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Remnants of ancient genetic diversity preserved within captive groups of scimitar-horned oryx (*Oryx dammah*)

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Abstract

Scimitar-horned oryx, now considered extinct in the wild, persists in large numbers in captivity. In this first molecular genetic study on this species, we explore the patterns of genetic diversity across European, North American, and a few other captive groups using microsatellite markers and mitochondrial control region sequencing. Strong population structure was not evident from microsatellite data but we discovered deep divergence within the mitochondrial DNA haplotypes from a network analysis where three disconnected networks were obtained, with estimated divergence times of *c.* 2.1–2.7 million years. Mismatch distribution analyses suggest population expansions *c.* 1.2 and 0.5 million years ago. We discuss our findings in the context of historical climatic changes in North Africa and use information obtained on current patterns of genetic diversity within captive groups to make recommendations for future captive management and reintroduction strategies.

Keywords: antelope, captive breeding, conservation genetics, Hippotragini, North Africa, oryx

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Introduction

Captive breeding is seen to play an increasingly important role in the conservation of threatened species. Several species such as the scimitar-horned oryx (*Oryx dammah*), the Przewalski's horse (*Equus ferus przewalskii*), and the black-footed ferret (*Mustela nigripes*) have been successfully retained in captivity after extinction in the wild. This trend is likely to continue in the future since thousands of threatened species are thought to require captive breeding over the next few hundred years in order to prevent them from going extinct (Tudge 1995). Regional and international programs now exist for many endangered species in captivity where coordinated breeding and management is practised. Efforts are made to preserve the genetic variation of the wild population from which founders were drawn, to minimize loss of this initial diversity as a consequence of inbreeding, and to produce appropriate animals for reintroduction to the species' former range (Russello & Amato 2004).

The scimitar-horned oryx (SHO) belongs to the Hippotragini tribe within the Antilopinae subfamily of Bovidae, along with addax (*Addax nasomaculatus*), roan (*Hippotragus equinus*), sable (*Hippotragus niger*), and two other oryx species, the Arabian oryx (*Oryx leucoryx*) and the Plains oryx or Gemsbok (*Oryx gazella*). During the middle ages, SHO is known to have spanned right across North Africa, from Mauritania on the Atlantic coast to Sudan on the Red Sea, along the interface between true desert and the less arid 'North Saharan/Mediterranean' habitat and the 'Sahelian' habitat (region bordering the Sahara to the south and varying in width from several hundred kilometres to over 1000 km) (Newby 1978, 1980). Populations on the northern fringe of the Sahara are thought to have disappeared by the beginning of the 20th century, with the southern Sahelian range remaining almost continuous until the 1960s (Fig. 1). Continued fragmentation eventually led to the extermination of the species from across this region, with the last confirmed sightings made in Chad in the mid-1980s (Newby 1988). Reasons for the decline include drought, loss of habitat, over-hunting, and competition with domestic

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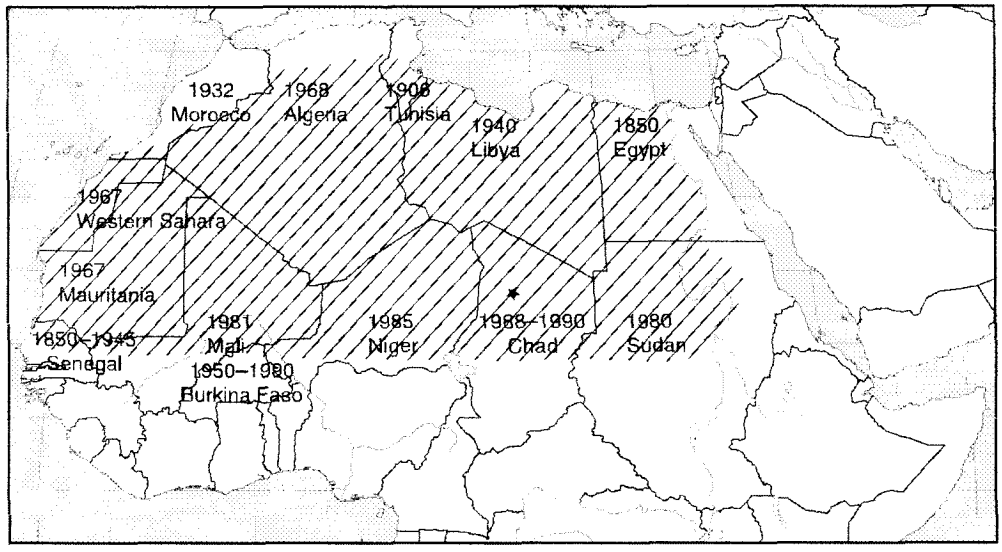

Fig. 1 Historical distribution of SHO across North Africa with approximate extinction times within regions. The star in Chad indicates the approximate location of the base camp in 1967 from which SHO were captured within a 200-km radius. Outline map obtained from BYU Geography Department (www.geog.byu.edu/outlinemaps.dhtml). Information obtained from Wakefield *et al.* (2004).

livestock (Jackson 1978; Newby 1988; Dixon *et al.* 1991). SHO is now officially classified as extinct in the wild (IUCN 2006) but exists in large numbers in captivity, and there may be as many as 6000 animals held in zoos, private collections, and ranches worldwide (Gilbert 2005). Although there are records from the 1930s of a small number of individuals that may have contributed to the modern captive groups, the vast majority of founders were captured in Chad in the 1960s (Wakefield *et al.* 2004). This consisted of three animals caught in 1963 and taken to the USA, and a greater number (*c.* 44) captured in 1967 (Fig. 1), of which *c.* 26 were taken to USA, *c.* 18 were brought to Europe, and a handful of individuals were sent to zoos in South Africa and Japan (A. Rost, personal communication). Descendants of the North American and European animals are now managed within the Species Survival Plan (SSP) of the American Association of Zoos & Aquaria (AZA), and the European Endangered Species Programme (EEP) of the European Association of Zoos & Aquaria (EAZA), respectively. Other coordinated captive breeding programmes exist in Australasia and Japan, and there are also SHO in parts of the world that are not covered by any such programme.

There is considerable interest in the re-introduction of SHO to parts of its former range and as many as 13 North African countries have become signatories to the *'Action plan for the conservation and restoration of Sahelo-Saharan antelope and gazelle'* (Beudels-Jamar *et al.* 1998). Captive-bred SHO have already been successfully released into protected areas in Tunisia, Morocco and Senegal (Gordon & Gill 1993; Wakefield *et al.* 2004). In this first genetic study on the SHO, we explored the extent and patterns of genetic variation within the EEP and SSP groups which represent the most significant captive management programmes in terms of size (*c.* 460 and 230 individuals, respectively) and numbers of founders (Gilbert 2005). Small numbers of additional samples from other captive groups and from museums were also used for comparison. We report on the current mitochondrial and microsatellite diversity in these captive groups, and based on our findings, make inferences on historical patterns of demography and suggest future management strategies for this species.

