *lelwel*, *tora*, *swaynei* and *cokei*) which appears to be of relatively recent origin (< 200,000 year bp; Flagstad *et al.,* 2001). Phylogenetic analysis of mitochondrial DNA sequences supported the latter classification (Arctander *et al.,* 1999, Flagstad *et al.,* 2001) in that the northern and southern clades are reciprocally monophyletic. Within each of these clades, lineages tend to cluster according to morphotype, but not exclusively, such that groups defined by morphotypes are polyphyletic.

Hartebeest were among the most widely distributed antelopes in Africa, but have declined rapidly in number and distribution, and seem especially vulnerable to local extinction. For example, *A. b. cokei* became locally extinct in Kenya's Amboseli National Park and Mwea National Reserve within the last decade. Two subspecies with severely restricted ranges in northern east Africa are critically endangered (*A. b. tora* and *A. b. swaynei*). A third, *A. b. lelwel*, is declining across range states that include Central African Republic, southern Sudan, northern D.R. Congo, northern Uganda, and central Kenya. All but the latter have severely limited conservation means or potential.

Central Kenya marks the transition between *A. b. lelwel*, at the south-eastern extremity of its range, and *A. b. cokei,* which is patchily distributed across southern Kenya and adjoining regions of northern Tanzania (Fig. 1). Hartebeest were once abundant across this zone but have been extirpated by settlement over large areas.

Hartebeest with *A. b. lelwel* morphology (Fig. 2) remain at only two locations in Kenya. Ruma National Park near Lake Victoria holds a declining population of a few hundred. Ruma NP is small (140 km^2) and surrounded by dense humanity, and may not endure as a protected area. The other population is in Laikipia District, north-west of Mount Kenya, numbering less than 1000. While nowhere formally protected, many of the properties that comprise the hartebeest range in Laikipia are currently well-protected in the functional sense.

Resolving the systematics of the Laikipia hartebeest is important because they warrant exceptional conservation status, for two reasons. First, as a 'flagship' species for a spectacular and biologically diverse area that hosts more wildlife than all of Kenya's protected areas except the Masai Mara Reserve, but lacks formal protection status. Second, given the precarious status of *A. b. lelwel* elsewhere, the Laikipia population has potential to be one of the most secure populations of this morphotype remaining in Africa. The Meru NP population has dwindled to a few dozen and may be supplemented by translocation in the near future. Genetic data that might assist in the decision to supplement this population, and select the appropriate source population, were lacking.

To address these systematic and conservation genetic issues, we used mitochondrial DNA sequences and a suite of nuclear (microsatellite) markers to describe the structure of hartebeest populations straddling the transition zone between *A. b. lelwel* and *A. b. cokei* in Kenya, against the wider context of population genetic variation in hartebeest across eastern Africa.

Materials and Methods

Samples were collected as skin using the biopsy dart method (Karesh *et al.*, 1989), as fragments cut from salted skins, or as fresh dung. DNA was extracted from skin using the standard proteinase-K-phenol-chloroform method (Sambrook *et al.,* 1989), or from dung using the *Dynabeads DNA Direct Kit* (Dynal AS, Oslo; Flagstad *et al.,* 1999). One pellet from each individual was placed in a plastic bag, 400 µL phosphate-buffered saline (PBS, pH 7.4) added, and the surface of the pellet gently massaged to wash off epithelial cells. The supernatant was transferred to a 1.5 ml eppendorf tube and extracted using the magnetic beads procedure described by Rudi *et al.* (1997). DNA extracts were eluted in 40 µL of Tris-EDTA buffer (TE) for 5 minutes at 65°C and diluted 10 times in distilled water prior to amplification.

Building on the results of Arctander *et al.* (1999) and Flagstad *et al.* (2001) who analysed D-loop sequences from 134 individuals representing 7 locations in Africa, we added 75 homologous hartebeest mitochondrial DNA sequences from 4 new locations in Kenya (Laikipia, Ruma NP, Naivasha, and Meru NP; the latter were collected too late to feature in the mitochondrial analysis) and 2 new locations in Tanzania (Mara-Serengeti NP and Ngorongoro Crater; Table 1). Included were an additional 8 sequences to 6 already available from Nairobi NP. Six sequences available from the Masai Mara Reserve in Kenya, and 9 from the Maswa region in Tanzania were pooled with 14 new samples from Serengeti NP. Eleven *A. b. lelwel* sequences from Uganda, Sudan and DR Congo, available from Arctander *et al.,* (1999) and Flagstad *et al.* (2001), were pooled, as

were 5 from the western sector of the *lelwel* range from Central African Republic (CAR) and Chad.

mtDNA PCR amplification and sequencing: A 475 bp region of the mitochondrial D-loop adjacent to the $tRNA^{pro}$ gene was targeted for PCR and solid-phase DNA sequencing (Hultmann *et al.,* 1989) using the primers applied by Arctander *et al.,* 1999 and Flagstad *et al.,* 2001. The primers employed were 5'-AATAGCCCCACTATCAGCACCC-3' (L15394; specific to hartebeest) paired with 5'-TATGGCCCTGAAGTAAGAACCAG-3' (H15947; "mammalian" primer targeting CSB-D; Southern *et al.,* 1988). Primers are light- and heavy-strands corresponding to the 5' primer end in *Homo sapiens* mitochondrial genome as given in data base entry HSMITG, accession number X93334.

DNA samples were extracted and amplified at Mpala Research Centre in a 50 µl reaction volume containing ~10 ng of target DNA, 0.5 µl (10 µM) of each primer, 200 µmol dNTPs, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl (pH 8.3) and 1 U of Taq polymerase. A Hybaid OMN-E thermalcycler was used with 35 cycles, consisting of a 30-second denaturation at 94°C, a 30-second annealing at 60°C and a 45-second extension phase at 72°C. A 4-minute pre-denaturing step at 94°C and a 7-minute post-cycle extension temperature at 72°C were employed.

Two different amplification approaches were applied for the dung extracts. The first approach was identical to the amplification of skin tissue extracts except for the use of 45 cycles instead of 35. A second approach was employed when PCR products were insufficient for sequencing. Thirty cycles of amplification under the same conditions as

for the skin tissue extracts were followed by a second step with 5 µl of the amplified product subjected to 40 cycles. The second step was run with the same temperature profiles as above. The PCR conditions, however, were changed to 50 mM KCl, 10 mM Tris-HCl, 2.85 mM $MgCl_2$, 12.5 pmol of each primer, 400 µM of each nucleotide, and 1 U of Taq polymerase. All PCR products were run on a 1% agarose gel and viewed using ethidium bromide staining and ultraviolet transillumination.

Enzymatic clean-up of PCR Products: PCR products were prepared for sequencing by removing excess primers and dNTPs with Exonuclease I (*Exo I*) and shrimp alkaline phosphatase (SAP; Amersham Pharmacia Biotech, Greece). Five µl of the enzyme mix (SAP 0.74 µl, Exo I 0.38 µl and dH_2O, 3.88 µl) were added to 20 µl of PCR product, spun down and incubated through successive steps of 37°C for 30 minutes and 80°C for 15 minutes, for digestion of excess primers and dNTPs, and to permanently inactivate enzymes, respectively. Enzyme-purified PCR products were sequenced using the BigDye Terminator system (ABI; Sanger, 1977).

Microsatellite DNA

Samples used for microsatellite DNA analysis: A total of 84 samples obtained from 5 localities in Kenya (Ruma NP, Laikipia, Nairobi NP, Naivasha and Meru NP) and 2 localities in Tanzania (Ngorongoro and Mara-Serengeti NP) were used in this analysis (Table 1, Figure 1).

PCR amplification of microsatellite loci: A total of 7 pairs of fluorescent-labeled primers were used to genotype hartebeest DNA samples on an ABI automated sequencer (Table 2; sequences obtained from O. Flagstad). A Perkin Elmer 9700 thermalcycler was used with a 10 µl reaction containing 1 µl of template DNA, 50 mM KCl, 10 mM Tris-HCl, 1.35 mM of $MgCl_2$, 200 µM of each nucleotide, 5 picomoles of each primer and 0.5 U of Taq polymerase. Hot start was initiated through a 5 minute denaturing step at 94°C. This was followed by 35 cycles of 95°C for 30 seconds, annealing temperature ranging from 50-57°C for 30 seconds and elongation at 72°C for 45 seconds (Flagstad *et al.,* 1999). The cycles were followed by a final elongation temperature of 72°C for 10 minutes.

