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A recent bottleneck in the warthog and elephant populations of Queen Elizabeth National Park, revealed by a comparative study of four mammalian species in Uganda national parks

Vincent B. Muwanika[1], Hans R. Siegismund[2], John Bosco A. Okello[1], Charles Masembe[1], Peter Arctander[2] and Silvester Nyakaana[1]

[1]Institute of Environment and Natural Resources, Makerere University, PO Box 7298, Kampala, Uganda
[2]Zoological Institute, Department of Evolutionary Biology, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark

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Abstract

Until 1972, Uganda's national parks boasted of large numbers of large mammal species. Following the breakdown of law and order between 1972 and 1985, large-scale poaching led to an unprecedented decline in numbers of most large mammals in Uganda's national parks. However, the extent of decline varied in the different parks across different animal species. We have investigated the genetic effects of these reductions in four mammalian species (the common warthog, African savannah elephant, savannah buffalo and common river hippopotamus) from the three major parks of Uganda using both microsatellite loci (for elephant and warthog populations) and mitochondrial control sequence variation in the warthogs, elephants, buffaloes and hippopotamuses. Queen Elizabeth National Park showed extreme reduction in nucleotide diversity for two species, the common warthog ($\pi = 0.0\%$) and African elephant ($\pi = 0.4\%$); no such decrease was found for the two other species, the buffalo ($\pi = 3.7$–5.4%) and hippopotamus ($\pi = 1.7$–1.9%), in the three parks. Nuclear microsatellite markers on the other hand showed high gene diversity in all populations in the common warthog (mean H_e 0.66–0.78) and the African savannah elephant (mean H_e 0.68–0.72). We interpret these results in terms of varying poaching pressure in the different parks, susceptibility of different species to poaching and differences in effective population sizes at the mitochondrial and nuclear loci.

INTRODUCTION

Before 1972, Uganda had large herds of elephants (*Loxodonta africana*), buffaloes (*Syncerus caffer*), hippopotamuses (*Hippopotamus amphibius*), giraffes (*Giraffa camelopardalis*), warthogs (*Phacochoerus africanus*) and a variety of other large mammals (Douglas-Hamilton *et al*., 1980; Edroma, 1986). Uganda's three oldest and largest national parks, Murchison Falls (3840 km^2), Queen Elizabeth (1978 km^2) and Kidepo Valley (1440 km^2), held large mammalian concentrations. For instance, Queen Elizabeth National Park supported one of the highest biomasses of grazing animals in the world (Bourliere, 1965). Because of the large numbers of grazers, there were regular culling exercises so as to limit serious damage to the environment. However, despite these cropping exercises, animal numbers still remained high (Laws, Parker & Johnstone, 1970; Thorton, 1971). Following the breakdown of law and order during the political turmoil of the 1970s and early 1980s, Uganda's national parks and game reserves were encroached upon, and their wildlife slaughtered in huge numbers (Edroma, 1986; Lamprey & Michelmore, 1996). As anarchy and economic chaos continued in the country, there was illegal acquisition of automatic rifles and poaching of virtually every game species became uncontrollable. By 1979, Uganda's once large herds of game became threatened with extinction (Edroma, 1981). For instance, in Queen Elizabeth National Park, elephant population size fell from 4139 in 1967 to 150 in 1980 (Eltringham & Malpas, 1980), while in Murchison Falls National Park, the population fell from 8313 to only 128 during the same period (Edroma, 1986). A similar trend in fall of population size to that among elephants was experienced in other large mammals in Uganda's national parks. Population size of buffaloes, hippopotamuses and warthogs fell by more than 70% while some mammals such as rhinos were exterminated during the same period (Edroma, 1986; Lamprey & Michelmore, 1996).

However, the drastic decline in Uganda's mammal populations did not occur with the same magnitude in all parks across all species. In Kidepo Valley national park, a comparison of animal censuses between 1972 and 1980 indicated that although there was a decline in animal

All correspondence to: Vincent B. Muwanika. Tel.: 256 41 532789; Fax: 256 41 530134; E-mail: muienr@muienr.mak.ac.ug/ vmuwanika@yahoo.co.uk.

numbers, the decline was not as drastic as that registered for Queen Elizabeth and Murchison Falls (Ross, Field & Harrington, 1976; Eltringham & Malpas, 1980; Edroma, 1981, 1986). Among the large mammals the African elephant, having been reduced by 99%, was probably the worst affected. Relative calm returned to Uganda in the mid-1980s and many of these impacted populations have increased although they have not reached the pre-decline population sizes.