Materials and methods

Samples

We obtained faecal, blood, and ear/muscle biopsy samples. Three to six fresh faecal pellets were placed in 50 mL tubes containing *c.* 30 g silica gel (Type III indicating, Sigma) with a small piece of filter paper separating the faecal material from the silica gel (Wasser *et al.* 1997). The tubes were held at ambient temperature for several weeks prior to being stored long term at 4 °C. Blood was collected by zoo veterinarians and shipped within 24 h for processing, or held at –20 °C and shipped frozen. All tissue samples were placed in 100% alcohol and shipped at ambient temperature. A total of 122 faecal and 35 blood/skin samples were obtained from EEP participating zoos (UK, Spain, France, Greece, Holland, Germany, Portugal, Denmark, Poland, Croatia, Czech Republic, and Israel). Sixty-nine faecal samples could be allocated to specific

individuals but the remaining 53 were unidentifiable. An additional 6 and 19 faecal samples were also obtained from Pretoria zoo, South Africa, and Dubai Desert Conservation Reserve (DDCR), United Arab Emirates, respectively (total faecal sample $n = 147$). The 35 blood/skin samples included two samples from Marwell zoo, which were from animals that were translocated to Australia in 1987. Forty-eight tissue samples were obtained from various zoos/ranches within the USA, which consisted largely of muscle biopsies taken using remote injection darts by trained professionals, and a smaller number of blood/necropsy samples taken during veterinary procedures/autopsies. A small number of museum samples were also obtained, which consisted of pieces of pelt from animals collected in Sudan (1824, $n = 1$, 1911, $n = 3$) and Chad (1925, $n = 1$), and a tooth from an animal collected in Chad (1960s).

DNA extraction

DNA was extracted from faecal samples in a dedicated area using the QIAamp DNA stool mini kit (QIAGEN) according to manufacturer's instructions but with the following modifications: *c.* 100 mg dried faecal material was placed in an Eppendorf tube with 1.8 mL ASL buffer, mixed thoroughly, and allowed to incubate at 37 °C for 12–24 h, and the final postextraction elution step was carried out for 30 min. A maximum of 15 samples were processed at one time with 1–2 negative controls. DNA was extracted from blood using the protocol described in Bruford *et al.* (1998), and from skin biopsies using a standard phenol–chloroform protocol (Milligan 1998). In the case of museum samples, pieces of pelt were cryopulversied in liquid nitrogen using an MM300 mixer mill (Retsch) and *c.* 200 mg of powder placed in 5 mL of extraction buffer (0.45 M EDTA pH 8, 1% sarcosyl, 0.4 mg/mL proteinase K), followed by the procedure described in Vigilant *et al.* (2001). For DNA extraction from teeth, a hand-held drill was used to make a hole in the root and a small amount of material collected and used as described above. All these procedures were carried out in contamination-free areas with appropriate negative controls.

Microsatellite analyses

A set of six microsatellite loci previously described in sheep (MAF46, MAF50, OarFCB304, OarAE119, OarCP26) or cattle (RBP3) and found to amplify polymorphic alleles in Arabian oryx (Marshall *et al.* 1999) was used. Polymerase chain reactions (PCR) were carried out in a 15-μL volume containing 2 μL template, 1× PCR buffer (ABgene, 75 mM Tris-HCl, pH 8.8, 20 mM $(NH_4)_2SO_4$, 0.01% (v/v) Tween 20), 1.0–3.0 mM $MgCl_2$, 12 μg BSA (Roche), 200 μM each dNTP, 200 nM each primer and 0.4 U DNA polymerase (ABgene). Amplification conditions consisted of initial denaturation for 4 min, followed by 30–45 cycles (blood and faecal DNA, respectively) at 94 °C for 30 s, 54–62 °C annealing temperature for 30 s, and 72 °C for 30 s, followed by a final extension at 72 °C for 10 min. The 5′ end of the forward primer was fluorescently labelled and the products were separated using gel electrophoresis on an ABI PRISM 377. Alleles were sized relative to an internal standard (HD400 with ROX label) and scored using GENESCAN 3.0 and GENOTYPER software (Applied Biosystems). In the case of faecal samples, heterozygous genotypes were accepted once confirmed in two separate amplifications, but homozygous genotypes were repeated 4–6 times to ensure high levels of accuracy.

Mitochondrial DNA sequencing

PCR amplification of either the complete control region (1.24 kb) (primers *SHODLOOPFOR* 5′-TCAAGGAAGA-AGCTATAGCC and *SHODLOOPREV* 5′-CATCTAGGC-ATTTTCAGTGA described in Iyengar *et al.* 2006) or a shorter 353-bp product (primer *SHODLOOPFOR* and primer *SHODLOOP350REV* 5′-TGTGGTACGTCGGTTTGC) was carried out in a 30-μL volume containing 2 μL template, 1× PCR buffer (ABgene), 2.5 mM $MgCl_2$, 24 μg BSA, 200 μM each dNTP, 200 nM each primer and 0.5 U *Taq* DNA polymerase (ABgene). The large product was amplified from all tissue samples but with faecal samples, upon failure to amplify the large product, attempts were made to amplify the smaller 353-bp product. Amplification conditions were as follows: initial denaturation for 4 min, followed by 30–40 cycles (blood and faecal DNA, respectively) of 94 °C for 30 s, 55 °C for 30–60 s (0.35-kb and 1.2-kb products, respectively) and 72 °C for 30–60 s (0.35- and 1.2-kb products, respectively), followed by a final extension at 72 °C for 10 min. In a few cases ($n = 4$), amplification of the larger product was carried out using both blood and faecal DNA in order to confirm identical sequences from both sources. In the case of museum samples, two separate PCRs were carried out for each sample amplifying overlapping products of 129 bp and 125 bp using primers *SHOMUSFOR1* (5′-GAAGCACTATCAATATATCCC) and *SHOMUSREV1* (5′-GTTATGAAATTTCCGCGTGC); and *SHOMUSFOR2* (5′-TCAACACAAACTTTCCACCC) and *SHOMUSREV2* (5′-GTTGGTTCATGTGCAGTAAG), respectively. Amplification conditions were as described above for the 0.35-kb product and sequencing was carried out on an ABI PRISM 377.

Statistical analyses

Microsatellites. Polymorphism within management groups (EEP, SSP, SA — South Africa, UAE) and overall in the entire captive population, measured as the total number of alleles, mean number of alleles per locus, and mean

observed heterozygosity was calculated using GENEPOP (Raymond & Rousset 1995). Allelic richness estimates were made using FSTAT (Goudet 2001). Tests for deviation from Hardy–Weinberg expectations and genotypic linkage disequilibrium were performed in GENEPOP followed by sequential Bonferroni correction (Rice 1989). Estimates of within-group F_{IS} were obtained using GENETIX version 4.04 (Belkhir *et al.* 2003). STRUCTURE 2.0 (Pritchard *et al.* 2000; Falush *et al.* 2003) was used to look for the presence of genetic structure among the samples. This software uses a Bayesian clustering approach to infer the number of populations (K) in a data set without a priori assignment of samples to populations. We used the population admixture model (where each individual is assumed to have inherited a proportion of its ancestry from each population) with correlated allele frequencies among populations. Ten replicates (to check for consistency) were run at each estimated group size (from $K = 1$ to $K = 5$) using a burn-in of 50 000 iterations and collection of data over 500 000 iterations. Values for the log likelihood of data across runs and values for individual membership within groups were then evaluated. Posterior probability values were calculated for the maximum log-likelihood value obtained using the formula given in Pritchard & Wen (2003). Pairwise estimates of the coefficient of relatedness (r) for all individuals were calculated using the Lynch & Ritland (1999) measure within the program IDENTIX (Belkhir *et al.* 2002). A recent study found that the Lynch and Ritland estimate (r_{xyLR}) more accurately depicted true relatedness between individuals compared to the Queller & Goodnight (1989) estimate (r_{xyQG}) (Russello & Amato 2004). Pairwise kinship coefficients were calculated as half the pairwise relatedness coefficients (Hardy 2003), and mean kinship (mk) values were then obtained for every individual as the average of pairwise kinship coefficient values to all other individuals including itself (Russello & Amato 2004).