For electrophoresis, the PCR products from each DNA were pooled and run together with an internal size standards (TAMRA 350, Applied Biosystems). The software GENESCAN and GENOTYPER (Applied Biosystems) were used to automate measurement of allele length and to quantify peak heights in samples containing multiple alleles per locus. Data from samples that amplified poorly for each particular locus were discarded.

Data Analysis - Mitochondrial DNA

Phylogeny reconstruction and choice of DNA substitution model*:* Since previous studies (Arctander *et al.* 1999, Flagstad *et al.,* 2001) showed the 'southern' hartebeest (*lichtensteinii* and *caama*) form a monophyletic clade, one *caama* sequence and two *lichtensteini* sequences were used as outgroups in this phylogenetic analysis. Sequencher 3.1 (Gene Codes Corporation, Ann Arbor MI) was used to examine sequence quality, and

the nucleotide sequences were compared to registered sequences in GenBank and confirmed as a fragment of the hartebeest mitochondrial DNA. Mitochondrial DNA D-loop sequences were aligned using CLUSTAL X (Thomson *et al.,* 1997) and Se-Al (Rambaut, 2002) excluding 11 base pairs of uncertain alignment. Final analyses were based on a 489 base pair alignment of mitochondrial D-loop.

To select a model of DNA substitution that best fits the data, a maximum likelihood ratio test implemented in the program Modeltest ver 3.06 (Posada & Crandall, 1998) was used. The model selected by hierarchical likelihood ratio tests (K81 uf+I+G) was implemented in PAUP*4.0b10 using the Modeltest generated likelihood settings: base frequencies, A = 0.3517, C = 0.2540, G = 0.1262, T = 0.2681; number of substitution types = 6; substitution rate matrix = (1.00 67.17 3.40 67.17); shape parameter of the gamma distribution = 0.41; and assumed proportion of the invariable sites = 0.40. These settings were used for Neighbor Joining (NJ) and for Maximum Likelihood (ML) analyses. For Maximum Likelihood analysis, a starting tree obtained by random addition underwent TBR branch swapping. Bootstrap resampling support was based on 100 (ML), 500 (MP) or 1, 000 (NJ) replicates, with TBR (MP) or NNI (ML) branch swapping of starting trees obtained by NJ (for ML bootstrap) or simple stepwise addition.

Population genetic structure was analysed using ARLEQUIN software version 2.001 (Schneider *et al.,* 2000), with Tamura Nei distances (Gamma correction, A = 0.01; allowing for unequal mutation rates among sites), and 0.05 as the allowed level of missing data. Mismatch distribution used 100 bootstrap replicates, while neutrality tests used 16,000 simulated samples. Analysis of Molecular Variance (AMOVA; Excoffier *et*

al., 1992) was used to examine the extent of differentiation among 'subspecies' and sampling locations. The significance of Φ-statistics was tested using 1,000 replications. The exact test of population differentiation used 100,000 steps in Markov chain and 3,000 dememorization steps. Haplotype diversity within populations (H) was estimated using the equation of Nei and Tajima (1981). Estimates for nucleotide diversity (π, the average number of differences between two DNA sequences at each nucleotide site) used Nei & Li (1979).

Mismatch frequency distributions were used to draw inferences about historical demography of hartebeest populations. Departures between observed and expected distributions under the expansion hypothesis (Rogers and Harpending, 1992) were tested using the chi-square test of goodness of fit in the program ARLEQUIN.

Microsatellite DNA analysis

Genetic variation: Genetic variability in hartebeest from 8 sampling locations was determined by examining the mean number of alleles per locus, allele frequencies at each locus, observed heterozygosity (*Ho*), 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).

Heterozygosity: An unbiased estimate of gene diversity or expected heterozygosity (H_E) was derived for each locus per population combination using the equation $H_E = 2n(1-$

$\sum p_i^2) / (2n-1)$, Where n is the number of individuals sampled and p_i is the frequency of each of the alleles at a particular locus (Nei, 1987).

Hardy-Weinberg Equilibrium (HWE): The test for deviations from HWE was performed using the program GENEPOP ver 3.4 (Raymond and 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, 1,000 iterations and 1,000 dememorizations.

Genotypic linkage disequilibrium was examined for all pairwise combinations of loci in each population, also using the program GENEPOP ver 3.4. The null hypothesis (H_o) 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 analyzed using the Markov chain method in a similar manner to the test for HWE expectations described above (Raymond and Rousset, 1995).

Population tree: Genetic similarity (p) was estimated using the proportion of shared microsatellite alleles, which has been shown to be appropriate for closely related populations (Bowock *et al.,* 1994). The distance measure *1-p* was used to reconstruct a UPGMA population tree using the program NEIGHBOUR, from the package PHYLIP ver 3.5 (Felsenstein, 1993). Bootstrap values for the observed tree topology were based on 1,000 re-samples.

Results

Mitochondrial DNA

Phylogenetic analysis was based on a 489 bp alignment of 89 unique mitochondrial D-loop sequences, combining 12 unique sequences added from this study in Kenya and Tanzania with 77 previously published sequences (Arctander *et al.,* 1999; Flagstad *et al.,* 2001). A maximum number of sequences were included in the phylogenetic analysis to enhance accuracy (Pollock *et al.,* 2002).

All new sequences generated in this study clustered within the 'Eastern Lineage' of Flagstad *et al.* (2001), and bootstrap support was enhanced (Fig. 3). All haplotypes from individuals with *A. b. cokei* morphology clustered within the '*swaynei-cokei* clade', with one exception. This was cokei784, which fell within the '*lelwel* group' (Flagstad *et al.,* 2001) of the '*lelwel-cokei* clade' (Figure 3), and was shown by this study to be distributed widely across the *A. b. cokei* range, but was not found in populations having *lelwel* morphology.

Two closely related haplotypes with high frequencies in Laikipia (H17 and H18) clustered with a haplotype from the Uganda/Kenya border (lelwel43) to form a clade distinctive from both the '*lelwel* group' (Flagstad *et al.,* 2001) and the '*swaynei-cokei* clade' (Figure 3). The remaining haplotypes from Laikipia fell within the '*swaynei-cokei* clade', often on the same branch as haplotypes from populations having *cokei* morphology. The sole haplotype from Ruma NP (H13), another population with *lelwel* morphology, also fell within the '*swaynei-cokei* clade', forming a relatively long branch that lacked representation in any other population. These results confirm and compound the mitochondrial polyphyly of hartebeest subspecies comprising the 'Eastern Lineage'.

Genetic variation: A slightly different set of sequences was used in the population genetic than the phylogenetic analysis because several published hartebeest sequences were of uncertain provenance, and several others differed by indels that were not used as characters in the phylogenetic analysis. Among 75 samples added by this study from 6 locations, a total of 23 unique haplotypes was found, 16 not encountered in previous studies. Seven of the 16 new haplotypes were found only in one population (Laikipia), and the remaining new haplotypes did not feature in more than 2 populations. Added to those previously published, the total was 82 haplotypes among 178 samples from 11 locations (Table 3).

The observed transition/transversion ratio was 8.00, and among-site variation was moderate, with $\alpha = 0.01$. Compared to results from previous studies (Wilhelmus *et al.,* 1999; Flagstad *et al.,* 2001; Arctander *et al.,* 1999; Flagstad *et al.,* 1999), moderate to high haplotype and nucleotide diversity was recorded in all populations except Ruma NP (Table 4).

Population Genetic Subdivision: A hierarchical analysis of molecular variance (AMOVA) revealed significant Φ statistics, with the highest proportion of genetic variation observed within populations (50.55%; d.f. = 166; $\Phi_{ST} = 0.497$). A substantial proportion of the variance was distributed among morphotypes (36.76%; d.f. = 5; $\Phi_{CT} = 0.366$), leaving the lowest proportion distributed among populations within morphotypes (12.70%; d.f. = 6; $\Phi_{SC} = 0.207$; $P < 0.001$ in all cases).

Estimates of genetic subdivision (F_{st}) revealed significant structure between all but 4 population pairs, including some with the same morphotype (Table 5). The only exceptions were between Mara-Serengeti NP and two other populations with *A. b. cokei* morphology, Ngorongoro and Naivasha. The Uganda and Chad populations (both of *A. b. lelwel* morphotype) were also not significantly subdivided, as were the *A. b. tora* and Chad populations. The latter was the only instance of lack of subdivision between morphotypes, but samples sizes were low.

