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Population Genetic Structure of Savannah Elephants in Kenya: Conservation and Management Implications

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Abstract

We investigated population genetic structure and regional differentiation among African savannah elephants in Kenya using mitochondrial and microsatellite markers. We observed mitochondrial DNA (mtDNA) nucleotide diversity of 1.68% and microsatellite variation in terms of average number of alleles, expected and observed heterozygosities in the total study population of 10.20, 0.75, and 0.69, respectively. Hierarchical analysis of molecular variance of mtDNA variation revealed significant differentiation among the 3 geographical regions studied ($F_{CT} = 0.264$; $P < 0.05$) and a relatively lower differentiation among populations within regions ($F_{SC} = 0.218$; $P < 0.0001$). Microsatellite variation significantly differentiated among populations within regions ($F_{SC} = 0.019$; $P < 0.0001$) but not at the regional levels ($F_{CT} = 0.000$; $P > 0.500$). We attribute the high differentiation at the mitochondrial genome to the matrilineal social structure of elephant populations, female natal philopatry, and probably ancient vicariance. Lack of significant regional differentiation at the nuclear loci vis-a-vis strong differences at mtDNA loci between regions is likely the effect of subsequent homogenization through male-mediated gene flow. Our results depicting 3 broad regional mtDNA groups and the observed population genetic differentiation as well as connectivity patterns should be incorporated in the planning of future management activities such as translocations.

African elephant (*Loxodonta africana*) populations face increasing fragmentation and isolation across the continent (Said et al. 1995; Blanc et al. 2003). In part, this fragmentation is a result of ivory poaching during the 1970s and 1980s, which drove severe declines in central and eastern Africa (Ottichilo et al. 1987). Kenya's elephant populations were particularly hard hit during the 1970s–1980s, with estimates of a 90% decline in the 20 years prior to the ivory ban. Recent censuses, however, demonstrate that populations in Tsavo, Amboseli, and Laikipia–Samburu areas are increasing by 3–4% per year (Kahumbu et al. 1999; Omondi, Bitok, et al. 2002; Thouless et al. 2003; Wittemyer et al. 2005). The international ban on trade in elephant products by the Convention on International Trade in Endangered Species in 1989 acted to reduce the pressures of ivory trade. Currently, 19% of Kenya is elephant range, but only 8% is under protective status (Blanc et al. 2003). The country's second largest population occurs in the predominantly unprotected Samburu–Laikipia region of northern Kenya (Poole et al. 1992). The management and

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conservation of Kenya's elephants are further complicated by increasing range fragmentation and isolation and accelerated land conversion, for which solutions can be well served by scientifically informed management options.

Although African savannah elephants have been the subject of intense conservation study and debate in recent years, most molecular studies on this species are mainly concentrated at the continental and regional levels (Roca et al. 2001; Comstock et al. 2002; Eggert et al. 2002; Nyakaana et al. 2002; Roca and O'Brien 2005), with few attempting to assess finer scale population structure (Georgiadis et al. 1994; Siegismund and Arctander 1995; Essop et al. 1996; Coetzee et al. 1999; Nyakaana and Arctander 1999; Nyakaana et al. 2001). Studies on population genetics of other large African mammals have revealed interesting patterns important to the management of these species, ranging from a gradual isolation by distance in buffalos (Simonsen et al. 1998), a moderate amount of differentiation among African hippo populations (Okello, Nyakaana, et al. 2005), to extreme levels of differentiation in oryx (Masembe et al. 2006), Grant's gazelles (Lorenzen et al. 2007), and sable antelopes (Pitra et al. 2002). A previous phylogeographic study of some East African elephant populations revealed the degree of population subdivision as measured using mitochondrial DNA (mtDNA) was approximately twice that measured from microsatellites (Nyakaana et al. 2002). Detailed population genetic structure in elephant range countries including Kenya remains largely unknown, despite the importance of this information in guiding the management of this species.

Management activities by the Kenya Wildlife Service to alleviate human–elephant conflict and the ecological impacts of high elephant density include large-scale translocations of elephants from high-density parks to low-density areas (Omondi, Wamba, et al. 2002). Although such management intervention is increasingly necessary, it is preferable that such undertakings consider the genetic integrity and population genetic structure of the species in addition to the often difficult logistical constraints. In this study, we used variation at the mtDNA control region and 20 nuclear microsatellite loci to assess the 1) detailed population genetic structure of elephants in Kenya and 2) genetic diversity and extent of differentiation among these populations. In addition to providing the relevant scientific information regarding the status of this keystone species, the results presented here are directly useful for guiding the conservation and management of elephants in Kenya.