Mitochondrial DNA. Sequences were checked by eye, edited, and aligned using BIOEDIT 5.0.9 (Hall 1999). Numbers of haplotypes, private haplotypes, polymorphic sites, and haplotype and nucleotide diversity were determined using DNASP 4.0 (Rozas *et al.* 2003). The model of DNA substitution that best fitted the data was selected using MODELTEST, version 3.06 (Posada & Crandall 1998). The Tamura-Nei + I + γ model [proportion of invariable sites (I) = 0.7786; $\alpha = 0.7361$] was selected by the Akaike information criterion (AIC). Model selection by the AIC has been reported to offer several advantages over hierarchical likelihood-ratio tests (Posada & Buckley 2004). Sequences were analysed using maximum-parsimony (MP) and maximum-likelihood (ML) approaches in PAUP, version 4.0b10 (Swofford 2002). A heuristic search with the tree-bisection–reconnection (TBR) branch swapping algorithm with 100 random taxon addition replicates was used in both cases. Node support was assessed using 1000 bootstrap replicates with 10 random taxon addition replicates in the case of MP, and 100 bootstrap replicates with one random taxon addition replicate in the case of ML (due to insufficient computer power). We also used a Bayesian likelihood approach in MRBAYES, version 3.1 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) using a general GTR + I + G model, allowing MRBAYES to estimate the various model parameters. The analysis was carried out using the default setting of four Markov chains (three heated and one cold) for 3 000 000 generations, sampling once every 100 generations. Four separate analyses were carried out simultaneously starting with different random trees. Post-burn-in trees (22 500) from all four analyses were used to estimate posterior probabilities. A homologous sequence from the Arabian oryx (GenBank: AJ235326) was used as the outgroup in all analyses.

Mean uncorrected p distances between groups of haplotypes were measured in MEGA version 2.1 (Kumar *et al.* 2001). Genealogical relationships among sequences were determined by a minimum spanning network using the statistical parsimony method (Templeton *et al.* 1992) implemented in TCS 1.18 (Clement *et al.* 2000). The algorithm within this program estimates the 95% statistical confidence limit for the maximum number of nucleotide substitutions between two haplotypes (the parsimony limit) and sequentially connects taxa into networks within this limit. A mismatch distribution of pairwise substitutional differences among haplotypes and a range of neutrality statistics capable of detecting the genetic traces of population growth, decline, or stability, were examined using DNASP version 4.0 (Rozas *et al.* 2003). Values for Fu's F-statistic (F_S, which specifically tests for population growth and detects excesses of low-frequency alleles) and Fu and Li's F^* and D^* statistics were obtained (Fu 1997). Observed values of F_S were compared with values obtained upon 1000 simulations in order to determine 99% confidence intervals. To estimate time since expansion (t), we used the formula $\tau = 2ut$ where $u = 2\mu k$, where μ is the mutation rate per site per million years and k is the length of the sequence. For estimation of recent population size, we used estimates of theta (θ) and the formula $\theta = 2N_{ef}\mu$, where N_{ef} is the female effective population size and μ is the mutation rate in substitutions/site/generation. This estimate is representative of recent rather than historical population size since genealogical information is not used (Crandall *et al.* 1999).

Results

Historical demography

Forty haplotypes (two indels considered) were identified among 141 SHO samples upon sequencing the entire

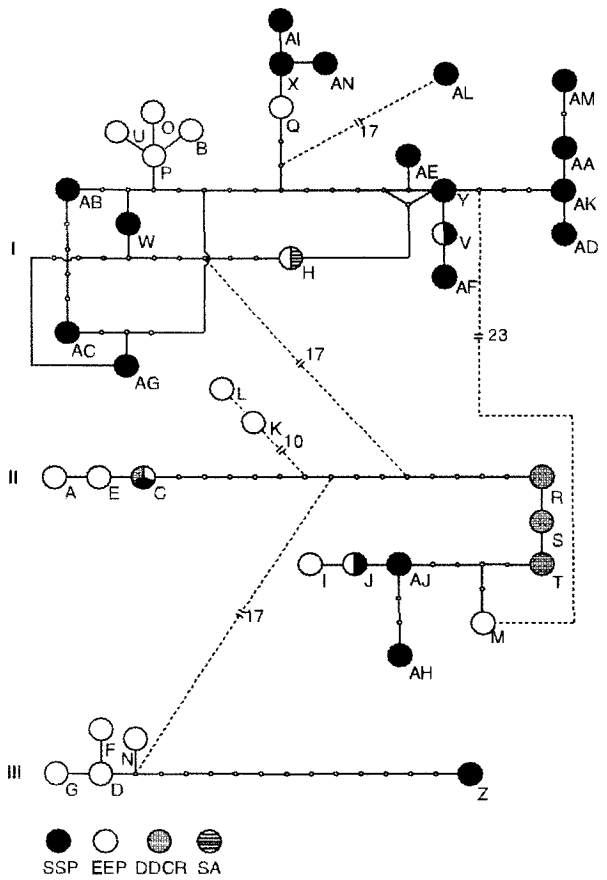

Fig. 2 Statistical parsimony network for haplotypes A–AN. Each single line represents a mutation step and small circles represent missing haplotypes. Numbers of mutation steps required for connecting the three networks and two sets of unconnected haplotypes are also shown.