Geographical proximity was weakly related to mtDNA distance between populations. To depict this effect, the genetic distance between each pair of populations was plotted against the geographic distance separating them (Fig. 4). A positive but scattered trend was observed overall that rose steeply and then leveled off at geographic distances greater than about 500 km. In general, genetic distances between populations having similar morphology (*cokei-cokei* and *lelwel-lelwel* pairs) were low, relative to genetic distances between populations of different morphology. That this was true irrespective of geographic distance is suggestive of genetic disjunction between *lelwel* and *cokei* morphotypes. However, genetic distances between Ruma NP and other *lelwel* populations were among the highest, emphasizing the anomalous mitochondrial status of the Ruma NP samples.

Mismatch Frequency Distributions: Distributions of the number of nucleotide differences between each pair of haplotypes were examined within the 6 populations for which samples were added in this study (Ruma NP was omitted because there was no nucleotide variation). All populations exhibited multimodality (not shown), and Chi-

square tests invariably indicated a significant departure between observed frequencies and unimodality expected under the population expansion hypothesis (Nee *et al.*, 1996; $P < 0.0001$ in all cases). Although multimodal patterns are consistent with long-term population stability (Rogers and Harpending, 1992), in this case the incidence of > 22 pairwise differences between haplotypes within a population was more likely due to the presence of haplotypes representing both the *swaynei-cokei* and *lelwel* clades of the mtDNA phylogeny. Both were present in all populations except Nairobi NP.

Microsatellite DNA

Allelic variation and linkage: Among 84 hartebeest samples from 7 populations a total of 55 alleles were recorded at 7 microsatellite loci (Table 6). No significant differences were observed in allele frequency distribution at each of the seven microsatellite loci using Fisher's exact test ($P < 0.05$; data not shown). Exact tests for genotypic linkage disequilibrium between pairs of microsatellite loci across all populations revealed no evidence for linkage ($P > 0.05$ in all cases; data not shown). Allelic variation among populations was also not significantly different. Observed heterozygosity was relatively low in Meru NP and Laikipia, and high in Ruma NP (Table 6), but there was no significant difference between observed and expected heterozygosities in all the populations tested (Table 6).

There were significant departures from Hardy-Weinberg Equilibrium in Nairobi NP, Ngorongoro and Mara-Serengeti NP, all due to apparent heterozygote deficiency. In tests on individual loci only FCB304, which had the most alleles, was not in HWE (P <

0.001), also showing heterozygote deficiency in four populations (Nairobi NP, Naivasha, Ngorongoro and Mara-Serengeti NP).

Population Genetic Subdivision: Pairwise analysis of population subdivision revealed generally low F_{ST} values (Table 5). None of the estimates involving both populations with *A. b. cokei* morphology was significantly greater than zero. By contrast, all other population pairs were significantly subdivided, including those in which both populations had *A. b. lelwel* morphology. Private alleles were found in Naivasha (2), Meru NP (3), and Ruma NP (1).

Population tree: When individuals for which allelic information was available for all 7 loci were used to derive a distance-based UPGMA tree, populations with *A. b. cokei* morphology clustered together with strong bootstrap support. However, clustering did not accord with the degree of their geographic separation (Fig. 5). Basal to this *A. b. cokei* group were populations with *A. b. lelwel* morphology at Laikipia and Ruma NP, but only the branch to the former was well supported. Despite its *A. b. cokei* morphology, the position of the Meru NP population was not well supported. When all individuals were included for which allelic information was available for at least 6 loci, a tree with similar topology was obtained but with lower bootstrap support (Fig. 5).

Discussion

A survey of rapidly evolving genetic markers revealed relatively little distinction and varying levels of subdivision among hartebeest populations in Kenya that straddle the transition zone between two morphotypes. The analysis enhanced results of previous studies focusing solely on mtDNA, showing the majority of genetic variation to exist within populations, and less variation partitioned between morphotypes. However, our analysis of populations in such close geographical proximity reduced the tendency for mtDNA haplotypes to cluster phylogenetically according to morphotype. Populations with *lelwel* morphology shared divergent mitochondrial haplotypes with populations that are morphologically *cokei* in ways that suggest both retention of shared ancestral polymorphism, and more recent gene flow. Shared ancestral polymorphism is consistent with the observed pattern of a haplotype in the *lelwel* clade (cokei784) being absent from populations with *lelwel* morphology, but widespread among *cokei* populations. Conversely, gene flow was invoked by the pattern of more derived haplotypes belonging to the cokei clade being present not only in populations with *cokei* morphology but also in populations with *lelwel* morphology. For example, cokei796, cokei747, cokei786, cokei783, and cokei46 were widespread in Serengeti, Nairobi, Naivasha and Ngorongoro, and also present in Laikipia. The reciprocal pattern was not observed, that is, haplotypes belonging to the lelwel clade were not found in both *cokei* and *lelwel* populations.

Microsatellite distinctions between populations and morphotypes were more subtle than mitochondrial distinctions. Alleles showed little tendency to be abundant within one morphotype and absent from the other. While lower indices of population subdivision were observed for biparentally inherited microsatellites than for maternally

inherited mitochondrial markers, the haploid, uniparental mode of inheritance of mtDNA would have contributed to this effect (Avise, 1994), as would greater philopatry among females than males. Interpretation of these patterns as to the degree of hybridization between *lelwel* and *cokei* is limited by lack of microsatellite information from populations further from the contact zone, in the 'core' of the *lelwel* range.

The observed patterns are consistent with the refugium hypothesis of Arctander *et al.* (1999) and Flagstad *et al.* (2001), which holds that present day hartebeest morphotypes originally diverged in isolation of each other during recent periods of climatic change. However, our results further suggest that *cokei* and *lelwel* later resumed contact, and that maternal gene flow was predominantly from populations with *cokei* morphology to those with *lelwel* morphology. Such a biased pattern would result, for example, if in fights between males, the more robust horns of *A. b. lelwel*, with the long pedicel providing superior leverage (J. Kingdon, pers. comm.), conferred an advantage over males with *cokei* horn morphology.

Our genetic results also agree with the assignment by Stuart and Stuart (1963) and East (1998) of all hartebeest in the transitional zone as hybrid between *A. b. lelwel* and *A. b. cokei*. This includes the Laikipia, Ruma NP, and Meru NP populations. Although limited variation in horn shape and size was evident within populations, our field observations of gross morphology showed the hartebeest at Ruma NP and Laikipia to resemble *A. b. lelwel* in horn shape, with more frequent appearance of intermediate morphology in the latter, and the Meru NP hartebeest to resemble *A. b. cokei* (a quantitative analysis of skull morphology is in progress). Thus, there was a sharp disparity between the subtle, apparently seamless genetic transition between morphotypes

and the marked disjunction in gross morphology over short distances. For example, about 100 km separates southern Laikipia from Naivasha, eastern Laikipia from Meru NP, and Ruma NP from the northern Masai Mara, yet morphological distinctions and affinities to their respective 'parental' phenotypes were readily apparent within each of these pairs. These patterns differed fundamentally from those in wildebeest, also an Alcelaphine, in which mitochondrial differences were marked between morphologically distinct populations in close geographic proximity (Templeton and Georgiadis, 1995; Arctander *et al.,* 1999).

Earlier observations, documented when hartebeest distribution was less fragmented than today, support the notion that the transition zone between morphotypes was remarkably narrow. For example, 'Laikipia hartebeest' were consistently distinguished from 'hybrids' in early game reports of the British administration in Kenya. G. H. Goldfinch (1914) noted that 'Laikipia hartebeest appeared to share the same ground, but not the same herds, with hybrids near Lake Olbolossat' [in south-west Laikipia] on a seasonal basis. He also noted 'there appears to be a [hybrid] form…near Meru'. And the last remaining individual of a population considered to be hybrid between *lelwel* and *cokei*, that went extinct in the Nakuru area of the Rift Valley in 1967, had the appearance of a more equal mix between the two (Gosling 1969). Some individuals on Solio Ranch in southern Laikipia have a similar appearance (Fig. 2C).

Consistent with their emergence in the last 140,000 years (Flagstad *et al.,* 2001), subtle genetic differences among populations suggest that gross morphological distinctions between *lelwel* and *cokei* evolved recently, and entail relatively few loci.

Sexual selection and hybrid inferiority may have played a role in maintaining morphotypic distinctions, despite historical gene flow.