Materials and Methods

Sampling and DNA Extraction

A total of 100 elephant biopsy tissue and/or fresh dung samples were collected from 8 populations in Kenya. In addition, we included data on mitochondrial control region sequences from 59 individuals previously sequenced by Nyakaana et al. (2002) giving a total of 159 individuals analyzed in this study. Samples covered all major elephant populations in the country, including Marsabit (MA; $n = 23$), Samburu (SA; $n = 40$), Mt Kenya (MK; $n = 12$), Meru (ME; $n = 14$), Amboseli (AM; $n = 27$), Tsavo (TS; $n = 14$), Shimba Hills (SH; $n = 9$), and Maasai Mara (MM; $n = 20$) but excluding the relatively inaccessible remnant Mt Elgon, Kerio Valley, Boni, and Dodori populations (see Figure 1 for geographic distribution of populations and their acronyms). Samples were collected as described in Karesh et al. (1987) and Okello, Nyakaana, et al. (2005) and stored in 25% dimethyl sulfoxide saturated with sodium chloride (Amos and Hoelzel 1991) at ambient temperature while in the field and −80 °C in the laboratory. Total genomic DNA was extracted from each sample using the DNeasy protocol for animal tissues (Qiagen, Hilden, Germany), following the manufacturer's instructions.

mtDNA Control Region Amplification and Sequencing

For each DNA extract, the 5′ hypervariable segment of the control region was polymerase chain reaction (PCR) amplified (Saiki et al. 1988) with primers *LafCR1* and *LafCR2* (Nyakaana and Arctander 1999; Nyakaana et al. 2001). The cycling parameters were slightly modified as follows: one cycle of enzyme activation at 95 °C for 10 min; followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 46 °C for 30 s, and extension at 72 °C for 45 s; and a final extension at 72 °C for 15 min. The PCR was performed in a 50 μl reaction volume containing approximately 2–5 ng of genomic DNA, 50 pmol of each primer, 1.5 mM $MgCl_2$, 5 μl of 10X PCR gold buffer (Applied Biosystems, Foster City, CA), 50 pmol deoxynucleotide triphosphates, 13 μl of sterile double-distilled water, and 1.25 units of *AmpliTaq Gold* DNA polymerase (Applied Biosystems) using a Hybaid Express Thermal Cycler (Thermo Hybaid, UK). PCR controls without DNA template were included in all amplifications to check for any possible contaminations.

The double-stranded PCR products were purified using QiaQuick PCR purification kit (QIAGEN) and cycle sequenced using 25 cycles of denaturation at 96 °C for 10 s, annealing at 46 °C for 5 s, and extension at 60 °C for 4 min. Each reaction was done in a 10 μl reaction volume containing 2 μl of ready mix (Applied Biosystems), 1 μl reaction buffer, 2 μl sterile double-distilled water, 1.2 μl of one primer, and 3 μl of purified double-stranded PCR product. The cycle-sequenced products were ethanol precipitated using 3 M sodium acetate (pH 4.6) and 96% ethanol, followed by 70% ethanol, and finally dried at 65 °C. Both strands of the template DNA were sequenced following the dideoxy chain–termination method of Sanger et al. (1977) with the BigDye terminator cycle sequencing kit (Applied Biosystems), according to the manufacturer's instructions. The products were electrophoresed using 4% polyacrylamide gels on an ABI 377 prism (Applied Biosystems) and analyzed using the program SEQUENCHER version 4.7 (Gene Codes, Ann Arbor, MI). The sequences were assembled in BIOEDIT 7.0.5 (Hall 1999) and automatically aligned using CLUSTALW program (Thompson et al. 1994), inbuilt in the genetic analysis package MEGA