Population genetic theory makes clear quantitative predictions of reduction in genetic variability expected from population declines or bottlenecks. Populations that are reduced to small sizes experience loss in genetic variation (Nei, Maruyama & Chakraborty, 1975; Maruyama & Fuerst, 1985; Frankham, 1995). Genetic drift in such populations may facilitate the fixation of deleterious genes. In addition, future population fitness may be undermined by allele loss and lowered heterozygosity. Some studies suggest that populations that have recently gone through a bottleneck experience loss in number of alleles and heterozygosity at polymorphic loci (Maruyama & Fuerst, 1985). Often, the loss in number of alleles is faster than the reduction of heterozygosity resulting in an observed gene diversity that is higher than the expected equilibrium gene diversity (Luikart & Cornuet, 1996). Molecular markers have been used in a variety of studies to investigate the genetic effects of population bottlenecks in natural populations and, in most of these, a correlation between population bottleneck and sharp reduction in genetic variation has been reported (Bonnell & Selander, 1974; Goldsworthy *et al*., 2000; Weber *et al*., 2000).

In this study, we assess genetic diversity using both nuclear microsatellite loci (in the common warthog and African elephant) and mitochondrial control region sequence variation in four large mammals, the common warthog, the African savannah elephant, the savannah buffalo and the common hippopotamus sampled from the three national parks of Uganda. The aim was to study the extent to which declines in population sizes affected genetic diversity in the different mammals from the different parks of Uganda.

MATERIALS AND METHODS

Species, populations studied and sampling strategy

Warthog and buffalo samples were collected from three major parks of Uganda: Queen Elizabeth (QE), Murchison Falls (MF) and Kidepo Valley (KV) (Fig. 1). There are no hippopotamuses in KV, hence hippopotamus samples were obtained from only two parks, QE and MF. Samples were obtained as either: (1) skin biopsies obtained by darting free-ranging animals using a crossbow (Karesh, Smith & Frazier-Taylor, 1987), (2) dry skin scrapings from carcass remains or (3) fresh dung droppings. Samples were taken from animals randomly, irrespective of age and sex. Table 1 shows the number and types of samples from the three localities used in this study. All samples were stored in 25% dimethylsulphoxide (DMSO) saturated with sodium chloride (Amos & Hoelzel, 1991) and stored at ambient temperatures in the field and at –80°C in the laboratory.

DNA extraction

Total genomic DNA was extracted using two procedures: (1) standard procedures, involving treatment with SDS and proteinase K, followed by phenol/chloroform extraction (Sambrook, Fritsch & Maniatis, 1989); (2) Dneasy tissue kit (QIAGEN) following the manufacturer's protocol (QIAGEN, 1999).

Mitochondrial DNA amplification and sequencing

The warthog

Approximately 370 base pairs of the 5′ end segment of the control region was PCR amplified with primers *MT4* (5′-CCTCCCTAAGACTCAAGGAAG–3′) (Arnason, Gulberg & Widegren, 1993) and *PeaR* (5′-AGTTCATAATTGAAACCCCCA-3′). *PeaR* is located upstream of the D-loop and was specifically designed to amplify the 5′ part of warthog D-loop in conjunction with *MT4*. Amplification was carried out in 50 micro litre reaction volumes containing 10 ng of total genomic DNA, 50 pmol of each of the primers, 5X PCR reaction buffer (Boehringer Mannheim GMBH), 50 pmol dNTPs and 1 unit of *Taq* polymerase (Boehringer Mannheim GmbH). The PCR temperature profile was: 1 cycle of denaturation at 94°C for 5 minutes followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 52°C for 1 minute and extension at 72°C for 1 min 30 sec. Blank extractions and blank PCR reactions were included as contamination controls. One primer in each reaction was 5′-end biotinylated, and the double-stranded PCR product was separated into single strands using streptavidin-coated paramagnetic beads (DYNAL). Single-stranded DNA was dissolved in distilled water and used as template for sequencing by dideoxy chain-termination method (Sanger, Nicklen & Coulson, 1977) using sequenase kit version 2.0 (Amersham Pharmacia Biotech, Inc.), [α-^{35}]-dATP (Amersham Pharmacia Biotech, Inc.) and a primer complementary to the template. Both strands were sequenced. Products of the sequencing reaction were electrophoresed in 6% polyacrylamide/ 7M-urea gel. The gel was fixed, dried, exposed on a Kodak film for 24–48 hours and read manually.

The savannah buffalo

Approximately 504 base pairs of the 5′ end segment of the control region was PCR amplified using primer pair *Dsca1* (5′-AATATAAAGAGCCTCCCCAG-3′) and *Dsca2* (5′-CGGCCATAGCTGAGTCC-3′) (Simonsen, Siegismund & Arctander, 1998). Amplification was carried out as described above for the warthog. The PCR temperature profile was: 1 cycle of denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 2 minutes. One primer was 5′-end