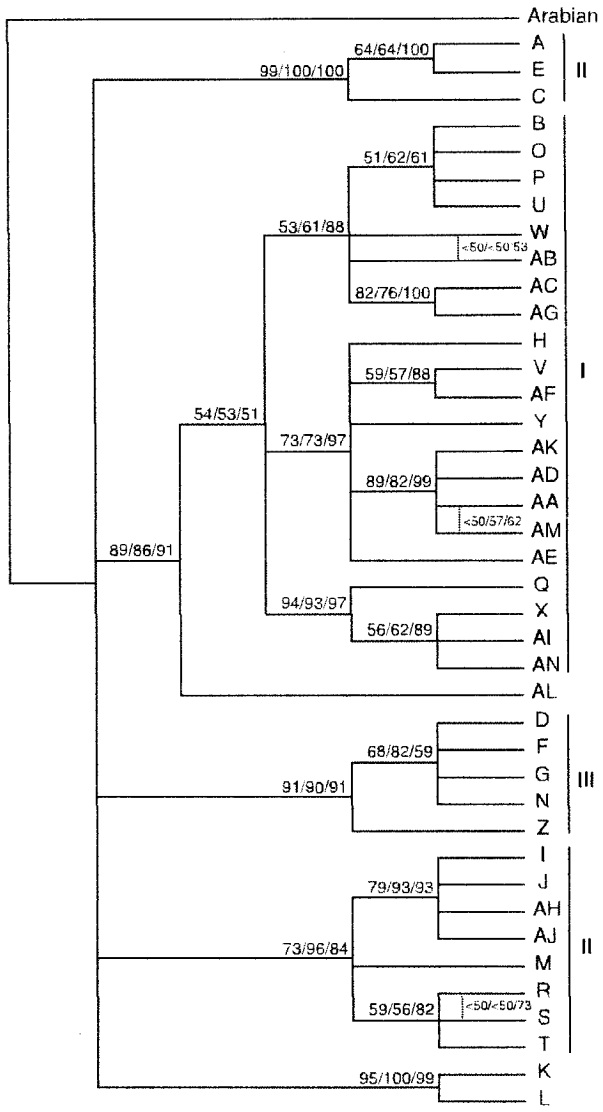

Fig. 3 Consensus tree depicting haplotype relationships. Numbers above nodes are bootstrap support for the node using ML and MP, and posterior probabilities using Bayesian analysis (ML/MP/Bayesian). Weak associations found in one/two analyses are shown in a smaller font between haplotypes W & AB, AA & AM, and R & S). Network groupings of the haplotypes are also shown.

control region (Table 1). Sequencing of the smaller 353-bp product from a further 45 samples where amplification of the 1.2-kb product was unsuccessful, did not provide additional information. The sequences reported are likely to represent cytoplasmic mtDNA (*cymt*) and not nuclear copies of mtDNA (*numt*) because we designed species-specific primers for this study and obtained identical sequences from both blood and tissue samples in a few individuals. A network analysis produced three disconnected networks with 95% confidence (connection limit = 15) indicating deep divergence between haplotypes (Fig. 2). Three unconnected haplotypes were also observed (K-L and AL). Numbers of mutation steps required for connecting haplotypes between the three separate networks and for connecting the unconnected haplotypes are shown in Fig. 2. A minimum of 24 mutation steps were necessary to connect haplotypes between networks. Four most parsimonious trees were produced using maximum parsimony (MP), with topologies very similar to those obtained with maximum likelihood (ML) and Bayesian analyses. A consensus tree is shown in Fig. 3 with MP and ML bootstrap support values and Bayesian posterior probabilities. Haplotypes within networks I and III were found to group together with fairly high bootstrap and posterior probability values, but network II haplotypes grouped into two separate clades (A, E, C) and (I, J, AH, AJ, M, R, S, T). Mean uncorrected p distances between haplotypes in networks I and II, I and III, and II and III were 2.62%, 2.35%, and 3.01%, respectively. From a previous study on the