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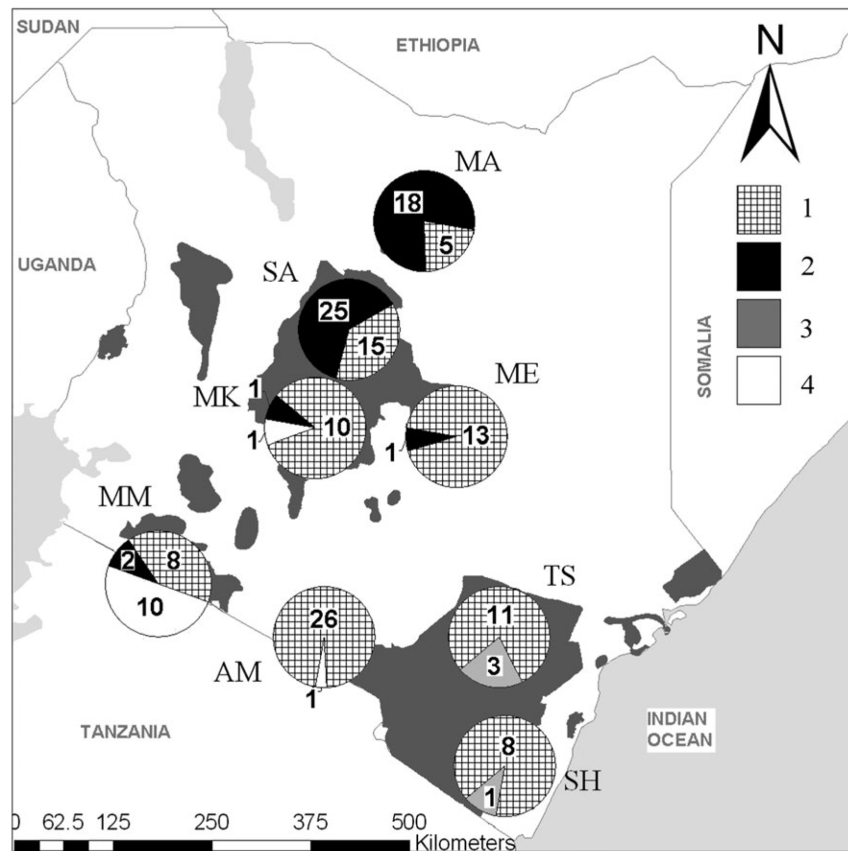

Figure 1. Map of Kenya showing the current distribution of elephants (gray-shaded areas). The pie charts show proportionately the percentage of individuals in each population sampled belonging to the 4 respective clades observed based on mtDNA. The sampling locality (Marsabit = MA; Samburu = SA; Mt Kenya = MK; Meru = ME; Amboseli = AM; Tsavo = TS; Shimba Hills = SH; and Maasai Mara = MM) is approximately in the center of each pie chart, and the number of individuals sharing the haplotype group for each population is indicated either inside or next to the slices of each pie chart.

version 4.0 (Tamura et al. 2007). The new mtDNA sequence haplotypes have been submitted to the GenBank (accession numbers EU525654–EU529675).

Microsatellite Amplification

All samples collected during the present study (see Table 1 for sample sizes for each population) were genotyped for polymorphism at 20 microsatellite loci: LaT05, LaT06, LaT07, LaT08, LaT13, LaT16, LaT17, LaT18, LaT24, LaT25, LaT26 (Archie et al. 2003), FH1, FH39, FH40, H67, FH103 (Comstock et al. 2000), LA4, LA6 (Eggert et al. 2000); LafMS02 (Nyakaana and Arctander 1998), and LafMS06 (Nyakaana et al. 2005). In each case, the forward primer was dye labeled and PCR amplified following procedures described in Okello, Wittemyer, et al. (2005). In brief, the PCR products were electrophoresed on 4% polyacrylamide gels using an ABI Prism 377 DNA Sequencer (Applied Biosystems) and ROX 500 as an internal standard. The alleles were analyzed and scored using the computer programs GENESCAN version 3.0 and GENOTYPER version 2.1 (Applied Biosystems), respectively.

In microsatellite genotyping of the dung samples, we adopted a stringent process that included multiple tubes approach and parent–offspring Mendelian checks to confirm the compatibility of alleles, the latter for Samburu population with individually known elephants. Each individual locus was genotyped at least twice to confirm the genotypes, and 2 more repeat genotyping were done for the few inconsistent genotypes and a majority consensus genotype taken (Taberlet et al. 1996). This approach yielded a high genotyping success in a previous study, with only around 2% genotyping error rate (Okello, Wittemyer, et al. 2005), including an average null allele frequency of 0.011, below the 20% level suggested by a simulation-based study to bias individual-based genetic analyses (Dakin and Avise 2004).

mtDNA Control Region Sequence Analysis

Nucleotide diversity and the average number of nucleotide differences per site between 2 sequences (Nei 1987) within the total sample and individual populations were estimated using the computer program DNASP version 4.10.9 (Rozas

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Table 1. Comparison of Kenya elephant populations' pairs in terms of F_{ST} based on chosen Kimura-2-parameter distance method for mitochondrial control region sequences (below diagonal) and alleles at nuclear microsatellites (above diagonal)