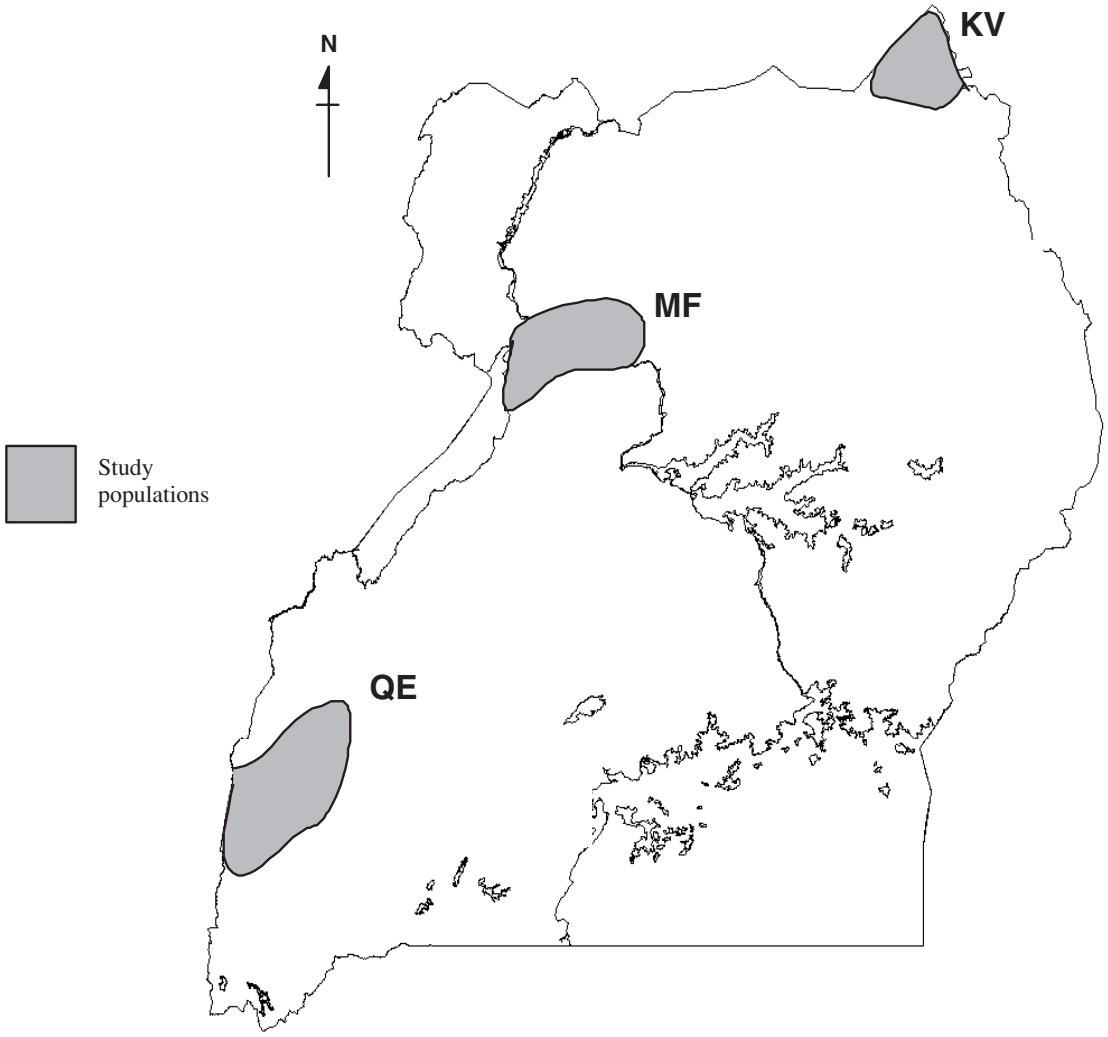

Fig. 1. Schematic map of Uganda showing the location of the study populations. QE, Queen Elizabeth; KV, Kidepo Valley; MF, Murchison Falls National Parks.

Table 1. Number and types of samples for the three mammals from three parks used in this study. Cells are arranged with parks as KV/MF/QE. Of the 102 elephant mtDNA sequences included in the analysis, 72 are originally from Nyakaana & Arctander (1999), and 30 from Nyakaana *et al*. (2002); all elephant microsatellite data used in this study are from Nyakaana & Arctander (1999).

	Biopsy	Dry skin	Dung	Total
Warthog	2/15/26	1/0/0	17/5/0	20/20/26
Buffalo	19/22/21	-/-/-	-/-/-	19/22/21
Hippopotamus	0/28/8	0/0/15	-/-/-	-/28/23

biotinylated, and the double-strand PCR product was separated into single strands, sequenced, electrophoresed and visualized as described for the warthog above.

The common hippopotamus

A pair of primers *Ham-1* (5′-CCACCATCAG-CACCCAAA-3′) and *Ham-2* (5′-CGAGATGTCTT-ATTTAAGGGGAA-3′) was used to PCR amplify approximately 400 base pairs of the 5′ end of the mtDNA control region. Double-strand amplifications were carried out as described for warthogs. The thermal cycling parameters used were: 1 cycle of initial denaturation at 94°C for 5 minutes, followed by 32 cycles of denaturations at 94°C for 1 minute, annealing at 46°C for 1 minute, and extension at 72°C for 2 minutes. One primer was 5′-end biotinylated, and the double-strand PCR product was separated into single strands, sequenced, electrophoresed and visualized as described for the warthog above.