Conservation implications

In this case, gross morphology superficially appears to provide a superior basis than genetics for management action intended to conserve evolutionary diversity in hartebeest. An interpretation of the genetic data alone might have concluded that a subset of the populations in this study is isolated by distance, but not distinct from each other in fundamental or qualitative ways. In the case of the Ruma NP population, analysis based solely on mtDNA would have been further misleading in two senses. First, in implying phylogenetic affinity to the *cokei* clade rather than the lelwel clade. And second, in implying that genetic variation in the population was low. Low mtDNA variation at Ruma NP suggests the population has 'bottlenecked' in the past, but given that it had the highest microsatellite heterozygosity, nuclear variation appeared unaffected (in all other populations, levels of nuclear and mitochondrial variation were moderate to high, compared to other African bovid species; Alpers *et al.,* 2004, and references therein).

But gross morphology could not – as genetic data can – illuminate the processes that underlie the observed morphological patterns. In this case, both morphological and genetic data were required to define the targets of conservation. Hartebeest populations in Laikipia, Ruma NP and Meru NP are important in that they provide the only remaining examples, each different from the other, of what appears to be resumed contact between two morphotypes that diverged in allopatry, a process that has been ongoing across a vast area for thousands of generations.

Yet all these populations are threatened. Dwindling numbers in Meru NP prompted conservation managers to propose supplementing the population from elsewhere. Contrary to East (1998), in which the Meru NP population is incorrectly grouped with Laikipa and Ruma NP on morphological grounds, similarities in gross morphology would suggest a more suitable source population to be Naivasha or Nairobi NP. While this would be unlikely to result in reproductive incompatibilities, our microsatelle results suggest the Meru NP hartebeest to be genetically subdivided from the 'core' *cokei* populations, and the only remaining hybrid population with *cokei* morphology. A preferable strategy, aiming to conserve the products of hybridization, would be to breed up the remaining individuals at Meru in a large area surrounded by a predator-proof fence. The Laikipia population is also declining and is nowhere formally protected. The property holding the highest densities (Solio Ranch in the extreme south) is unlikely to remain intact. The Solio hartebeest should be moved to a more permanently secure location. Similarly, suitable refuges for the Ruma NP hartebeest should be identified should this protected area not survive pressures from surrounding humanity.

Conservation protocols and practices are ill-defined for hybrids between species (IUCN 2004) morphotypes. By emphasizing recognizable species, this inevitably relegates hybrids and variants to a lesser status, forcing much of the attention on the important but narrow question of whether or not products of inter-breeding might be viable. In practice, conservation managers do well to evaluate reproductive compatibility before merging two populations. Too rarely considered are the implications of disrupting more subtle evolutionary processes. Often there is no option (particularly in captivity),

but in many African countries, including Kenya, where there has been little mixing of populations by translocation, opportunities to conserve ongoing evolutionary processes persist, and should be pursued. If these more subtle but valuable evolutionary processes are not defined as soon as possible, and explicitly integrated into national conservation strategies, they will not be conserved. Defining evolutionary processes is possible through partnerships between national and international agencies, and with funding from zoos and conservation non-government organizations (NGO's).

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References

Alpers DL, van Vuuren BJ, Arctander P, Robinson TJ (2004) Population genetics of the roan antelope (*Hippotragus equines*) with suggestions for conservation. Mol Ecol 13: 1771-1784

Amos W and Balmford A (2001) When does conservation genetics matter? Heredity 87: 257-265

Arctander P, Johansen C, Coutellec-Vreto M-A (1999) Phylogeography of three closely related African Bovids (Tribe Alcelaphini). Mol Biol Evol 16: 1724-1739

Barendse W, Armitage SM, Kossarek LM et al., (1994) A genetic linkage map of the bovine genome. Nature Genetics 6:227-235

Bowock AM, Ruiz-Linares A, Tomfohrde J, Minch E, Kidd JR, Cavalli-Sforza (1994) High resolution of human evolutionary trees with polymorphic microsatellites. Nature 368:455-457

Buchanan FC and Crawford AM (1992) Ovine dinucleotide repeat polymorphism at the MAF209 locus. Anim Genet 23:183

Buchanan FC and Crawford AM (1993) Ovine microsatellites OarFCB11, OarFCB128, OarFCB193, OarFCB266 and OarFCB304. Anim Genet 24:145

Buchanan FC, Galoway SM and Crawford AM (1994) Ovine microsatellites OarFCB5, OarFCB19, OarFCB20, OarFCB48, OarFCB129 and OarFCB266 loci. Anim Genet 25:60

Cillie B (1987) Mammals of Southern Africa. South Africa: Frandsen Publishers

Crandall KA, Bininda-Edmonds ORP, Mace GM, Wayne RK (2000) Considering evolutionary processes in conservation biology. Trends in Ecology and Evolution 15:290-295

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

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

Felsenstein J (1993) Phylogenetic inference using Parsimony (PHYLIP) 3.5. Washington University, Seattle

Flagstad O, Olsaker I, Roed KH (1999) The use of heterologous primers for analysing microsatellite variation in hartebeest Alcelaphus buselaphus. Hereditas 130:337-340

Flagstad O, Syvertsen PO, Stenseth NC, Jakobsen KS (2001) Environmental change and rates of evolution: the phylogeographic pattern within the hartebeest complex as related to climatic variation. Proc R Soc Lond B Biol Sci. 268:667-677

Goldfinch GH (1914) Report of the Game in Western Kenia District. Appendix 1 in Annual Report of the Game Warden for the year 1913-1914. East African Protectorate, Government Printer, Nairobi

Gosling LM (1969) The last Nakuru hartebeest. Oryx 10:173-174

Greig JC (1979) Principles of genetic conservation in relation to wildlife management in Southern Africa. S Afr J Wildl Res 9:57–78

Groth DM and Wetherall JD (1994) Dinucleotide repeat polymorphism within the ovine major histocompatibility complex class I region. Anim Genet 25:61

Hulme DJ, Silk JP, Redwin JM, Barendse W and Beh KJ (1994) Ten polymorphic ovine microsatellites. Anim Genet 25:434-435

Hultman T, Stahl S, Hornes E, Uhlen M (1997) Direct solid—phase sequencing of genomic and plastid DNA using magnetic beads as solid support. Nucleic Acids Research 17:4937-4946

IUCN 2004 (2004) IUCN Red List of Threatened Species. IUCN, Gland. Switzerland and Cambridge, UK

Karesh W, Smith F, Frazier-Taylor H (1989) A remote method for obtaining skin biopsy samples. Cons Biol 1:261-262

Kingdon J (1997). The Kingdon guide to African Mammals. Academic Press, London

Kingdon J (1982) East African Mammals: An Atlas of Evolution in Africa. Vol. III Part D (Bovids). University of Chicago Press, Chicago

Matthee CA, Robinson TJ (1999) Mitochondrial DNA population structure of roan and sable antelope: implications for the translocation and conservation of the species. Mol Ecol 8:227-238

Minch E, Ruiz-Linares A, Goldstein D, Feldman M, Cavalli S (1996) MICROSAT: a computer program for calculating various statitstics on microsatellite allele data ver 1.5d, Stanford University Medical Centre, Stanford.

Nee S, Holmes EC, Rambaut A, Harvey PH (1996) Inferring population history from molecular phylogenies. Pp. 66-80 in Harvey PH, Leigh Brown AJ, Maynard Smith J & Nee S (Eds) New uses for new phylogenies. Oxford University Press, New York

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonuclease. Proc Natl Acad Sci USA 76:5269-5273

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

Nei M & Tajima F (1981) DNA polymorphisms detectable by restriction endonucleases. Genetics 97:145-163

Pitra C, Kock RA, Hofmann RR, and Lieckfeldt D (1998) Molecular phylogeny of the critically endangered Hunter's antelope (Beatragus hunteri Sclater 1889). J Zool Syst Evol Res 36:179-184

Pollock DD, Zwickl DJ, McGuire JA, Hillis DM (2002) Increased taxon sampling is advantageous for phylogenetic inference. Syst Biol 51:664-671

Posada D, Crandall KA (1998) MODEL TEST: testing the model of DNA substitution. Bioinformatics14:817-818

Rambaut A (2002) Sequence Alignment Editor http://evolve.zoo.ox.ac.uk/software/Se-Al/main.html Oxford, University of Oxford

Raymond M and Rousset F (1995) GENEPOP (ver 3.3): population genetics software for exact tests and ecumenicism. J Hered 86:248-249

Rogers AR, Harpending HC (1992) Population growth makes waves in the distribution of pairwise genetic differences. Mol Biol Evol 9:552-569

Rudi K, Kroken M, Dahlberg OJ et al. (1997) Rapid Universal method to isolate PCR-ready DNA using magnetic beads. Biotechniques 22:506-511

Sambrook J, Fritsch EF, Manniatis T (1989) Molecular cloning: A Laboratory Manual, 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA

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

Schneider S, Roessli S, Excoffier L (2000) Arlequin: software for population genetic data analysis. Ver. 2.000. Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva

Sidney J (1965) The Past and Present Distribution of some African Ungulates. Trans Zool Soc Lond 30:1-396

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

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463-7

Southern SO, Southern PJ, Dizon AE (1988) Molecular characterization of a cloned dolphin mitochondrial genome. J Mol Evol 28:32-42

Stewart DRM & Stewart J (1963) The distribution of some Large Mammals in Kenya. J E Afr Nat Hist Soc 24:1-52

Templeton AR and Georgiadis NJ (1996) A landscape approach to conservation genetics: Conserving evolutionary processes in the African Bovidae. In: Avise JC and Hamrick JL (eds), pp. 378-430 Conservation Genetics: Case histories from nature. Chapman and Hall, New York

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

Vrba ES (1995) The fossil record of African antelopes (Mammalia, Bovidae) in relation to human evolution and paleoclimate. In: Vrba ES, Denton GH, Partridge TC, Burckle LH (eds) pp 385-424 Paleoclimate and evolution with emphasis on human origins. Yale University Press, New Haven and London

Wilhelmus R, Harley (1999) Phylogenetic relationships in the bovid subfamily Antilopinae based on mitochondrial DNA sequences. Mol Phyl & Evol 12:87-94

Young AG and Clarke GM (2000) Genetics, Demography and Viability of Fragmented Populations pp 1-113. Cambridge University Press, United Kingdom

Fig. 1. Distribution of hartebeest in Africa (after East 1998), with different symbols denoting morphotypes. Symbols are filled grey if mtDNA sequences from that location were used in this analysis, and black where populations were added by this study (see legend below).

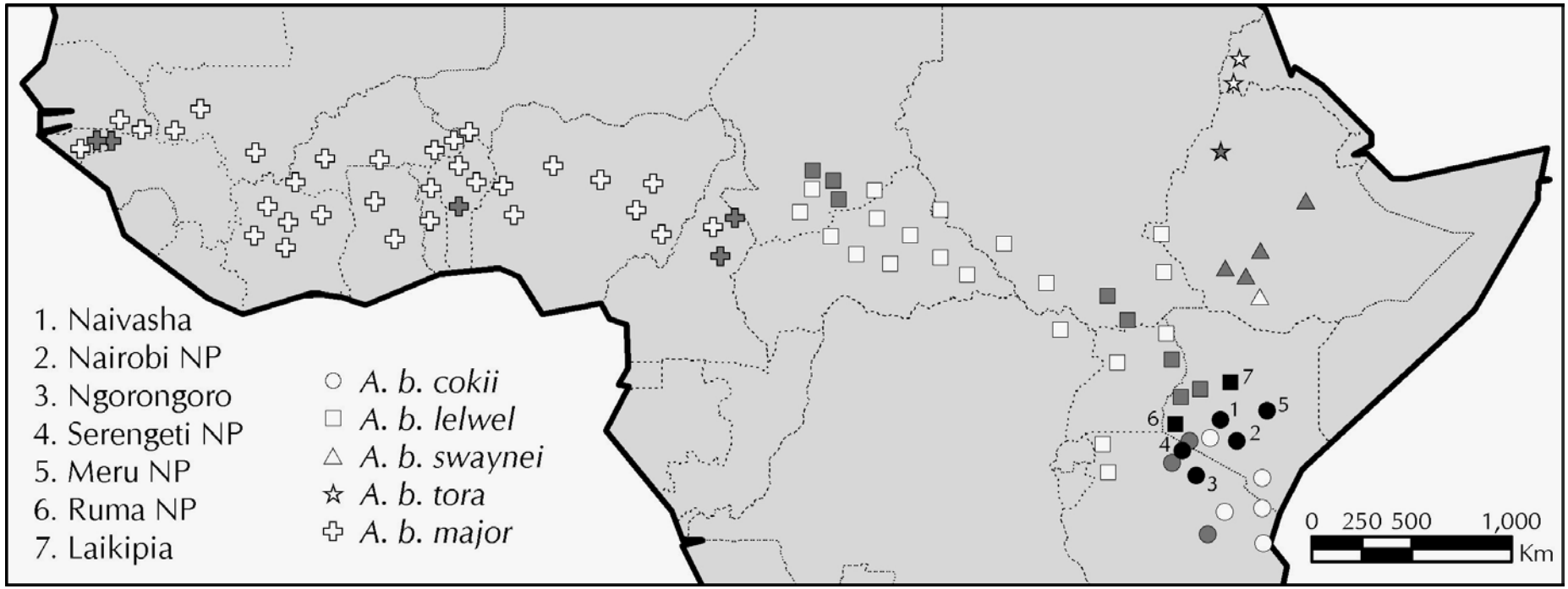
Fig. 2. Frontal and lateral views of male horn morphology in Nairobi NP (A; *A. b. cokei*), Meru NP (B; resembles *A. b. cokei*), Solio Ranch, Laikipia District (C; greater resemblance to *A. b. lelwel*), and Ruma NP (D; resembles *A. b. lelwel*). (Photos by N. Georgiadis.)

Fig. 3. Maximum likelihood based on 489 characters of mtDNA D-loop
(-ln L. = 3434.0). Bootstrap values shown only for groups indicated at right: ML/NJ/MP

Fig. 4. Plots of genetic distance versus geographical distance (km) for pairs of populations based on mtDNA data (A, using standard genetic distance of Nei, 1987) and microsatellite DNA data (B, using *1-b*; Bowock et al. 1994). Symbols distinguish pairs in which both populations had *cokei* morphology (triangles), both populations had *lelwel* morphology (circles), and one population had *cokei* morphology, the other *lelwel* (squares). Values for all remaining pairs are symbolized by crosses. Filled symbols in A distinguish pairs involving the Ruma NP population. Filled symbols in B distinguish pairs involving the Meru NP population.

Fig. 5. An UPGMA population tree derived from genetic distances based on microsatellite data. Bootstrap values are indicated for trees based on individuals in which data from 7 microsatellite loci, and at least 6 microsatellite loci, were available.

(Fig. 1)


1. Naivasha
2. Nairobi NP
3. Ngorongoro
4. Serengeti NP
5. Meru NP
6. Ruma NP
7. Laikipia
A. b. cokii
A. b. lelwel
A. b. swaynei
A. b. tora
A. b. major
0 250 500 1,000
Km

(Fig. 2)