Table 1 Variable sites within the mtDNA control region haplotypes

	111122223	3333333344	4444444444	4444444444	5555555555	5555566666	6666677777	7888990000	1111 0000000000	1111111111 0000000011	1 1	SAMPLES
	3007723570	2367889900	1334455666	7778888999	2233555668	8899900122	4556735556	9268580122	2223344445	5678999901	3	Captive group
	0785650085	5616494717	2240178019	0353457358	6706679240	5614704246	8199872399	9179369701	2344834561	4390245733	9	(location, number)
A	CCTTATGCAT	AGTAGCGTTC	GGAACTCCTC	CGACAGTTTC	ACCCAGTCTG	ATTATAATAT	TAACCTCAAA	CTTGGTACTA	TAGAGAATCA	TTTATTTTTT	T	EP (Mar,1)
B	TTCCG...G.	.....T.CC.	....T.TTCT	...T.....	GTTT....A	..C...G...	.....A..G	...A..G...	C....G..T.	A.C...C..C	.	EEP (Mar,2; Sel,2; LeP,3; Leip,2)
C	.....	.....	.....	.....	.....	.....	.....	.....	....A.....	A.....	.	EEP (Mar,8; Ches,2; Karls,1; War,2; Wob,3; Edin,3; DDCR,2; Mad,1); SSP (Fos Rim,1; Bam, 1)
D	T..C....G.	...G.TA...	.....	...TGA....	.TT.....A	G...C.....	..G...T...	...A...TC.	C..GA..C..	A.C.....C.	.	EEP (Mar,1; Whip,1; Wob,1)
E	.....	.....	.....	.....	.....	.....	.....	.....	....A.....	.....	.	EEP (Mar,1)
F	T..C....G.	...G.TA...	.....	...TGA....	.TT.....A	G...C.....	..G.T.T...	...A...TC.	C..GA..C..	A.C.....C.	.	EEP (Mar,1)
G	T..C....G.	...G.TA...	...G.....	...TGA....	.TT.....A	G...C.....	..G...T...	...A...TC.	C..GA..C..	A.C.....C.	.	EEP (Mar,1)
H	TTCCG...G.	.....T.CC.	....T.TTCT	...T.....	.TT.....CA	..C.....	.....A...	T..A..G...	C.A..G..T.	A.....C	.	SA (Pret,2) EEP (Cots,2; Manor,2; Sel,4)
I	T..C..A.G.	..C..T....	A.....T..	.....T	..TT....C.	C	...T.C....	...A.....	C...A..C..	A.....C	.	EEP (Artis,1)
J	T..C..A.G.	.....T....	A.....T..	.....T	..TT....C.	C	...T.C....	...A.....	C...A..C..	A.....C	.	EEP (Artis,4); SSP (Bam,2; Wilds,1)
K	T..C....GC	G....T....	.....	T..T.....	.TT..A....	..C...G.GC	.....	.....	CG..A.....	A.....	.	EEP (LaPalm,1; Aal 3; Lun,1; Karls,3; Ber, 3; Leip,3; Zag, 3)
L	T..C...AGC	G....T....	.....	T..T.....	.TT..A....	..C...G.GC	.....	.....	CG..A.....	A.....	.	EEP (LaPalm, 1)
M	T..C....G.	.....T....	A.....T..	.....	.....T	..T.....C.	C	...T.C....	...A..G...	C...A.....	A.C.....C	. EEP (Aal,2)
N	T..C....G.	...G.TA...	.....	...TGA..C.	.TT.G...A	G...C..C..	..G...T...	...A.C.TC.	C..GA..C..	A..G....C.	.	EEP (Cots,1)
O	TTCCG...G.	.....T.CC.	....T.TTCT	...T.....	.GTT.....A	..C...G...	.....A..G	...A..G...	C....G..T.	A.C...C..C	.	EEP (Whip,2)
P	TTCCG...G.	.....T.CC.	....T.TTCT	...T.....	.TTT.....A	..C...G...	.....A..G	...A..G...	C....G..T.	A.C...C..C	.	EEP (War,1; Pra,5; Lis,1; Zag,1)
Q	TTCCG...G.	.....CC.	....T.TTCT	...T....C.	.TT.....A	..CG.....	.....A..G	T..A..G...	C....G....	A.C.....	.	EEP (Sel,1; Ber,1; Pra,1)
R	T..C....G.	.....T....	A.....T..	.....C.T	..T.....C.	.....	...T.....	...A.....	C...A.....	A...C....C	.	UAE (DDCR,1)
S	T..C....G.	.....T....	A.....T..	.....C.T	..T.....C.	C	...T.....	...A.....	C...A.....	A...C....C	.	UAE (DDCR,1)
T	T..C....G.	.....T....	A.....T..	.....C.T	..T.....C.	C	...T.C....	...A.....	C...A.....	A...C....C	.	UAE (DDCR,1)
U	TTCCG...G.	.....T.CC.	....T.TTCT	...T.....	.TTT.....	..C...G...	.....A..G	...A..G...	C....G..T.	A.C...C..C	.	EEP (Ber,1)
V	TTCCG...G.	.....T.CC.	....T.TTCT	...T.....	.TT.....CA	..C.....	.G....A...	T..A.....	C.A..G..T.	A.C.....C	.	EEP (Amers,1), SSP (SDiego,3; Mem,1)
W	TTCCG...G.	.....T.CC.	....T.TTCT	...T.....	.TTT.....A	..C...G...	.....A...	...A..G...	C....G....	A.....C..C	.	SSP (Bam,2)
X	TTCCGC..G.	.....CC.	....T.TTCT	...T....C.	.TT.....A	..CG.....	.....A..G	T..A..G...	C....G....	A.C.....	.	SSP (Bam,4; HDZ,12)
Y	TTCCG...G.	.....T.CC.	....T.TTCT	...T.....	.TT.....CA	..C.....	.G....A...	T..A..G...	C.A..G..T.	A.C.....C	.	SSP (Bam,1)
Z	T..C....G.	...G.TA...	.....C....	.AGT.AC...	.TT.....A	GC..C.G...	..G....G..	..CA....C.	C..GA.GC.G	ACC.....-	.	SSP (Bam,1; Wilds,2)
AA	TTCCG...G.	.....T.CC.	...GT.TTCT	...T.....	.TT...C.CA	..C.....	.G....A...	T.....G...	C.A..G....	A.C..C...C	.	SSP (Bam,1)
AB	TTCCG...G.	.....T.CC.	....T.TTCT	...T.....	.TTT.....A	..C...G...	.....A...	...A..G...	C....G....	A.C...CCCC	.	SSP (Fos Rim,1)
AC	TTCCG...G.	.....T.CC.	....T.TTCT	...T....C.	.TT.....A	..C...G...	.....A..G	...A..G..G	C....G....	A.C...CCCC	C	SSP (Fos Rim,1)
AD	TTCCG...G.	.....T.CC.	...GT.TTCT	...T.....	.TT...C.CA	..C.....	.....A...	T.....G...	C.A..G....	A.C.....C	.	SSP (HDZ,1)
AE	TTCCG...G.	.....T.CC.	....T.TTCT	...T.....	.TT.....A	..C.....	.G....A...	T..A..G.C.	C.A..G..T.	A.C.....C	.	SSP (Bam,1)
AF	TTCCG...G.	.....T.CC.	....T.TTCT	...T.....	.TT.....CA	..C.....	.G....A...	T..A.....	C.A..G..T.	A.C..C...C	.	SSP (SDiego,1)
AG	TTCCG...G.	.....T.CC.	....T.TTCT	...T....C.	.TT.....A	..C...G...	.....A..G	...A..G..G	C....G....	A.....C..C	C	SSP (Nat,3)
AH	T..C..A.G.	.....T....	A.....T..	.....T	..TT....C.	.....G...C	...T.C..G.	...A.....	C...A..C..	A....C...C	.	SSP (Fos Rim,1)
AI	TTCCGC..G.	.....CC.	....T.TTCT	...T....C.	.TT.....A	..CG.....	.....A..G	T..A..G...	C....G....	A.C..C....	.	SSP (HDZ,2)
AJ	T..C..A.G.	.....T....	A.....T..	.....T	..TT....C.	C	...T.C....	...A.....	C...A..C..	A.....C	.	SSP (Bam,1)
AK	TTCCG...G.	.....T.CC.	...GT.TTCT	...T.....	.TT...C.CA	..C.....	.G....A...	T.....G...	C.A..G....	A.C.....C	.	SSP (HDZ,1)
AL	TTCCG...G.	.A..AT..CT	.A..T.T.C.	...T....C.	.TT....T.A	.C..C..C..	C.....A...	TC.AA.G...	CG...G....	A.....	.	SSP (HDZ,1)
AM	TTCCG...G.	.....T.CC.	...GT.TTCT	...T.....	.TT...C.CA	..C.....	.G....A...	T.....G...	C.A..G....	A.C..C.CCC	.	SSP (HDZ,1)
AN	TTCCGC..G.	.....CC.	....T.TTCT	...T....C.	.TT.....A	..CG.....	.....A..G	T..A..G...	C....G....	A.C....C..	.	SSP (HDZ,1)

Mar = Marwell, Sel = Selwo, LeP = Le Pal, Leip = Leipzig, Ches = Chester, Karls = Karlsruhe, War = Warsaw, Wob = Woburn, Edin = Edinburgh, DDCR = Dubai Desert Conservation Reserve, Mad = Madrid, Fos Rim = Fossil Rim, Bam = Bamberger, Whip = Whipsnade, Pret = Pretoria, Cots = Cotswold, Wilds = The Wilds, La Palm = La Palmyre, Aal = Aalborg, Lun = Lunaret, Ber = Berlin, Zag = Zagreb, Pra = Prague, Lis = Lisbon, Amers = Amersfoort, SDiego = San Diego, Mem = Memphis, HDZ = Henry Doorly zoo, Nat = National zoo. GenBank Accession nos are DQ159406–DQ159445 for haplotypes A-AN.

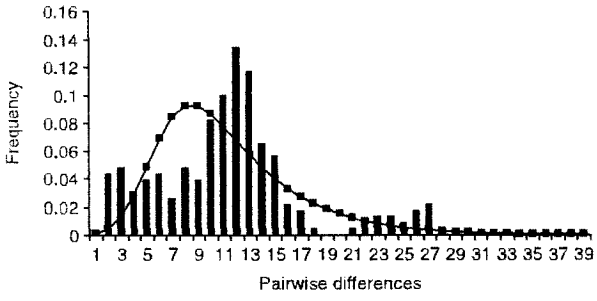

Fig. 4 Pairwise mismatch distribution for network I haplotypes. The bars represent observed values and the solid line represents expected values under a population growth-decline model.

characterization of the control region in *Oryx* species (Iyengar *et al.* 2006), a mean divergence rate for the entire sequence was estimated to be approximately 1.13% per million years. Using this estimate, divergence times of *c.* 2.3 million years between networks I and II, 2.1 million years between networks I and III, and 2.7 million years between networks II and III were obtained.