Sequence analysis

Nucleotide sequences were aligned by eye using the program SeqApp ver. 1.9 (Gilbert, 1993), with insertions/deletions introduced so as to minimize transversions. To estimate genetic variation, we calculated basic statistics of sequence variation, including number of distinct haplotypes, haplotype diversity and nucleotide diversity (π, Nei, 1987, eqn 10.5). The extent of population subdivision was quantified using F_{ST} statistic calculated using the program ARLEQUIN version 2.0 (Schneider *et al*., 2000).

Analysis of microsatellite loci

The common warthog

Several microsatellite loci originally described in studies of the domestic pig (e.g. Rohrer *et al*., 1994, 1996, 1997; Alexander, Rohrer & Bettie, 1996; Troyer *et al*., 1996) were screened. Seven of them were chosen on the basis of their polymorphism, clarity of alleles on gels and possibility of multiplexing. The seven that were chosen and optimized for the common warthog were SW607, S0289, SW1682, SW1301, SW403, SW2419 and S0331. All loci were dinucleotide repeats. The loci were PCR amplified in a 10 micro litre reaction volume containing 10 ng of total genomic DNA, 2mM $MgCl_2$, 2 mM PCR GOLD buffer (Boehringer Mannheim GMBH), 0.2 pmol of each of the dNTPs, 0.2 pmol of each primer and 0.4 units of AmpliTaq GOLD DNA polymerase (Boehringer Mannheim GMBH). PCR profiles comprised of 94°C for 10 minutes of initial DNA denaturation and enzyme activation followed by 28–34 cycles of 30 seconds at 94°C, 60 seconds at the corresponding annealing temperature (Table 2), 30 seconds at 72°C. Details of annealing temperature and cycles for each locus are shown in Table 2. A final extension phase of 10 minutes at 72°C followed all reactions. All PCR reactions were run using dye-labelled primers (one primer in each primer set). The products were run on 4% acrylamide gels on an ABI 377 (Perkin Elmer) using ROX 500 as an internal standard. Where amplification was possible, the same individuals typed at mtDNA sequences were typed at microsatellite loci.

The African savannah elephant

Microsatellite data for four nuclear microsatellite loci of individuals sampled from the three parks were obtained from Nyakaana & Arctander (1999).

Statistical analysis of microsatellite data

Microsatellites were tested for genotypic disequilibrium using an exact test based on a Markov chain algorithm as implemented in GENEPOP version 3.1 (Raymond & Rousset, 1997). The test was performed for each population and the statistical significance was assessed using Bonferroni correction (Rice, 1989). Deviation of observed genotypes from Hardy–Weinberg proportions was tested for, using the same program.

Table 2. PCR conditions for microsatellite loci

Locus	Annealing temperature	Number of cycles	Reference
SW607	52°C	30	Rohrer *et al*., 1994
S0289	57°C	28	Rohrer *et al*., 1996
SW1682	58°C	30	Rohrer *et al*., 1996
SW1301	58°C	32	Rohrer *et al*., 1996
SW403	50°C	32	Rohrer *et al*., 1994
SW2419	58°C	34	Rohrer *et al*., 1996
S0331	55°C	30	Rohrer *et al*., 1994

Genetic variation was quantified by calculating number of alleles per locus (K), observed heterozygosity per locus (H_o) and expected heterozygosity per locus (H_e) under the Hardy–Weinberg expectation (Nei, 1987). Total number of alleles for each population, and the average observed and expected heterozygosity for each population, were also calculated. Inbreeding coefficient (F_{IS}), as a measure of heterozygote deficiency or excess (Wright, 1978), was estimated for each locus in each population. Extent of population differentiation was quantified using Weir & Cockerham's (1984) analogue of Wright's F_{ST} computed using ARLEQUIN version 2.0 (Schneider *et al*., 2000).

Evidence of expected heterozygote excess (Cornuet & Luikart, 1996) from microsatellite allele frequency data was tested for, using the program BOTTLENECK (Piry, Luikart & Cornuet, 1999). Calculations were performed for each population and locus under the Two Phase Model (TPM). In a recently bottlenecked population, the observed gene diversity is higher than the expected equilibrium gene diversity which is computed from the observed number of alleles under the assumption of an equilibrium population (Piry *et al*., 1999). One statistical test, the Wilcoxon sign-rank test (Luikart & Cornuet, 1998), was conducted to determine whether populations exhibit significant number of loci with gene diversity excess. The test provides relatively high power of resolution and it can be used with few loci and individuals.