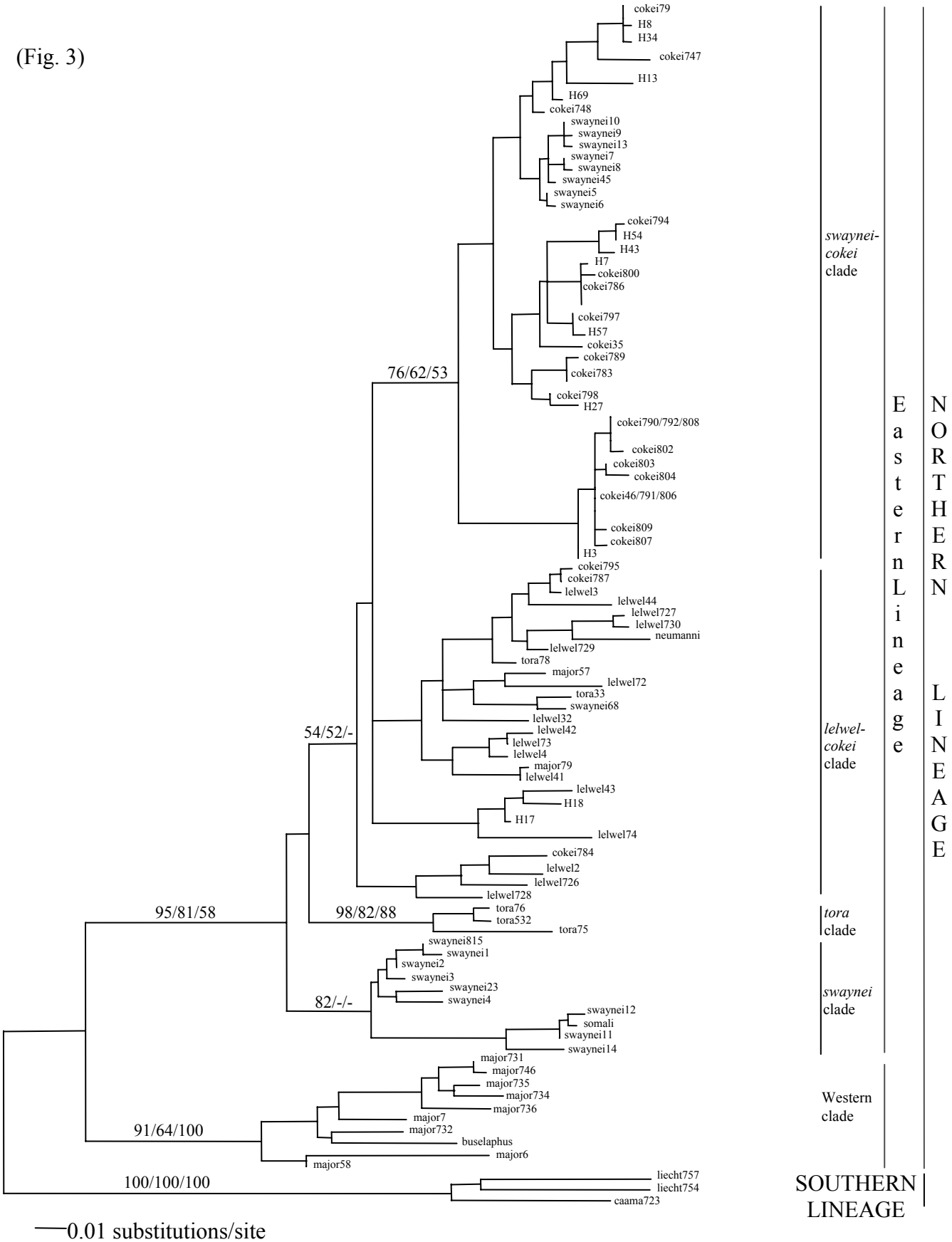
A

B

C

D

(Fig. 3)


cokei79
H8
H34
cokei747
H13
H69
cokei748
swaynei10
swaynei9
swaynei13
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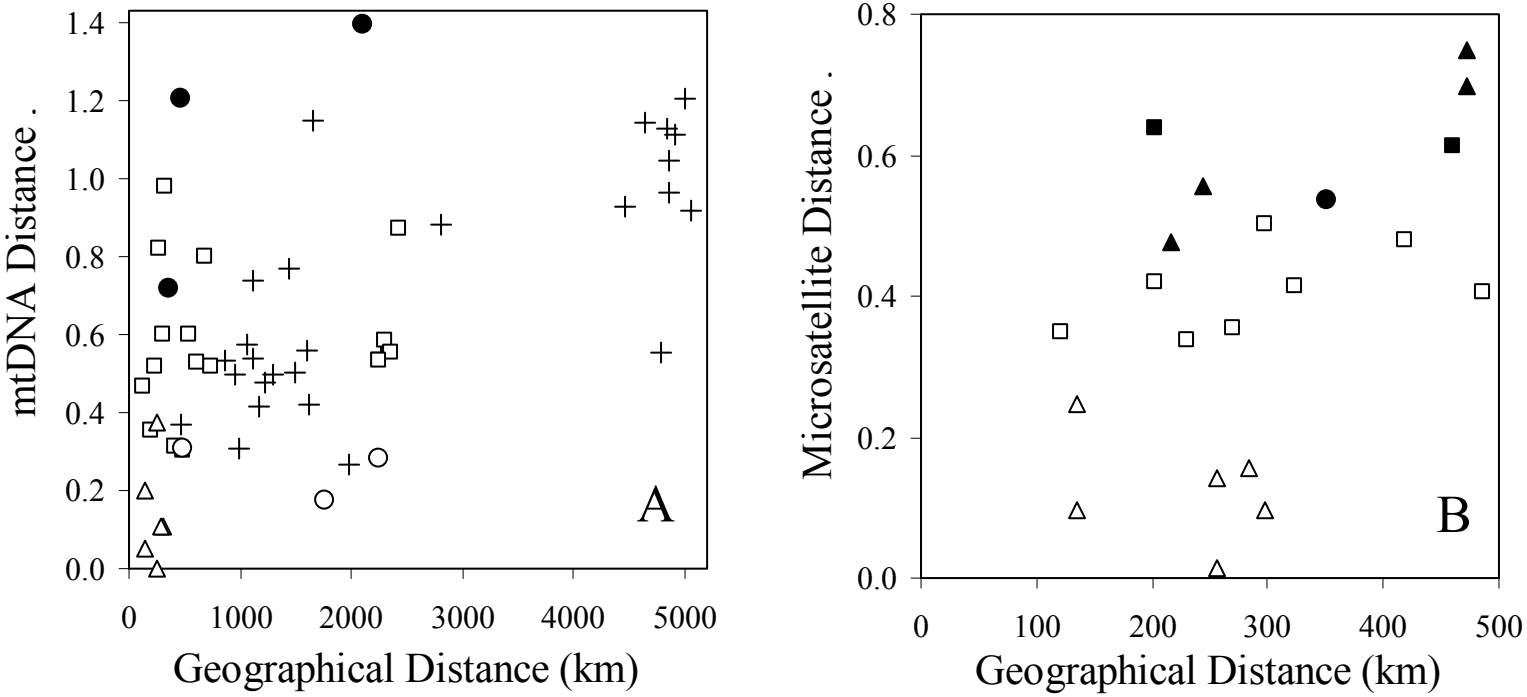
swaynei8
swaynei45
swaynei5
swaynei6
cokei794
H54
H43
H7
cokei800
cokei786
cokei797
H57
cokei35
cokei789
cokei783
cokei798
H27
cokei790/792/808
cokei802
cokei803
cokei804
cokei46/791/806
cokei809
cokei807
H3
cokei795
cokei787
lelwel3
lelwel44
lelwel727
lelwel730
neumanni
lelwel729
tora78
major57
lelwel72
tora33
swaynei68
lelwel32
lelwel42
lelwel73
lelwel4
major79
lelwel41
lelwel43
H18
H17
lelwel74
cokei784
lelwel2
lelwel726
lelwel728
tora76
tora532
tora75
swaynei815
swaynei1
swaynei2
swaynei3
swaynei23
swaynei4
swaynei12
somali
swaynei11
swaynei14
major731
major746
major735
major734
major736
major7
major732
buselaphus
major6
major58
liecht757
liecht754
caama723
76/62/53
54/52/-
95/81/58
98/82/88
82/-/-
91/64/100
100/100/100
swaynei-cokei clade
lelwel-cokei clade
tora clade
swaynei clade
Western clade
Eastern Lineage
NORTHERN LINEAGE
SOUTHERN LINEAGE
0.01 substitutions/site

(Fig. 4)


mtDNA Distance .
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1000
2000
3000
4000
5000
Geographical Distance (km)
A
Microsatellite Distance .
0.8
0.6
0.4
0.2
0.0
0
100
200
300
400
500
Geographical Distance (km)
B

(Fig. 5)

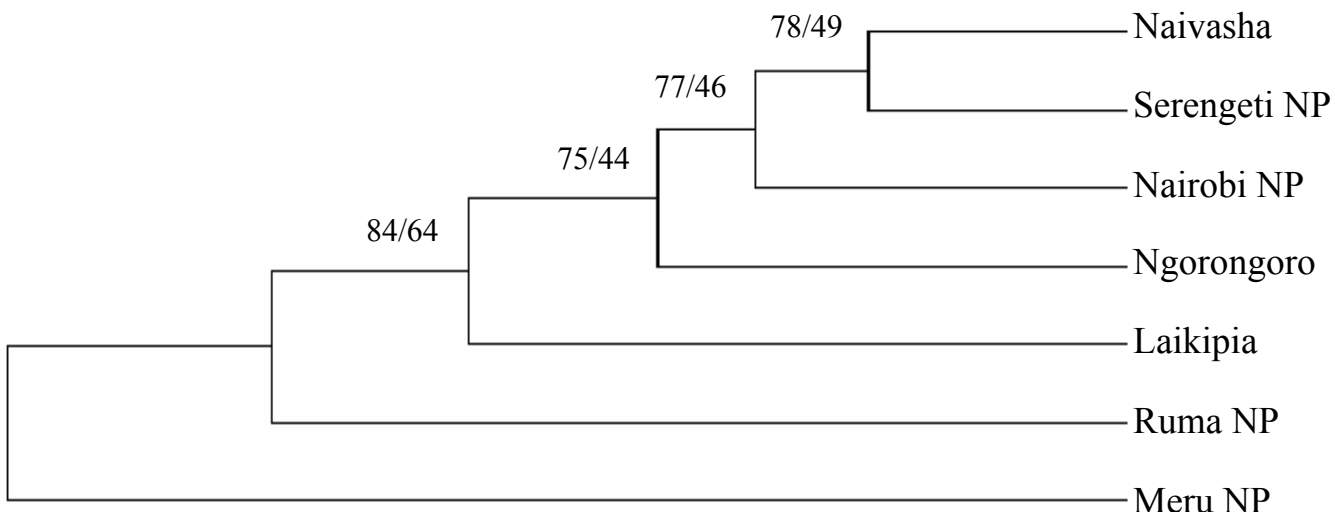

78/49
77/46
75/44
84/64
Naivasha
Serengeti NP
Nairobi NP
Ngorongoro
Laikipia
Ruma NP
Meru NP

Table 1. Summary of provenance and number of samples used in this analysis. Numbers in bold denote new samples added by this study to samples from which results have been published previously (Arctander *et al.,* 1999; Flagstad *et al.,* 2001).