	SA	MA	ME	MK	AM	TS	SH	MM
SA	**1.153**	0.019^{*}	$-0.005^{\text{n.s.}}$	NA	0.020^{*}	$-0.005^{\text{n.s.}}$	0.0197^{*}	0.014^{*}
MA	$0.051^{\text{n.s.}}$	**0.676**	$0.011^{\text{n.s.}}$	NA	0.028^{**}	0.034^{**}	0.047^{***}	0.025^{**}
ME	0.326^{***}	0.559^{****}	**0.573**	NA	$-0.008^{\text{n.s.}}$	$0.001^{\text{n.s.}}$	$0.006^{\text{n.s.}}$	$0.004^{\text{n.s.}}$
MK	0.321^{***}	0.552^{****}	$0.035^{\text{n.s.}}$	**1.179**	NA	NA	NA	NA
AM	0.423^{****}	0.654^{****}	$0.045^{\text{n.s.}}$	0.190^{***}	**0.766**	$0.013^{\text{n.s.}}$	0.039^{*}	$0.010^{\text{n.s.}}$
TS	0.330^{***}	0.554^{****}	$0.033^{\text{n.s.}}$	$-0.015^{\text{n.s.}}$	0.163^{**}	**0.578**	0.034^{**}	$0.011^{\text{n.s.}}$
SH	0.334^{**}	0.598^{****}	$-0.034^{\text{n.s.}}$	$0.049^{\text{n.s.}}$	$-0.025^{\text{n.s.}}$	$-0.008^{\text{n.s.}}$	**1.257**	0.053^{****}
MM	0.455^{****}	0.528^{****}	0.331^{***}	0.258^{**}	0.393^{**}	0.290^{**}	0.289^{**}	**2.514**

Percentage nucleotide diversity for each of the individual populations is shown bolded along the diagonal. Statistical significance at respective levels is denoted with asterisk as: $^{****} < 0.0001$, $^{***} < 0.001$, $^{**} < 0.01$, $^{*} < 0.05$. Bolded asterisks are ones that remained significant on Bonferroni corrections for multiple comparisons. The symbol "$^{\text{n.s.}}$" and NA, respectively, represent nonsignificant F_{ST} at 5% level, and cases where comparisons could not be made because microsatellite genotyping failed in that population.

et al. 2003). We used the computer program MRMODELTEST version 2.2 (Nylander 2004), a modification of MODELTEST version 3.6 (Posada and Crandall 1998), to determine the nucleotide substitution model that best suited the data set. We then estimated phylogenetic relationships among mtDNA control region haplotypes using the neighbor-joining algorithm (Saitou and Nei 1987), incorporating the chosen Kimura-2-parameter model of DNA evolution (Kimura 1980) using the program MEGA version 4.0 (Tamura et al. 2007). The reliability of the nodes defined by this tree was assessed using 1000 bootstrap replications.

Microsatellite Genetic Variation Analysis

Potential genotyping errors in the data set, arising from failure of alleles to amplify such as allelic dropout (Miller and Waits 2003), short allele dominance (Wattier et al. 1998), or slippage during PCR amplification (Shinde et al. 2003), were checked using the software MICRO-CHECKER 2.2.1 (Van Oosterhout et al. 2004) based on 10 000 randomizations in each of the 7 analyzed elephant populations. We quantified genetic diversity in terms of average number of alleles, observed and expected heterozygosities across the 20 loci using the computer program GDA version 1.1 (Lewis and Zaykin 2001). Tests for deviations from Hardy–Weinberg proportions were carried out using the Markov chain method as implemented in GENEPOP version 4.0 (Raymond and Rousset 1995; Rousset 2007). Bonferroni corrections (Rice 1989) were used to compensate for multiple tests in estimating significance levels.

Testing for Populations and Regional Genetic Structure

We organized the 8 sampled populations into 3 groups based on contiguity of range (Blanc et al. 2003) to test for genetic evidence of regional structuring. The northern region consists of the Samburu, Marsabit, Mt Kenya, and Meru populations; the southern region includes the Amboseli, Tsavo, and Shimba Hills populations; and with Maasai Mara being the only sampled western region population (Figure 1). We used the hierarchical analysis of molecular variance (AMOVA) (Excoffier et al. 1992; Michalakis and Excoffier 1996) to test for the overall significance of genetic differentiation among these 3 regional groups (F_{CT}), among populations within these regions (F_{SC}), and among populations (F_{ST}) using the computer program ARLEQUIN version 3.11 (Excoffier et al. 2005). We conducted these analyses based on the chosen Kimura-2-parameter model and number of different allele distance methods for mitochondrial control region and microsatellite data sets, respectively, and obtained statistical significance level based on 10,000 permutations using the computer program ARLEQUIN. We quantified the extent of genetic differentiation between populations based on population pairwise F_{ST} (Kimura-2-parameter distances) using the program ARLEQUIN version 3.1. Finally, we tested for the presence of isolation by distance using Mantel tests (Mantel 1967) by assessing for correlations between pairwise genetic and interpopulation land distances using the computer program ARLEQUIN version 3.1. Statistical confidences of the tests were evaluated based on 100,000 random permutations.