A mismatch distribution of pairwise differences among all haplotypes did not show a unimodal pattern which is generally interpreted as a signature of population expansion (Rogers & Harpending 1992). Instead, it showed a multimodal, erratic ('ragged') pattern. Similar patterns were obtained when haplotypes from networks II and III were analysed independently (haplotypes K,L excluded from network II, data not shown). However, when samples from network I alone were analysed (haplotype AL excluded), the mismatch distribution fitted a unimodal curve with a low raggedness statistic ($r = 0.012$, $P < 0.05$) (Fig. 4). A test for Fu's F_S, a powerful test for population expansion, was found to be highly significant when all haplotypes ($F_S = -19.22$ (99% CI: −14.87–3.49) or network I haplotypes alone were analysed (haplotype AL excluded) ($F_S = -14.16$, 99% CI: −8.00–9.06), nonsignificant when network II haplotypes alone were analysed (haplotypes K,L excluded) ($F_S = -2.57$, 95% CI: −4.24–4.33), and very marginally significant when network III haplotypes alone were analysed ($F_S = -0.51$, 95%CI: −0.48–4.43). A significant value of F_S rejects population stasis/neutrality indicating an excess of recent mutations, and thus, population expansion and/or selection. Range expansion can be distinguished from the effects of selection by the patterns of significance of F_S, F^* and D^* (Fu 1997). A range expansion is indicated when F_S is significant and F^* and D^* are not, while the reverse suggests selection. Nonsignificant values were obtained for F^* and D^* when either all haplotypes, network I haplotypes, or network II haplotypes were analysed, but a marginally significant value for D^* (−1.24, $P < 0.05$) and a nonsignificant value for F^* were seen in the case of network III haplotypes. Estimates of time since expansion were generated from the mismatch distributions for network I haplotypes and for all haplotypes, resulting in values for τ of 5.76 and 12.90, respectively. Using the average mutation rate of 1.13% per million years for the control region sequence, and an estimated generation time of 5 years for SHO, time since expansion was calculated to be approximately 0.5 million years ago (Ma) and 1.2 Ma for network I and all haplotypes, respectively.

In order to obtain some idea of the recent population size of SHO in the wild prior to its catastrophic decline, we estimated recent effective population size from the formula theta (θ) = $2N_{ef}\mu$ where N_{ef} is the female effective population size and μ is the mutation rate. Using the divergence rate estimate of 1.13% per million years and a generation time of 5 years, we obtained a value for μ of *c.* 0.56×10^{-7} substitutions/site/generation. Applying the value of θ (0.0198) to this formula and assuming that 50% of the populations were adults, and that roughly 70% of these adults reproduced, a census size of 1 million animals was obtained. A number of factors such as migration, population structure, and fluctuations in population size are known to affect the value of θ (Crandall *et al.* 1999), and consequently, this value provides only a rough estimate of population size.

Approximately 200 nucleotides were successfully sequenced from two of the museum samples used: tooth (Chad, 1960s), and pelt (Dongola, Sudan, 1911). Based on the short stretch of sequence obtained, the sequence from the tooth sample was found to be identical to several other haplotypes, but that obtained from the pelt sample from Sudan was found to contain one novel transition (data not shown).

Current mtDNA diversity in captive groups

The SSP group showed a higher number of polymorphic sites (6.9%) and a higher number of private haplotypes (18) compared to the EEP group which showed 6.1% variation and 15 private haplotypes, but haplotype diversity and nucleotide diversity values were very similar in both these groups (Table 2). Three haplotypes were found to be private to the UAE group. Mean uncorrected *p* distance across all 40 haplotypes was 1.96%. When only the 5′ variable section of the control region was considered, this value rose to 2.22%. Four haplotypes were found to be shared between captive groups: haplotype C, widespread among EEP individuals, was also seen within UAE and SSP samples; haplotype H was found in both EEP and SA groups; and haplotypes J and V were found in both EEP and SSP groups (Table 1, Fig. 2). While haplotypes from all three networks were represented in the SSP group, the majority of network I sequences (15/22) were found within this group. The highest uncorrected *p* distance values were seen between haplotypes AK and Z, AA and Z, and AM

Table 2 Genetic diversity within captive SHO

	EEP	SSP	SA	UAE	TOTAL
Mt Dloop haplotypes (1188nt)					
Sample size	86	48	2	5	141
No. of haplotypes	19	21	1	4	40
No. of private haplotypes	15	18	0	3	—
No. of polymorphic sites (% variation)	72 (6.1)	82 (6.9)	0	17 (1.4)	99 (8.3)
Haplotype diversity (SD)	0.880 (0.018)	0.875 (0.040)	0	0.900 (0.161)	0.926 (0.01)
Nucleotide diversity (SD)	0.017 (0.0004)	0.015 (0.0019)	0	0.008 (0.002)	0.018 (0.0005)
Microsatellites					
Sample size	120	48	4	6	178
Total no. of alleles	27	35	11	9	82
No. of private alleles	0	7	0	0	—
Allelic richness	2.92	3.34	2.20	1.71	—
Average no. of alleles/locus	5.4	7.0	2.2	1.8	16.4
Observed heterozygosity (%)	54	57	32	27	42
Average range	6.2	8.0	3.4	4.0	8.0
F_{IS} (95% CI)	0.128 (0.065–0.185)	0.020 (−0.082–0.101)	0.348 (−0.500–0.600)	0.222 (−0.250–0.390)	0.134 (0.083–0.179)

SD, standard deviation; average range, average range of allele size expansion in repeat motif number.

and Z (3.63%, 3.71%, and 3.79%, respectively), all found within the SSP group. A number of haplotypes representing all three divergent networks (X, W, AE, Y, and AA from network I, C, J, and AJ from network II, and Z from network III) were detected within Bamberger Ranch in Texas, USA. Both haplotypes AA and Z showing one of the maximal *p* distance values were found within Bamberger. Another large ranch in Texas (Fossil Rim) was found to contain haplotypes spanning two networks (AB and AC from network I, and C and AH from network II). Within HDZ, although only network I haplotypes were detected (X, AI, AN, AL, AM, AK, AD), the majority (all except X) were found to be unique to individuals from this zoo. Haplotypes from across all three networks were also found in the EEP group but network I haplotypes were underrepresented. Haplotypes E and D were found in the two individuals translocated to Australia from Marwell zoo in 1987. Haplotypes K and L, which were disconnected to the rest of the networks, were found in several individuals, including two individuals sent in 1999/2000 to Sidi Toui National Park, Tunisia, from La Palmyre zoo, France, as part of a reintroduction programme (Table 1). UAE samples consisted of three closely related haplotypes R, S, and T, and one slightly more distant haplotype, C, which was shared with both EEP and SSP groups. Only one haplotype (H) was found between two individuals from Pretoria zoo and was shared with individuals from the EEP group.

Current microsatellite diversity in captive groups

We successfully amplified six microsatellite loci in 106 out of 147 faecal samples (72%). Thirteen repeat samples were identified from the multilocus genotypes obtained and were deleted, leaving 93 samples for all analyses. One locus each within the EEP and SSP group (MAF46 and FCB304, respectively) was found to deviate significantly from Hardy–Weinberg equilibrium after Bonferroni correction. Both loci demonstrated heterozygote deficits but since the same locus was not found to consistently deviate across both groups, this is not thought to be a consequence of null alleles. Tests for linkage disequilibrium after Bonferroni correction revealed one association that remained significant (OarCP26 and MAF50). Both loci, mapped to sheep chromosome 4, are separated by 30 cM (www.thearkdb.org/, Roslin Institute), a distance considered adequate to ensure linkage equilibrium by some authors (e.g. Luo *et al.* 2004). However, given the significant result, we eliminated locus MAF50 from all analyses.