RESULTS

Mitochondrial DNA variation

The common warthog

The first 370 base pairs of the 5′ end of mitochondrial control region were successfully amplified and sequenced in 57 warthogs. In the alignment of the sequences, a total of 20 variable sites (two deletions, three transversions and 15 transitions) was observed defining seven haplotypes in the total sample. The estimated nucleotide diversity in the total sample was 1.5%. Within populations, nucleotide diversity varied considerably from as low as zero (0.0%) in QE, 0.4% in MF to 1.4% in KV (Table 3). Haplotype diversity in the total sample was 0.78. Within populations, QE showed the lowest haplotype diversity (0.0), while the highest was observed in KV (0.63). Moderate haplotype diversity was observed in MF (0.5). Estimates of genetic diversity for the warthog populations are summarized in Table 3. The F_{ST} values computed for the pairwise comparisons are shown in Table 4. These values and their corresponding P values indicate that a significantly large proportion of genetic diversity (41–75%) is found between populations.

The African savannah elephant

In the alignment of 102 sequences, a total of 31 variable sites (one deletion and 30 transitions) was observed defining 12 haplotypes. Estimates of genetic diversity for the three populations are summarized in Table 3. Four

Table 3. Summary statistics for the control-region sequence variation in the warthog, elephant, buffalo and hippopotamus populations from the three major national parks of Uganda. Sample size *N*; percent nucleotide diversity π; number of detected haplotypes in each population *K*; haplotype diversity *H*.

	N			π			*K*			*H*		
	QE	MF	KV	QE	MF	KV	QE	MF	KV	QE	MF	KV
Warthog	20	19	18	0.0	0.4	1.4	1	3	4	0.00	0.50	0.63
Elephant	66	9	27	0.4	2.4	1.1	4	4	6	0.12	0.81	0.74
Buffalo	21	22	19	5.4	4.1	3.7	19	21	18	0.99	0.99	0.99
Hippopotamus	23	28	–	1.9	1.7	–	22	27	–	1.0	1.0	–

Table 4. F_{ST} values between populations based on mtDNA analysis. The level of significance shown as * denotes significance at *$P < 0.5\%$, ** $P < 0.01\%$, *** $P < 0.001\%$.

	Warthog	Elephant	Buffalo	Hippopotamus
QE vs. MF	0.75***	0.75***	0.06**	0.08*
QE vs. KV	0.70***	0.67***	0.06**	–
MF vs. KV	0.41***	0.24***	0.07**	–

haplotypes were observed in QE. Ninety-four percent (62 out of 66) of all individuals sampled in QE had the same haplotype. The other three haplotypes in QE were encountered either once or twice. Six and four haplotypes were observed in KV amd MF respectively.

The estimated nucleotide diversity in the total sample was 1.1%. Within populations, nucleotide diversity varied from 0.4% in QE, 1.1% in KV to 2.4% in MF. Haplotype diversity for the total sample was 53%. A trend similar to that observed for nucleotide diversity is observed in haplotype diversity. KV's haplotype diversity (74%) is six times greater than that observed in QE (12%). All population pairs were found to be highly differentiated with F_{ST} values ranging from 0.24 to 0.75 (Table 4).

The savannah buffalo

The first 504 base pairs of the 5′ end of mitochondrial control region were successfully amplified and sequenced in 62 buffaloes from the three parks. In the alignment of these sequences, a total of 159 variable sites was observed defining 58 haplotypes in the total sample. Each of the haplotypes observed in the total sample was encountered once or twice, resulting in a high number of haplotypes for each of the three populations (Table 3). The estimated nucleotide diversity for the total buffalo sample is 4.7%. Within populations, nucleotide diversity varies from 3.7% in KV, 4.1% in MF to 5.4% in QE. Haplotype diversity for the total sample was 0.99. All the three buffalo populations exhibited a high haplotype diversity. Unlike the warthog and elephant, the extent of population subdivision in the buffalo was low with F_{ST} values ranging from 0.06 to 0.07.

The common hippopotamus

The first 400 base pairs of the 5′ end of mitochondrial control region were successfully amplified and sequenced in all the 51 hippopotamuses. In the alignment of these sequences, a total of 35 variable sites was observed defining 48 haplotypes in the total sample. With the exception of seven transversions all substitutions were transitions. Each of the 48 haplotypes observed in the total sample was encountered once or twice, resulting in a high number of haplotypes for each of the two populations (Table 3).

The estimated nucleotide diversity in the total sample was 1.84%. The nucleotide diversity for each of the two populations was, compared to that oberved in the warthog, moderate (QE = 1.9%; MF=1.7%). Haplotype diversity in the total sample and in each of the two hippopotamus populations was high (100%). In contrast to observation from the warthog and elephant populations, measurements of genetic diversity in the hippopotamus and buffalo populations (Table 3) show that there are no great differences between these parks.