Results

Mitochondrial and Microsatellite DNA Variation

From the total of 159 mtDNA control region sequences obtained for this study, we observed 22 haplotypes defined by 34 polymorphic sites based on a 401–base-pair fragment, 6 of the segregation sites were singleton variable, and 26 were parsimony informative. The overall mtDNA nucleotide diversity was low (1.68%), ranging from 0.58% in Tsavo (TS) to 2.51% in Maasai Mara (MM). Pairwise homogeneity tests revealed varying levels of population's pairwise differentiation patterns, with F_{ST} ranging from -0.033 ($P > 0.05$) to 0.654 ($P < 0.01$). Eight (29%) of the pairwise population comparisons showed no significant differentiation (for details, see Table 1) indicating a more recent divergence from a common ancestor for these particular population pairs relative to herds with divergent mtDNA lineages.

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A total of 100 individual elephants from 7 populations were successfully genotyped at 20 microsatellite loci, whereas the 12 samples from Mt Kenya failed to amplify at most microsatellite loci and were therefore excluded from microsatellite-based analysis. We observed no evidence for microsatellite scoring errors due to stuttering or large allele dropout. Evidence of null alleles (average frequencies quoted in brackets) was found at LaT07 and LaT16 in Shimba Hills (SH, $N_f = 0.032$); LaT25 in Marsabit (MA, $N_f < 0.001$); and MS02 in Maasai Mara (MM, $N_f = 0.032$), most likely due to a general excess of homozygotes for most allele size classes. However, we did not exclude these loci that showed signs of excess of homozygotes from the analyses because of the very low levels of null allele frequency suggested, which have been documented to cause minor bias in population structure analysis (Dakin and Avise 2004). The average number of alleles from the 20 microsatellite loci varied slightly across the 7 populations, ranging from 5.10 in Meru (ME) to 7.5 in Samburu (SA) and an overall of 10.2 in the total Kenya sample studied. Other diversity indices did not vary much across the focal populations (Table 2). Across all populations, 28 of 140 tests (population by locus) showed significant deviation from Hardy–Weinberg proportions, but only a single locus in one population (MS02) in Maasai Mara remained so after sequential Bonferroni corrections, and this was due to an excess of homozygotes.

Regional Genetic Structure

A radial unrooted neighbor-joining phylogenetic tree of the mtDNA control region clusters the haplotypes into 4 broad clades (Figure 2). An illustration of how the above observed genetic clades corroborate with the 3 geographical regions that elephants are known to inhabit in the country revealed that clade 1 is composed of haplotypes from all populations sampled, clade 2 is found in the northern and western populations, clade 3 represents only the southern populations, and clade 4 is composed of haplotypes that are frequent in the western population but found much less frequent in the other regions (Figure 1). A hierarchical AMOVA based on mitochondrial control region sequence variation revealed significantly high levels of genetic differentiation among the 3 regional groups tested ($F_{CT} = 0.264$; $P < 0.001$), among populations within groups ($F_{SC} = 0.218$; $P < 0.00001$), and among populations in the total sample ($F_{ST} = 0.424$; $P < 0.00001$). Contrary to the patterns observed from mtDNA, no evidence for regional differentiation was observed among the 3 regional groups of populations analyzed based on microsatellite variation ($F_{CT} = 0.000$; $P > 0.500$), but patterns of variation among populations within groups and among populations were corroborated (Table 3).

Pairwise analyses of population differentiation based on microsatellite data (populations' pairwise F_{ST}) revealed 2 out of 21 significant pairwise homogeneity tests compared with 13 out of 28 possible pairwise tests based on mitochondrial control region after sequential Bonferroni correction (Table 1). Some of the significantly differentiated pairwise F_{ST} tests based on mtDNA also occurred among populations within the same geographical regions, thereby underscoring higher substructure at this locus. Notably, there was an unexpectedly significant level of genetic difference between Shimba Hills and Tsavo that occurred at the nuclear loci and not at the mtDNA analyzed. Generally, a higher correlation was found between interpopulation genetic differences (Slatkin's linearized pairwise F_{ST}) and spatial proximity (land distances) at the mitochondrial control region (correlation coefficient, $r = 0.961$; $P < 0.00001$) than at microsatellite loci ($r = 0.481$; $P < 0.05$), thus showing a higher isolation-by-distance effect on the former than the latter marker system.