Total number of alleles, number of private alleles, allelic richness, average allelic range in repeat unit length and average heterozygosity were all once again, higher in the SSP than in the EEP group (Table 2). To check that this was not an effect of sampling large numbers of related individuals within the EEP group and not in the SSP group, we deleted one individual out of every known full-sib and parent–offspring relationship from both groups and re-analysed the remaining data (EEP $n = 102$; SSP $n = 43$). All values remained identical in both data sets (data not shown). The very small sample sets of SA and UAE showed much lower values for all these estimates but clearly require additional sampling. Within-group estimates for the inbreeding coefficient, F_{IS}, revealed the lowest value in the SSP group (0.020), with moderate levels in the EEP group (0.128).

Table 3 Results from STRUCTURE 2.0 for K values 1–5. The highest Ln $P(X/K)$ obtained in 10 independent runs, mean inferred assignment to clusters, and numbers of individuals with > 75% assignments within each cluster are shown

K	Ln $P(X/K)$	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
1	−2099.8					
2	−2035.8	0.454 (10EEP,36SSP,3UAE)	0.546 (73EEP,1SSP,1UAE,2SA)			
3	−1982.8	0.321 (2EEP,29SSP,1UAE)	0.338 (30EEP,1SSP,2UAE)	0.343 (37EEP,1SSP,4UAE)		
4	−1990.2	0.266 (20EEP,1UAE)	0.291 (25EEP,1SA)	0.205 (3EEP,5SSP)	0.238 (15SSP,1UAE)	
5	−2038.0	0.208 —	0.192 —	0.209 —	0.208 —	0.184 —

The results obtained using STRUCTURE 2.0 are summarized in Table 3. Results were highly consistent across the 10 independent repeat runs suggesting adequate numbers of iterations. The highest estimated log-likelihood value (in 10 runs) and the highest posterior probability (0.9991) was seen in the case of $K = 3$ and there was evidence of differential clustering of EEP and SSP samples, with the majority of SSP samples clustering separately to a large proportion of the EEP samples with > 75% assignment. However, overall proportions assigned to groups were fairly symmetric (~$1/K$ in each group) suggesting that there was no support for strong genetic structure across the sample set (Pritchard & Wen 2003). The F_{ST} value between EEP and SSP groups was also low, at 0.047 ($P < 0.001$).

Individuals important to global captive breeding based on mk *values*

Values obtained for *mk* in every individual were ranked from lowest to highest in order to prioritize the genetically most important animals, that is, those with the lowest *mk* values (Fig. 5). Almost all (44/48, 92%) SSP individuals had *mk* values lower than the median, while the majority (81/120, 68%) of the EEP individuals had *mk* values higher than the median. The UAE and SA samples fell on both sides of the median, with 4/6 lower and 2/6 higher than the median in the case of UAE and 2/4 lower and 2/4 higher than the median in the case of SA, respectively. The two genetically most important living animals were from Bamberger and possessed four of the eight SSP private alleles. Three SSP private alleles were found in six individuals from HDZ (ranked 2nd, 13th, 25th, 28th, 31st, and 38th). Fifteen of the HDZ individuals sampled were dead (marked with asterisks in Fig. 5), a number of which have produced no offspring (8th, 13th, 22nd, 28th, 45th, 55th). In Fossil Rim, three of the SSP private alleles were found in two individuals (ranked 14th and 44th). One individual from Fresno (ranked 7th) and two individuals each from San Diego and The Wilds (ranked 5th and 20th, respectively) had one SSP private allele each The highest ranked EEP individuals were from Selwo zoo, Spain (4th), and Artis zoo, Holland (11th), followed by the two animals sent to Sidi Toui from La Palmyre (18th and 23rd, respectively).

Discussion

Inferences on historical patterns of demography

Deep sequence divergence between the SHO mtDNA haplotypes was evident by the three disconnected networks, suggesting historical population isolation. Estimated divergence times between the networks ranged from *c.* 2.1–2.7 million years. These levels of divergence are likely to represent remnants of ancient divergence within SHO, since the genus *Oryx* along with a number of other arid-adapted bovid species first appear in the fossil record *c.* 2.7–2.5 Ma (Vrba 1995; Bobe & Eck 2001; deMenocal 2004), and molecular phylogenetic studies support a recent divergence of this genus within the Hippotragini tribe, with all three species consistently found as terminal taxa with very short branch lengths (Gatesy *et al.* 1997; Hassanin & Douzery 1999; Iyengar *et al.* 2006).

Significantly large negative values for Fu's F_S were obtained both when all haplotypes were analysed simultaneously, and when network I haplotypes were analysed separately. In addition, nonsignificant values for Fu and Li's F* and D* were seen in both these cases, a pattern that is highly suggestive of an ancient population expansion. A unimodal pattern of mismatch distribution characteristic of a population explosion was however, not observed when all haplotypes were considered. Results from a mismatch distribution are sometimes considered tentative since a number of factors such as the time of population expansion, population size before expansion, and subdivision of populations, have been found to affect the results (Marjoram & Donnelly 1994). It is possible that both population subdivision (clearly apparent from the three disconnected networks), and population size before

Fig. 5 Mean kinship (*mk*) values in every individual ranked from lowest to highest. Fifteen HDZ individuals that were dead are marked with asterisks.

expansion, are responsible for this observation. In the case of network I haplotypes however, a mismatch distribution showing a unimodal pattern was supported by a significantly low raggedness statistic (Harpending 1994). Times of population expansion were estimated to be *c.* 0.5 Ma and *c.* 1.2 Ma with network I or all haplotypes, respectively.

There is considerable evidence to suggest that glaciation events caused severe climatic changes within Africa. Throughout the Quaternary period which was dominated by ice ages that occurred every 41 000 years until 0.9 Ma, and every 100 000 years thereafter, it has been found that during glacial cycles, the climate was colder and drier in Africa with an increase in savannah and desert regions and a reduction in rainforests (e.g. deMenocal 2004; Hewitt 2004a). Studies have shown that at the last glacial maximum (LGM) *c.* 23 000–14 500 years ago, increased aridity in Africa resulted in the expansion of the Sahara desert zone hundreds of kilometre further south than at present, compressing the Sahelian zone equator wards (Thomas & Thorp 1995). However, other studies have found that at this time, areas in the northwest of the Sahara retained greater winter rainfall and consequently formed a belt of semidesert to the south of the present day desert margin (Hooghiemstra *et al.* 1992). Even in the central part of the Sahara, areas above 1500 m are thought to have resembled semidesert at the LGM since evidence suggests that winter rainfall occurred in these regions and maintained scattered vegetation (Maley 2000). Although many phylogeographical studies of large mammals across Africa have found evidence for the existence of glacial refugial areas within the west, east, and south of the continent (reviewed in Hewitt 2004b), there are very few studies that have investigated the evolutionary history of the fauna and flora of North Africa. Unsurprisingly, however, these studies have also found genetic evidence for conspicuous palaeoclimatic effects in this region (Brown *et al.* 2002; Cosson *et al.* 2005).