Microsatellite variation

The common warthog

Apart from locus SW403 where only two alleles in each of the two populations were detected, we observed a high degree of genetic variation within populations in terms both of number of alleles per locus (> 4 alleles per locus), and of observed and expected heterozygosity (Table 5). A total of 61 different alleles was observed at all seven loci in the two populations. Despite the relatively smaller number of individuals analyzed in MF, the total number of alleles scored in MF (44 alleles) was approximately the same as that scored in QE (45 alleles). Mean observed (H_o) and expected (H_e) heterozygosities in the two populations were high (Table 5).

In MF, no significant expected heterozygosity excess was found at any of the seven loci. In QE, a significant heterozygosity deficiency (as indicated by the positive F_{IS} values) was observed at three loci (SW607 $P < 0.01$, SW2419 $P < 0.01$ and S0331 $P < 0.01$) after the Bonferroni correction. The proportion of the genetic variation attributable to genetic differences between populations quantified using F_{ST} was 0.138 ($P < 0.001$), between QE and MF.

The expected gene diversity (H_e) was greater than expected equilibrium gene diversity (H_{eq}) at four out of seven loci in each of the two populations, QE and MF (Table 6). The Wilcoxon test, which tests for bottlenecks across loci, did not show significant effects of recent bottlenecks in either population ($P > 0.05$).

Table 5. Summary statistics of genetic variation at four microsatellite loci in the elephant and seven microsatellite loci in the warthog populations. Number of alleles detected in each population K; observed heterozygosity H_o; expected heterozygosity H_e, Wright's fixation index F_{IS}; single-locus P-values for Hardy–Weinberg tests $P_{(HW)}$; total number of alleles summed over all loci for each population $K(Total)$; average observed heterozygosity $H_o(Ave)$; average expected heterozygosity $H_e(Ave)$; average Wright's fixation index $F_{IS}(Ave)$ and total number of individuals analyzed for each population n. Details of locus-by-locus data at the four elephant microsatellite loci are found in Nyakaana & Arctander (1999: 1111).

		QE	MF	KV
Elephant	$K(Total)$	29	19	23
	$H_o(Ave)$	0.77	0.81	0.77
	$H_e(Ave)$	0.72	0.70	0.68
	$F_{IS}(Ave)$	–0.08	–0.15	–0.12
	n	42	9	21
Warthog SW607	K	8	7	
	H_o	0.615	0.600	
	H_e	0.905	0.811	
	F_{IS}	0.224	0.254	
	$P_{(HW)}$	<0.01	0.072	
S0289				
	K	8	6	
	H_o	0.500	0.773	
	H_e	0.585	0.786	
	F_{IS}	0.18	0.046	
	$P_{(HW)}$	0.22	0.27	
SW1682				
	K	7	8	
	H_o	0.423	0.867	
	H_e	0.565	0.848	
	F_{IS}	0.253	–0.022	
	$P_{(HW)}$	0.04	0.749	
SW1301				
	K	9	7	
	H_o	0.769	0.467	
	H_e	0.816	0.756	
	F_{IS}	0.058	0.378	
	$P_{(HW)}$	0.67	0.126	
SW403				
	K	2	2	
	H_o	0.307	0.067	
	H_e	0.329	0.131	
	F_{IS}	0.079	–	
	$P_{(HW)}$	1.0	–	
SW2419				
	K	6	8	
	H_o	0.308	0.667	
	H_e	0.673	0.860	
	F_{IS}	0.533	0.211	
	$P_{(HW)}$	<0.01	0.293	
S0331				
	K	5	6	
	H_o	0.538	0.600	
	H_e	0.757	0.766	
	F_{IS}	0.272	0.222	
	$P_{(HW)}$	0.005	0.126	
	$K(Total)$	45	44	
	$H_o(Ave)$	0.494	0.577	
	$H_e(Ave)$	0.661	0.747	
	$F_{IS}(Ave)$	0.218	0.182	
	n	26	15	

The African elephant

The African elephant data for all the four microsatellite loci in the three populations (QE, MF and KV) analyzed in this study were obtained from Nyakaana & Arctander (1999). All four loci exhibited high levels of polymorphism in each of the three populations (details of each locus in Nyakaana & Arctander, 1999). Average observed (H_o) and expected (H_e) heterozygosity in the three populations were high and had values close to each other (Table 4). The average F_{IS} in all populations was slightly negative. A total of 33 different alleles was observed for all four loci in the three populations. The highest number of alleles was observed in QE (31 alleles) and the lowest was observed in the MF population (19 alleles).The difference in allele numbers can be ascribed to varying sample sizes. In order to correct for this, we made ten random samples of nine individuals (the size of the Murchison Falls sample) from Queen Elizabeth and Kidepo Valley samples. This resulted in average number of alleles of 20.6 ± 1.6 and 19 ± 1.6 for Queen Elizabeth and Kidepo Valley, respectively. As for the warthog, tests of population subdivision perfomed by Nyakaana & Arctander (1999), showed that the three populations are highly subdivided.