Table 2. Measures of genetic variation based on 20 microsatellite loci in the 7 and combined total elephant populations analyzed for microsatellite variation from Kenya

Population	N	A	H_E	H_O
SA	19	7.50	0.741	0.735
MA	20	6.35	0.725	0.663
ME	7	5.10	0.743	0.712
AM	16	6.55	0.749	0.751
TS	14	6.00	0.725	0.637
SH	9	5.15	0.718	0.583
MM	15	6.05	0.730	0.734
Total	100	10.20	0.750	0.694

In each case, the number of samples analyzed (N), averages across loci of number of alleles (A), expected heterozygosity H_E, and observed heterozygosity H_O are indicated.

Discussion

This study presents fine-scale analysis of genetic relationships among elephant populations within Kenya. Fine-scale genetic studies of elephant population structure are rare (Nyakaana and Arctander 1999) despite their importance for regional management and as a complement to previous broader scale population genetic studies conducted on African elephants (Roca et al. 2001; Whitehouse and Harley 2001; Comstock et al. 2002; Eggert et al. 2002; Nyakaana et al. 2002; Wasser et al. 2004, 2007; Roca and O'Brien 2005) and recent behavioral genetic analyses (Hollister-Smith et al. 2007; Rasmussen et al. 2008). The focus of this study is assessing the detailed genetic population structure of elephants in Kenya, results of which should provide a benchmark for future conservation and management options such as translocation being carried out in the country.

Population Structure and Regional Differentiation

We observed approximately the same level of mitochondrial nucleotide diversity of African savannah elephants within Kenya (1.68%) to the continental wide level (2.0%)

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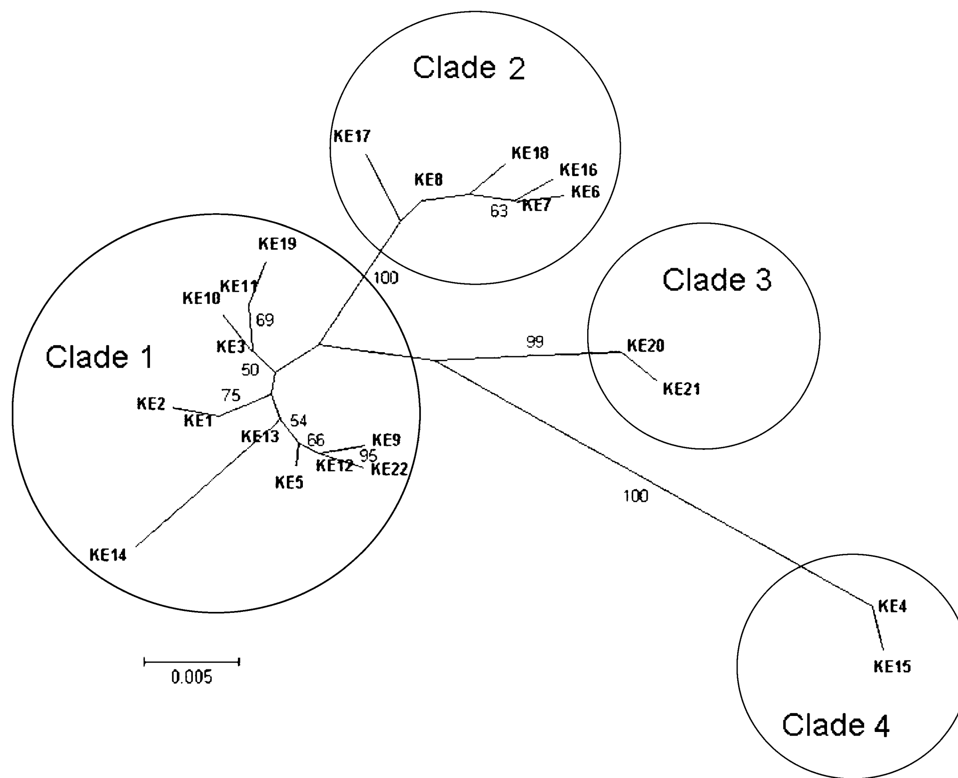

Figure 2. A radial neighbor-joining phylogenetic tree showing relationship among 22 mtDNA control region haplotypes obtained from 159 individual elephants analyzed from their populations in Kenya. Statistical support for the nodes in the tree was obtained based on 1000 bootstrap replications in the computer program MEGA 4.0 (only nodes with ≥50% replicates are indicated).