We propose therefore, that the population expansion signals detected at 1.2 Ma and 0.5 Ma in SHO are the result of the restriction of populations within suitable refugial areas during glacial cycles followed by expansion during favourable interglacial conditions. Since evidence already suggests that suitable semidesert habitat was available to the north and the south of the Sahara, and possibly even within the Sahara itself at higher altitudes, SHO populations may have become restricted into three or more such refugial areas during an ancient glaciation, resulting in the three divergent networks that are seen. A star-shaped topology characteristically seen when populations have undergone rapid range expansion following restriction into small refugia as seen in many species from temperate regions (e.g. Hull & Girman 2005) was, however, not seen in the networks. Perhaps refugial areas in this region were large, retaining sizeable numbers of diverse ancestral haplotypes. In this case, small numbers of ancestral haplotypes expanding into new haplotypes would then reveal small groups of star-shaped topologies as seen in network I (e.g. P, B, O, and U). Since all the samples used in this study are most likely to have originated from just one location (Chad), we are unfortunately unable to obtain a more complete picture of the various phylogeographical groups that may have existed, and an extensive survey of museum samples will prove useful in this context.

Following the ice ages, in the early part of the Holocene (9500–4500 years ago), there is extensive evidence to suggest that conditions were much more humid in the Sahara than it is at present ('the early Holocene pluvial episode'), with savannah extending right into the desert. Relict savannah plant species have been found in the Sahara and rock art left by early humans in the area suggest the existence of savannah species such as elephants and hippos during this time (e.g. Lézine 1989; deVivo & Carmignotto 2004). Thus, having undergone repeated population restriction and expansion during glacial cycles, the existence and maintenance of enormous numbers of SHO across North Africa in the past few thousand years is highly possible, providing support for the census estimate obtained in this study of *c.* 1 million individuals in the recent past. SHO was considered the most numerous large mammal of the Sahel during the middle ages, and as recently as 1936, herds of up to 10 000 animals were sighted in Chad (Bassett 1975; Newby 1988). High levels of genetic diversity must have been maintained within SHO populations since they were migratory, travelling large distances (over 600 km annual round trip recorded in Chad) in search of grazing (Newby 1988).

Genetic diversity preserved within captive groups

The SSP group was found to retain higher levels of genetic diversity with both mtDNA and microsatellites, reflecting the greater number of founder individuals taken to the USA from the initial captures made in Chad. Overall mean uncorrected *p* distance across all SHO haplotypes was 2.0%, a value that is comparable to those reported within other Hippotragines (1.9% in roan, Alpers *et al.* 2004) and the closely related Alcelaphines [1.7% in topi (*Damaliscus lunatus*), 2.4% in wildebeest (*Connochaetes taurinus*), Arctander *et al.* 1999]. However, the maximal levels of divergence between haplotypes reported in other Hippotragines are far greater than those seen in SHO. For example, in sable, Pitra *et al.* (2002) observed a mean sequence divergence of 14.6% between three clades representing regions in eastern and southern Africa, and in roan, Alpers *et al.* (2004) observed a maximal divergence of 27.5% between two haplotypes from Senegal and Botswana. In this study, a mean divergence of only 2.7% was observed between the three SHO networks with a maximum value of 3.8% between haplotypes AM and Z. Therefore, given

that SHO spanned across vast areas of North Africa, and that populations may have become isolated into ice age refugia resulting in highly divergent groups of haplotypes (networks), it appears that some of the range of diversity may have become lost. The detection of a novel transition within the museum sample from Sudan (1911) provides some evidence for the existence of greater diversity in the past, but more extensive sampling is required in order to elucidate historical patterns.

The lack of strong evidence for population genetic structure using microsatellites could be a result of the small number of loci used in this study since Evanno *et al.* (2005) have reported a drop in detection of signal of population genetic structure with five loci in comparison to 10 loci. However, other studies have successfully detected evidence for population structure using just five microsatellite loci (Pritchard *et al.* 2000; Hufbauer *et al.* 2004). Consequently, we interpret our finding of a lack of population structure as being a result of very large numbers of SHO existing largely in panmixia within the Sahelian region after the early Holocene pluvial episode. SHO are thought to have been highly nomadic, travelling vast distances on a regular basis (Newby 1988; Wacher 1988). Mean observed microsatellite heterozygosity in SHO across all groups was identical to that seen in wild populations of roan (42%, Alpers *et al.* 2004), and values seen within the SSP and EEP groups (57% and 54%) were very similar to that seen in captive populations of Arabian oryx (54% across six loci, four of which were the same as those used in this study) (Marshall *et al.* 1999).

Future captive breeding and reintroductions

Information from the SHO stud book database containing multigenerational captive breeding records suggests that the sample set used in this study includes, albeit to varying degrees, 80% and > 85% of the original founder lineages from the SSP and EEP groups, respectively (data not shown). Future captive breeding must maintain and actively manage the high levels of genetic diversity seen within the SSP group. Results suggest that Bamberger, HDZ, and Fossil Rim hold some of the most valuable global SHO genetic diversity. Demographic studies on SHO within the SSP has revealed an ageing population where far greater numbers of older rather than younger individuals are being held, and breeding is inadequate (also indicated by the very low F_{IS} value seen within SSP in this study), and it has been recognized that managed captive breeding is urgently required (Spevak 2004). Results from this study further highlight this need. Loss of some genetic diversity is already apparent in HDZ where a number of dead individuals of genetic importance have left no known progeny. Within the EEP group, although levels of overall genetic diversity are lower, mtDNA haplotypes from all networks are represented (except for a degree of under-representation of network I haplotypes), and levels of microsatellite allelic richness are high. Also, large numbers of younger individuals are held as a result of sustained managed captive breeding, rendering the population more 'stable' in the long term (Gilbert 2005). Although small sample sizes in the UAE and SA groups preclude conclusions, it is clear that management programmes running within individual countries need to keep in mind the requirement for animal import from other regions in order to prevent extensive inbreeding. Since all Australasian SHO are likely to have descended from a few individuals from Marwell, for example the two animals sampled in this study which had common EEP haplotypes and high *mk* values (ranked 147th and 149th), there is a need for future animal import from both within and outside the EEP into these regions. Two SHO sent to Sidi Toui National Park as part of 18 individuals sent from the EEP to various parks in Tunisia, possessed distinct haplotypes and low *mk* (ranked 18th and 24th), making them important in a global context. Studbook information indicates that the remaining animals sent to Tunisia are related at varying degrees to other animals held within the EEP. It is of interest to carry out further genetic analyses on these reintroduced animals and on animals to be re-introduced in the future in order to establish how best to maintain and supplement genetic diversity in these groups.

In conclusion, based on our findings, we recommend that a 'global' perspective for the captive genetic management of SHO is maintained, and that individuals across networks continue to be intermixed as currently practised. Individuals from various management programmes and regions need to be effectively utilized for sustained future captive breeding in order to ensure that the vital remnants of genetic diversity are retained and represented in future reintroduction programmes.

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