Although many loci had their observed gene diversity (H_e) higher than expected equilibrium gene diversity (H_{eq}) (Table 6), the overall result from the two bottleneck tests did not show significant effects of recent bottlenecks. All elephant populations are therefore in a mutation-drift equilibrium.

DISCUSSION

This paper represents the first attempt to assess the genetic effects of the population declines of large mammals in Uganda between 1970s and mid-1980s. Measures of genetic diversity based on mtDNA sequence variation in the warthog and elephant were concordant in showing that there is an extreme reduction of mtDNA variation in the Queen Elizabeth population compared to the Murchison Falls or Kidepo Valley populations. In Queen Elizabeth, only one haplotype was detected among the warthogs, while in the elephants a single haplotype was scored in 94% of all individuals sampled. Data from microsatellite variation among elephants and warthogs for the three populations differ from those of mtDNA sequence variation. At the nuclear loci, unlike mitochondrial loci, high levels of gene diversity were observed for both species in all the populations analyzed. Unlike in the warthog and elephant populations, moderate levels of mtDNA diversity were observed in the buffalo and hippopotamus populations in all the parks (Table 3).

Patterns of genetic diversity in the warthog and elephant populations

Our results show significantly reduced mtDNA diversity in the Queen Elizabeth warthog and elephant populations in comparison to the Murchison Falls or Kidepo Valley populations (Table 3). The observed mtDNA sequence variation in warthogs and elephants from QE is characteristic of populations that have undergone reduction in population size. Such populations are expected to show a rapid increase in the proportion of haplotypes that are identical or nearly so (e.g. Hoelzel *et al*., 1993; Weber *et al*., 2000). This arises because some

Table 6. Results of test for bottleneck by locus in each population. Probability is calculated to determine whether the expected gene diversity (H_e) is higher than the expected equilibrium gene diversity (H_{eq}) which is calculated from observed number of alleles for each locus in each population under the assumption of a mutation-drift equilibrium.

Elephant					Warthog				
Population	Locus	H_e	H_{eq}	Probability	Population	Locus	H_e	H_{eq}	Probability
QE	LafMS01	0.81	0.69	0.24	QE	SW1301	0.82	0.80	0.50
	LafMS02	0.84	0.75	0.30		S0289	0.56	0.78	0.01
	LafMS03	0.68	0.60	0.46		SW607	0.79	0.78	0.50
	LafMS04	0.51	0.53	0.17		SW1682	0.56	0.74	0.03
						SW403	0.29	0.24	0.41
MF	LafMS01	0.84	0.81	0.50		SW2419	0.65	0.69	0.28
	LafMS02	0.80	0.75	0.53		S0331	0.74	0.63	0.12
	LafMS03	0.54	0.47	0.54					
	LafMS04	0.62	0.47	0.27	MF	SW1301	0.74	0.78	0.23
						S0289	0.77	0.73	0.39
KV	LafMS01	0.70	0.65	0.39		SW607	0.80	0.82	0.29
	LafMS02	0.82	0.70	0.14		SW1682	0.85	0.84	0.47
	LafMS03	0.65	0.59	0.43		SW403	0.07	0.27	0.19
	LafMS04	0.56	0.58	0.18		SW2419	0.84	0.81	0.36
						S0331	0.77	0.73	0.38

haplotypes are lost through genetic drift so that subsequent individuals are descended from one or a few surviving ancestors. Genetic drift is also expected to lead to an increase in the between-population component of genetic diversity (Nei *et al*., 1975; Maruyama & Fuerst, 1985). The extreme reduction in mtDNA diversity in the QE warthog and elephant populations (Table 3) and the corresponding high component of the between-population genetic diversity (Table 4) are therefore largely attributed to a bottleneck in these mammals that followed the breakdown of law and order in Uganda between 1972 and 1985. During this period, Uganda's once large herds of game became threatened with extinction (Eltringham & Malpas, 1980). The elephant population size, having fallen by approximately 99%, was probably one of the worst affected. Although census data for the warthog in the same period and park are not available, genetic data in this study suggest that the warthog population in QE was similarly decimated. Results of this study are consistent with other studies that have reported a correlation between a population bottleneck and a sharp reduction in genetic variation, e.g. northern hairy-nosed wombat (Taylor, Sherwin & Wayne, 1994), black-footed rock-wallaby (Eldridge *et al*., 1999), northern elephant seal (Weber *et al*., 2000), and elephants in South Africa (Whitehouse & Harley, 2001).