(Nyakaana et al. 2002), the latter based on samples drawn from 11 localities in eastern, western, and southern Africa. The above observed level of diversity is somewhat the same as that previously obtained from a similar Uganda elephant study (Nyakaana and Arctander 1999), where the nucleotide diversity in the total sample was found at 1.4% level. We found a slightly lower level of mtDNA control region variation in this study representing the east Africa region (F_{ST} = 0.424; $P < 0.00001$) that corroborates variation among populations within each region in Africa (F_{SC} = 0.420; $P < 0.001$) reported by Nyakaana et al. (2002).

Previous research found significant subdivision between mtDNA haplotypes from elephant populations of eastern and southern Africa, with significant subdivision among populations both at the regional and continental levels (Siegismund and Arctander 1995). In this study, the mtDNA haplotype variation in Kenya inferred from hierarchical AMOVA showed high differentiation among elephant populations from different geographical regions (F_{CT} = 0.264; $P < 0.01$) and lower differentiation among populations within the same region (F_{SC} = 0.218; $P < 0.00001$). At the nuclear microsatellite loci, we observed very low but significant genetic differentiations among populations within regions but not among the 3 regions (for details, see Table 3). As found in Uganda (Nyakaana and Arctander 1999), we observed contrasting patterns between mitochondrial and nuclear DNA. Although morphological and nuclear genetic patterns are expected to reflect the overall

Table 3. Results of a hierarchical AMOVA based on mitochondrial and microsatellite DNA in Kenyan savannah elephant populations

	Mitochondrial D-loop			Microsatellite loci		
Variation source	Percentage	*F* index	*P* value	Percentage	*F* index	*P* value
F_{CT}	26.37	0.264	<0.01	0.04	0.000	>0.500
F_{SC}	16.04	0.218	<0.00001	1.87	0.019	<0.001
F_{ST}	57.60	0.424	<0.00001	98.09	0.019	<0.00001

Proportions of variation due to differences among regions (F_{CT}), among populations within regions (F_{SC}), and among populations (F_{ST}), their associated F indices and the P values are indicated for each genomic system analyzed.

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structure of a species, not surprisingly some discordant patterns exist between the 2 genomic systems. Microsatellite loci are highly variable, which can cause problems in the interpretation of the data (Hedrick 1999). The high mtDNA differentiation and the relatively low differentiation at the nuclear loci (see Table 1 for pairwise population comparisons) is most likely explained by the matrilineal elephant social structure characterized by female natal philopatry and male-biased gene flow (Nyakaana and Arctander 1999), as well as cytonuclear genomic dissociation of the 2 genomes (Roca and O'Brien 2005). Exceptions to this pattern occurred between Tsavo and Shimba Hills (TS–SH), Tsavo and Amboseli (SH–AM), and SH–TS population pairs, where significant genetic differences (before Bonferroni corrections) were observed for the nuclear loci and not the mtDNA locus (Table 1). This could be attributed to either some undocumented events or effects of low sample sizes. The life history of elephants is characterized by closely knit family units headed by matriarchs (Douglas-Hamilton 1972; Moss and Poole 1983), with males leaving as they attain puberty. This, combined with their large home ranges (Douglas-Hamilton 1972), increases outbreeding and random mixing of biparentally inherited genes relative to maternal ones (Nyakaana and Arctander 1999). It is therefore likely that a higher level of male-mediated gene flow contributed to the relatively low differentiation at the nuclear loci analyzed in this study, as compared with the patterns shown by mtDNA variation. Male-biased gene flow has previously been described from other populations of African elephants (Nyakaana and Arctander 1999). This phenomenon has also been documented in numerous other species, for example, geese and ducks (Greenwood 1980; Scribner et al. 2001), where females often display strong natal and breeding site fidelity whereas males migrate long distances (Avise et al. 1992; Blums et al. 2002), as well as in the Australian green turtles, *Chelonia mydas* (FitzSimmons et al. 1997) and the Australian red kangaroo, *Macropus rufus* (Clegg et al. 1998).