Morphotype	**Country**	**Locality**	**Sample Type**	**DNA Analysis**	
				mtDNA	**microsatellite**
cokei	Kenya	Naivasha	Skin	**16**	**14**
cokei	Kenya	Nairobi NP	Skin	6+**8**	**11**
cokei	Tanzania	Ngorongoro Crater	Skin	**11**	**13**
cokei	Tanzania	Mara-Serengeti NP	Skin	15+**14**	**17**
cokei	Kenya	Meru NP	Skin	-	**5**
lelwel	Kenya	Ruma NP	Skin / Dung	**2 / 6**	**2 / 6**
lelwel	Kenya	Laikipia	Skin / Dung	**6 / 12**	**4 / 12**
lelwel	Uganda + Congo+ Sudan			11	
lelwel	Chad			5	
swaynei	Ethiopia + Somalia			49	
tora				5	
major				11	
buselaphus				1	
lichtensteinii	Tanzania	Outgroup		2	
caama	South Africa	Outgroup		1	
		TOTAL		181	84

Table 2. Microsatellite primer sequences and sources.

Locus	Annealing Temp. (C)	Allele size range (bp)	F-Forward primer R-Reverse primer	Reference
ETH225	52	136-138	**F**-GATCACCTTGCCACTATTTCCT	Barendse *et al.*, 1994
			R-ACATGACAGCCAGCTGCTACT	
FCB48	57	146-164	**F**-GACTCTAGAGGATCGCAAAGAACCAG	Buchanan *et al.,* 1994
			R-GAGTTAGTACAAGGATGACAAGAGGCAC	
FCB304	57	129-161	**F**-CCCTAGGAGCTTTCAATAAAGAATCGG	Buchanan & Crawford 1993
			R-CGCTGCTGTCAACTGGGTCAGGG	
MAF209	57	121-145	**F**-TCATGCACTTAAGTATGTAGGATGCTG	Buchanan & Crawford 1992
			R-GATCACAAAAAGTTGGATACAACCGTGG	
MCM38	50	128-150	**F**-TGGTGAATGGTGCTCTTCATACCAG	Hulme *et al.*, 1994
			R-CAGCCAGCAGCCTCTAAAGGAC	
MCM58	52	164-178	**F**-CTGGGTCTGTATAAGCACGTCTCC	Hulme *et al.*, 1994
			R-CAGAACAATAAACGCTAAACCAGAGC	
SMHCC	57	177-213	**F**-ATCTGGTGGGCTACAGTCCATG	Groth & Wetherall 1994
			R-GCAATGCTTTCTAAATTCTGAGGAA	

Table 3. Frequency of mitochondrial haplotypes (with all 'duplicate' labels) within each population.

Haplotype	**Naivasha**	**Nairobi NP**	**Ngoro -ngoro**	**Mara-Serengeti NP**	**Ruma NP**	**Laikipia**	**Uganda Congo Sudan**	**Chad**	***A.b. swaynei***	***A.b. tora***	***A.b. major***	*A. b. buselaphus*	**Total**
Buselaphus	0	0	0	0	0	0	0	0	0	0	0	1	1
cokei796/H79/H8	1	0	0	1	0	1	0	0	0	0	0	0	3
cokei783/H2	0	0	6	5	0	1	0	0	0	0	0	0	12
H34	0	1	0	0	0	0	0	0	0	0	0	0	1
cokei747/H51/H20	1	0	0	0	0	1	0	0	0	0	0	0	2
H13/H32/H25/H14/H33/H37/H81/H82	0	0	0	0	8	0	0	0	0	0	0	0	8
H69/cokei748	0	0	0	0	0	1	0	0	0	0	0	0	1
swaynei10	0	0	0	0	0	0	0	0	2	0	0	0	2
swaynei9	0	0	0	0	0	0	0	0	1	0	0	0	1
swaynei13	0	0	0	0	0	0	0	0	2	0	0	0	2
swaynei7	0	0	0	0	0	0	0	0	1	0	0	0	1
swaynei8	0	0	0	0	0	0	0	0	1	0	0	0	1
swaynei45	0	0	0	0	0	0	0	0	1	0	0	0	1
swaynei5	0	0	0	0	0	0	0	0	1	0	0	0	1
swaynei6	0	0	0	0	0	0	0	0	1	0	0	0	1
cokei794/H61	0	0	0	2	0	0	0	0	0	0	0	0	2
H54	0	0	0	1	0	0	0	0	0	0	0	0	1
H43	0	1	0	0	0	0	0	0	0	0	0	0	1
H7	1	0	0	0	0	0	0	0	0	0	0	0	1
cokei800	0	0	0	1	0	0	0	0	0	0	0	0	1
cokei786/H5/H47/H11/H19/H41/H56/H6/H68/H74 /H10/H29/H44	5	1	1	4	0	2	0	0	0	0	0	0	13
cokei797/H58/H63	1	0	0	2	0	0	0	0	0	0	0	0	3
H57/H36/H53	2	1	0	0	0	0	0	0	0	0	0	0	3
cokei789783/H2/H21/H23/H26/H28/H30/H4/H55/ H65/H62/H64	0	0	0	1	0	0	0	0	0	0	0	0	1
cokei798	0	0	0	1	0	0	0	0	0	0	0	0	1
H27	0	0	1	0	0	0	0	0	0	0	0	0	1
cokei792/H12/cokii808/H39/H46/H66	1	2	0	4	0	0	0	0	0	0	0	0	7
cokei802/792/790	0	0	0	1	0	0	0	0	0	0	0	0	1
cokei803	0	1	0	0	0	0	0	0	0	0	0	0	1
cokei804	0	1	0	0	0	0	0	0	0	0	0	0	1
cokei791/H9/H35/H40/H42/H50/H52/H70 /cokei46/cokei806	3	4	0	1	0	1	0	0	0	0	0	0	9
cokei809	0	1	0	0	0	0	0	0	0	0	0	0	1
cokei807	0	1	0	0	0	0	0	0	0	0	0	0	1
H3	0	0	0	1	0	0	0	0	0	0	0	0	1
cokei795	0	0	0	1	0	0	0	0	0	0	0	0	1