All tests for a bottleneck in the common warthog and African savannah elephant for all populations were negative. This may be because the excess of observed heterozygosity in post-bottleneck populations is a transient feature, expected to last only a few generations (Luikart & Cornuet, 1998) and may therefore not be apparent in the warthog and elephant populations of Queen Elizabeth National Park. In general terms however, results from this study indicate that, unlike the mitochondrial genome, there is still a substantial amount of gene diversity at the nuclear loci in the warthog and elephant populations from QE. There are two possible reasons for this observation: (1) differences in effective population size at the mitochondrial and nuclear genomes; (2) preferential poaching of female animals. Because the effective population size of mtDNA is a quarter that of nuclear DNA, the recent reductions in population sizes could have reduced mitochondrial variability without appreciably affecting nuclear variability. This observation is consistent with many studies that have reported high levels of diversity at nuclear loci even when mtDNA is relatively impoverished (e.g. Palumbi & Baker, 1994; Nyakaana & Arctander, 1999; Walker *et al*., 2001). Tusk size would probably be the incentive for preferential poaching of female elephants. There is, however, no reported difference in size of tusks produced by the two sexes. Preferential female hunting is therefore unlikely to be the reason for the difference in variability observed at the two genomes.

Compared to the Queen Elizabeth elephant and warthog populations, these two species in Kidepo Valley and Murchison Falls show high variability. This is surprising considering the documented fall in population size of elephants in MF between 1967 and 1980. Three reasons could explain the observation: (1) the pre-decline population size in MF and KV was larger than that in QE; (2) the decline in population size was greater in QE than in either KV or MF; (3) there has been immigration from other populations that has restored part of the lost variation. Census surveys of large mammals for the three parks before 1972 and after 1980 support the first reason for MF and the second for KV. The pre-decline population size of elephants in MF was over 9000 individuals compared to approximately 3700 in QE (Olivier, 1991), and while there was a general decline of population sizes in all parks, KV experienced the least decline (Ross *et al*., 1976; Eltringham & Malpas, 1980; Edroma, 1981). There are no protected areas adjacent to KV or MF, and it is therefore highly unlikely that immigration could have restored genetic variability.

Genetic diversity in the buffalo and hippopotamus populations

Mitochondrial DNA is highly sensitive to severe reductions in population sizes (e.g. Hoelzel *et al*., 1993; Goldsworthy *et al*., 2000), but we found no evidence of this phenomenon

in the buffaloes and hippopotamuses of all the parks. All populations of the two species showed moderate to high levels of genetic diversity as measured in terms of number of haplotypes and nucleotide diversity (Table 3). Level of genetic diversity in the buffaloes observed in this study is similar to that reported among other buffalo populations in southern and eastern Africa (e.g. Simonsen *et al*., 1998; Van Hooft, 2001). Mitochondrial DNA diversity for hippopotamuses presented here cannot be directly compared to other hippopotamus populations in the region, because such equivalent data (D-loop sequence data) are not available. However, when compared to other large mammals of Africa such as buffaloes (Simonsen *et al*., 1998), elephants (Nyakaana & Arctander, 1999; Nyakaana, Arctander & Siegismund, 2002), impala and kudu (Nersting & Arctander, 2001), hippopotamuses show moderate levels of mtDNA diversity.

Comparison and interpretation of observed patterns in Queen Elizabeth

Unlike in the warthog and elephant populations, moderate levels of nucleotide diversity were observed within the buffalo and hippopotamus populations analyzed in this study (Table 3). There are two possible reasons for this observation: (1) that while the warthog and elephant populations were severely decimated, the buffalo and hippopotamus populations remained relatively undisturbed; (2) that immigration from neighbouring populations could in part have restored part of the lost diversity. If warthogs and elephants were more decimated than buffaloes and hippopotamuses, there would be greater reduction in mitochondrial diversity in the former than in the latter. Since it was poaching pressure that was responsible for decimating animal populations, such an interpretation may suggest varying poaching pressure for the different species during the period between 1972 and 1985 when Uganda's large mammals suffered the worst uncontrolled poaching (Edroma, 1986; Douglas-Hamilton, 1987). This is consistent with a scenario in which species that had a high commercial value (as elephant) and those that were easy to hunt for meat (as warthog) were severely reduced while those that were aggressive, difficult to hunt and had little or no commercial value (as buffalo and hippopatamus) were not severely reduced. This means that although buffaloes and hippopotamuses were poached, the intensity was relatively minimal compared to elephants and warthogs. Although census data of many large mammals are scanty, the few data available support this hypothesis. Elephants and rhinos, because of their tusks and horns, were more severely poached, with the rhino being exterminated, while buffaloes and hippos were only reduced by 70% during the same period (Edroma, 1986, Lamprey & Michelmore, 1996);

Although Queen Elizabeth is adjacent to Virunga National Park in the Democratic Republic of Congo, immigration is an unlikely explanation for the moderate nucleotide diversity in the buffaloes and hippopotamuses because it would be expected to affect the four species in the same manner.

CONCLUSION

Our results show that warthogs and elephants sampled from Queen Elizabeth exhibit extreme reduction in mtDNA sequence variation compared to those from either Murchison Falls or Kidepo Valley, while the buffaloes and hippopotamuses showed moderate levels of mtDNA sequence diversity in the three parks. The discordance in pattern of mtDNA diversity for the four species in the three parks may be interpreted as a result of varying poaching pressure on the different species in the parks.

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