The inferred discordant mitochondrial and nuclear genetic patterns in African elephants, also dubbed cytonuclear genomic dissociation (Roca et al. 2005, 2007; Roca and O'Brien 2005), could be attributed to different evolutionary histories of the nuclear and mitochondrial genomes. Previous phylogenetic and population genetic analyses of African elephants have revealed that mtDNA patterns in African elephants are neither congruent with morphology and traditional taxonomy (Debruyne 2005; Debruyne et al. 2003; Eggert et al. 2002; Nyakaana et al. 2002) nor with nuclear based genetic markers (Comstock et al. 2002; Roca et al. 2005, 2007; Roca and O'Brien 2005). The latter authors argued that African elephants are of 2 distinct species, forest and savannah, separated by a hybrid zone. Ancient episodes of hybridizations between forest female and savannah male elephants, which are larger and reproductively dominant to forest or hybrid males, led to incidences where in some savannah locations, like the study populations, occurrence of individuals with savannah-specific nuclear genotypes carry maternally transmitted forest elephant mtDNA. As a result, these 2 markers do not show the same population structure patterns.

Effects of genetic drift are often higher at the mitochondrial compared with nuclear genomes, caused by the smaller effective population size of the former. Although both markers should undergo the same basic evolutionary forces, they differ in that nuclear DNA has biparental, diploid inheritance, whereas mtDNA is haploid, predominantly uniparentally inherited and hence reveal female population history (Avise 1994). This fact makes the effective population size based on mitochondrial genes 4 times smaller than that from nuclear genome. On the other hand, one would theoretically expect that sex-related differences in mating strategies, especially high reproductive skew in males (Hollister-Smith et al. 2007; Rasmussen et al. 2008), will tend to increase the likelihood of male-mediated genetic drift at nuclear than mtDNA loci. As a result, N_E of nuclear loci would become lowered, resulting in a reduced difference in effective population size for the 2 marker types and therefore making the impact of genetic drift more equal.

Although the 2 markers differed in their resolutions, the genetic differences were significantly linked to distances between populations suggesting isolation-by-distance effect. This was particularly the case for the nuclear pattern. For the mtDNA pattern, the deep evolutionary differences we observed between the haplotype clades from the different regions may suggest some historical refugia followed by recent population expansion/emigration event; where elephants carrying clades 2, 3, and 4 migrated in from west and south into an original Kenyan population containing clade 1, which also exhibits the highest level of diversity (see Figure 1). Such admixture following Pleistocene refugia likely exacerbated by geographic barriers like the rift valley has also been suggested to explain continental wide patterns of elephant population structure (Nyakaana et al. 2002). Overall, the initial ancient mtDNA patterns may have been due to such above barriers and genetic differences accumulated became subsequently homogenized by male-mediated gene flow between herds and across regions.

Conservation and Management Implications

Conservation and management play an important role in the continued survival of several threatened species. With the rapid growth of human population and economic development in Africa, the implementation of a conservation plan for the continued survival of *L. africana* is imperative. One of the most serious issues facing African elephant conservation is human–elephant conflict, whose intensity is severe in many areas of agricultural and pastoral activities (Blanc et al. 2003). In Kenya, human–elephant conflict has attracted both local and international attention and in some cases has resulted in the translocation of "stray" elephants to other wild populations (Dublin and Niskanen 2003). Several translocations have taken place in Kenya in the past, and many are currently being planned. The decisions to move the elephants should consider genetic relationship among

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the populations involved as well as other logistic considerations. From the year 1990–2001, several elephants were moved from Shimba Hills National Park to Tsavo East National Park and from Lewa Downs Conservancy to Meru National Park (Omondi, Wamba, et al. 2002). The largest translocations of elephants ever were recently undertaken in Kenya, where 150 and 75 elephants were moved in 2 separate operations from Shimba Hills to Tsavo (Omondi P, personal observation). Although the mtDNA variation in this study demonstrates that Shimba Hills and Tsavo belong to the same mtDNA-based genetic region, future translocation programs should also strive to maintain the integrity between the regional mtDNA groups revealed in this study, as well as taking into consideration population pairs that are significantly differentiated.

Molecular genetic data offer important insight to the evolutionary history of population and hence should be integrated into the management decision-making processes for elephants and other wildlife species. In this study, we suggest that the observed genetic differences, especially the clear mtDNA pattern as well as homogeneity between population pairs within and among the different regions of elephants in Kenya, should be taken care of in future conservation planning and management options, such as elephant translocations. Overall, our results are informative to managers by depicting 3 differentiated regional groups of populations, which may be historical, and partly due to limited female dispersal, as well as demonstrating a lot of recent genetic mixing across these groups of populations. Significantly differentiated genetic groups at the mtDNA may be used to advocate for separate management (Moritz 1994), with the intent to preserve historic signatures that may have shaped the biogeographical patterns observed.

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