cokei787	0	0	0	1	0	0	0	0	0	0	0	0	1
lelwel3	0	0	0	0	0	0	1	0	0	0	0	0	1
lelwel44	0	0	0	0	0	0	1	0	0	0	0	0	1
lelwel727	0	0	0	0	0	0	1	0	0	0	0	0	1
lelwel730	0	0	0	0	0	0	1	0	0	0	0	0	1
lelwel729	0	0	0	0	0	0	1	0	0	0	0	0	1
tora78	0	0	0	0	0	0	0	0	0	1	0	0	1
major57	0	0	0	0	0	0	0	0	0	0	1	0	1
lelwel72	0	0	0	0	0	0	0	1	0	0	0	0	1
tora33	0	0	0	0	0	0	0	0	0	1	0	0	1
swaynei68	0	0	0	0	0	0	0	0	1	0	0	0	1
lelwel32	0	0	0	0	0	0	1	0	0	0	0	0	1
lelwel42	0	0	0	0	0	0	0	1	0	0	0	0	1
lelwel73	0	0	0	0	0	0	0	1	0	0	0	0	1
lelwel4	0	0	0	0	0	0	0	1	0	0	0	0	1
major79	0	0	0	0	0	0	0	0	0	0	1	0	1
lelwel41	0	0	0	0	0	0	1	0	0	0	0	0	1
lelwel43	0	0	0	0	0	0	1	0	0	0	0	0	1
H18/H76/H72/H75/H77/H78	0	0	0	0	0	4	0	0	0	0	0	0	4
H17/H73	0	0	0	0	0	7	0	0	0	0	0	0	7
lelwel74	0	0	0	0	0	0	0	1	0	0	0	0	1
cokei784/H1/H67/H22/H24/H31	1	0	3	2	0	0	0	0	0	0	0	0	6
lelwel2	0	0	0	0	0	0	1	0	0	0	0	0	1
lelwel726	0	0	0	0	0	0	1	0	0	0	0	0	1
lelwel728	0	0	0	0	0	0	1	0	0	0	0	0	1
tora76	0	0	0	0	0	0	0	0	0	1	0	0	1
tora532	0	0	0	0	0	0	0	0	0	1	0	0	1
tora75	0	0	0	0	0	0	0	0	0	1	0	0	1
swaynei815	0	0	0	0	0	0	0	0	1	0	0	0	1
swaynei1	0	0	0	0	0	0	0	0	9	0	0	0	9
swaynei2	0	0	0	0	0	0	0	0	6	0	0	0	6
swaynei3	0	0	0	0	0	0	0	0	1	0	0	0	1
swaynei23	0	0	0	0	0	0	0	0	1	0	0	0	1
swaynei4	0	0	0	0	0	0	0	0	2	0	0	0	2
swaynei11/swaynei12	0	0	0	0	0	0	0	0	16	0	0	0	16
Somali	0	0	0	0	0	0	0	0	1	0	0	0	1
swaynei14	0	0	0	0	0	0	0	0	1	0	0	0	1
major731	0	0	0	0	0	0	0	0	0	0	1	0	1
major746	0	0	0	0	0	0	0	0	0	0	1	0	1
major735	0	0	0	0	0	0	0	0	0	0	1	0	1
major734	0	0	0	0	0	0	0	0	0	0	1	0	1
major736	0	0	0	0	0	0	0	0	0	0	1	0	1

major7	0	0	0	0	0	0	0	0	0	0	1	0	1
major732	0	0	0	0	0	0	0	0	0	0	1	0	1
major6	0	0	0	0	0	0	0	0	0	0	1	0	1
major58	0	0	0	0	0	0	0	0	0	0	1	0	1
Total	**16**	**14**	**11**	**29**	**8**	**18**	**11**	**5**	**49**	**5**	**11**	**1**	**178**

Table 4. Summary of mitochondrial variation within each population.

Population	No. Samples	Polymorphic sites	No. of transitions	No. of transversions	Indels	Mean no. of pairwise differences	Haplotype diversity (H)	Nucleotide diversity (π)
Naivasha	16	55	52	3	1	22.66 ± 10.54	0.85	0.050 ± 0.026
Nairobi NP	14	32	23	5	4	11.21 ± 5.42	0.92	0.033 ± 0.018
Ngorongoro	11	47	38	3	9	25.26 ± 12.03	0.70	0.055 ± 0.030
Mara-Serengeti NP	29	61	43	5	19	19.53 ± 8.90	1.00	0.057 ± 0.029
Ruma NP	8	0	0	0	0	0.00 ± 0.00	0.00	0.00 ± 0.000
Laikipia	18	54	52	2	1	26.67 ± 12.26	0.83	0.063 ± 0.033
Uganda+Congo+Sudan	11	42	38	2	3	20.375 ± 9.765	1.00	0.063 ± 0.034
Chad	5	45	38	6	1	26.769 ± 14.214	1.00	0.058 ± 0.036
A.b. swaynei	49	76	66	2	9	31.77 ± 14.10	0.87	0.068 ± 0.034
A.b. tora	5	55	50	5	2	42.00 ± 22.11	1.00	0.090 ± 0.055
A.b. major	11	73	60	6	10	45.38 ± 21.35	0.97	0.129 ± 0.069

Table 5. Estimates of F_{st}, an index of genetic subdivision between pairs of populations, based on variation in mitochondrial (below diagonal) and microsatellite (above diagonal) DNA. Asterisk denotes significance at $p \leq 0.05$.

Population	**Naivasha**	**Nairobi NP**	**Ngoro-ngoro**	**Mara / Seren-geti NP**	**Meru NP**	**Ruma NP**	**Laikipia**	**Uganda / Sudan / Congo**	**Chad**	***A.b swaynei***	***A.b. tora***
Naivasha		0.024	0.016	0.016	0.095*	0.099*	0.071*	-	-	-	-
Nairobi NP	0.180*		0.018	0.083	0.166*	0.172*	0.139*	-	-	-	-
Ngorongoro	0.102*	0.313*		0.041	0.147*	0.091*	0.089*	-	-	-	-
Mara-Serengeti NP	-0.014	0.104*	0.050		0.204*	0.139*	0.112*	-	-	-	-
Meru NP	-	-	-	-		0.185*	0.161*	-	-	-	-
Ruma NP	0.403*	0.624*	0.560*	0.371*	-		0.182*	-	-	-	-
Laikipia	0.298*	0.452*	0.261*	0.268*	-	0.511*		-	-	-	-
Uganda / Sudan / Congo	0.435*	0.528*	0.395*	0.401*	-	0.660*	0.242*		-	-	-
Chad	0.425*	0.585*	0.400*	0.394*	-	0.798*	0.233*	0.086		-	-
A.b. swaynei	0.391*	0.417*	0.393*	0.379*	-	0.522*	0.438*	0.409*	0.409*		-
A.b. tora	0.427*	0.537*	0.342*	0.394*	-	0.683*	0.340*	0.252*	0.194	0.308*	
A.b. major	0.649*	0.700*	0.618*	0.681*	-	0.677*	0.672*	0.610*	0.554*	0.601*	0.4269*

Table 6. Summary of microsatellite variation within each population.

Locus		Naivasha	Nairobi NP	Ngorongoro	Mara-Serengeti	Meru NP	Ruma NP	Laikipia	All populations
ETH225	No. of individuals	14	11	13	11	5	8	17	
	No. of alleles	3	3	4	4	2	2	2	
	H_O	0.71	0.88	0.45	0.9	0.2	1	0.45	0.56
	H_E	0.83	0.77	0.73	0.86	0.51	0.66	0.4	0.65
FCB48	No. of individuals	14	11	13	11	5	8	16	
	No. of alleles	4	2	2	3	2	3	3	
	H_O	0.53	0.11	0.55	0.35	0.25	0.5	0.6	0.41
	H_E	0.49	0.11	0.5	0.36	0.53	0.42	0.68	0.44
FCB304	No. of individuals	14	11	13	11	5	8	17	
	No. of alleles	9	7	8	6	2	5	4	
	H_O	0.67	0.25	0.5	0.25	0.8	1	0.5	0.56
	H_E	0.89	0.86	0.88	0.84	0.53	0.82	0.77	0.79
MAF209	No. of individuals	14	11	13	11	5	8	16	
	No. of alleles	6	2	5	4	2	5	5	
	H_O	0.63	0.5	0.37	0.3	0.00*	0.42	0.8	0.43
	H_E	0.77	0.4	0.8	0.5	0.53	0.5	0.84	0.62
MCM38	No. of individuals	14	11	13	11	5	8	16	
	No. of alleles	6	5	6	6	2	4	4	
	H_O	0.83	0.63	1	0.91	0.2	0.87	0.69	0.8
	H_E	0.78	0.74	0.83	0.68	0.2	0.65	0.62	0.73
MCM58	No. of individuals	14	11	13	16	5	8	17	
	No. of alleles	5	5	4	4	3	3	2	
	H_O	0.75	0.6	0.25	0.15	1	1	0.00*	0.53
	H_E	0.76	0.78	0.82	0.78	0.71	0.67	0.53	0.72
SMHCC	No. of individuals	14	11	13	11	5	8	17	
	No. of alleles	8	6	6	7	3	3	5	
	H_O	0.71	0.88	0.45	0.75	0.8	1	0.45	0.72
	H_E	0.83	0.77	0.73	0.79	0.73	0.66	0.4	0.7
Mean[a]	No. of alleles	5.86 (2.12)	4.29 (1.98)	5.14 (1.95)	4.86 (1.46)	2.29 (0.49)	3.57 (1.13)	3.57 (1.27)	
	H_O	0.668(0.05)	0.507(0.06)	0.507(0.06)	0.611(0.05)	0.378(0.08)	0.743(0.06)	0.488(0.07)	
	H_E	0.714(0.05)	0.604(0.10)	0.745(0.04)	0.672(0.06)	0.511(0.05)	0.586(0.06)	0.618(0.06)	
	F_{IS}	0.064	0.16	0.319	0.09	0.26	-0.267	0.21	