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RESEARCH ARTICLE

Influence of habitat fragmentation on the genetic structure of large mammals: evidence for increased structuring of African buffalo (*Syncerus caffer*) within the Serengeti ecosystem

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Abstract Wildlife species exposed to habitat fragmentation are often in need of a conservation effort. The African buffalo (*Syncerus caffer*) is one of the key species in the Serengeti ecosystem as they form a large part of the herbivore biomass, providing ecotourism and valuable trophies. The ecosystem is a part of Tanzanias protected areas and is administrated under different management practices. Among these, we have analysed the genetic structure of buffalo ($n = 68$) from the Serengeti National Park (SNP), the Ngorongoro conservation area (NCA) and the Maswa game reserve (MGR). Both the sequence variation in a 493 base pair fragment of the mitochondrial D-loop and the allele frequency-distribution in 15 microsatellites suggest genetic structuring of the buffalo populations within the ecosystem. Both the allele frequency-distribution and the amount of genetic variation were high and similar in SNP and MGR, suggesting a high degree of gene flow between these locations. By comparison, the NCA buffaloes had significantly lower genetic variation and were genetically differentiated from SNP and MGR. Approximate Bayesian computation estimates suggest that the observed genetic structure is of a recent origin, indicating that the recent increases in developmental activity in the region may have influenced the genetic structure of the buffalo within the Serengeti ecosystem.

Keywords Genetic structure · Microsatellite · Mitochondrial DNA · Conservation · Tanzania

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Introduction

Historically, the vast populations of large wild herbivores in Africa provided a substantial resource, supplying local and regional communities with goods and economic income (Conover 1997; Loibooki et al. 2002; Ogutu 2002). However, during the past few decades, overexploitation, poaching, diseases and habitat loss have reduced many of these large herbivore species to the extent that they are now restricted to protected areas (Hilborn et al. 2006; Chantal et al. 2007). Unfortunately, many protected areas in Africa are currently experiencing size reduction and isolation due to increases in human populations and activities (Newmark

2008), which may disrupt or prevent dispersal and gene flow (Ralph et al. 2006; Heller et al. 2010). As a consequence, small or fragmented populations may experience inbreeding, loss of adaptive genetic variation and an increased risk of extinction (Allendorf 1986; Soulé and Mills 1992; Amos and Balmford 2001; Keller and Waller 2002). Tanzania has reserved more than 25% of its land areas to wildlife conservation and harbours a wide diversity of wild animals (Stuwart et al. 1990; Thirgood et al. 2004). Recently however, protected areas in Tanzania have suffered fragmentation and isolation resulting from human activities. More than two decades ago it was estimated that the country has lost about 43% of its original wildlife habitats (WRI 1989), a situation that requires particular conservation attention (Fynn and Bonyongo 2010).

The Serengeti ecosystem is a geographical region west of the Great Rift Valley in northern Tanzania, often defined as the area encompassed by the wildebeest migration (McNaughton and Campbell 1991). The ecosystem cover areas under different management practices with different degrees of allowed human impact, ranging from strict photographic tourism to trophy hunting and multiple usage of land with livestock keeping, agriculture and human habitation. In some parts of the ecosystem, human population growth, anthropogenic activities and fragmentation of the ecosystem have been rapid (Kurji 1981; Meertens et al. 1995; Kijazi et al. 1997; Estes et al. 2006), posing a serious threat to the long term viability of wildlife populations.

African buffalo (*Syncerus caffer*) is one of the key species in the Serengeti ecosystem; not only because they form a large part of the herbivore biomass in the area (Van Hooft et al. 2002, 2003; Iain et al. 2004), but also by providing ecotourism and valuable trophy hunting. A number of studies have been conducted on the species' ecology across the region (George 1975; Sinclair 1977; Runyoro et al. 1995; Estes et al. 2006; Metzger et al. 2010), providing information on its spatial and temporal population fluctuations. Historically, the species had a wide geographical distribution across nearly the whole of sub-Saharan Africa. However, the buffalo has declined in numbers across most of its historical range and its distribution has become highly fragmented (Sidney 1965; Sinclair 1977). In Tanzania the census population amounts to about 400,000 buffalos, but the population is increasingly fragmented and its distribution is becoming more restricted to protected areas (TAWIRI 2008).

Genetic studies have shown that the African buffalo harbours high levels of genetic diversity but low population differentiation throughout its ecological range (Simonsen et al. 1998; Van Hooft et al. 2000, 2002, 2003). However, to achieve sustainable conservation of the species, and to be able to identify potential management units and barriers to gene flow (De Young and Honeycutt 2005; Heller et al. 2010), it is necessary to attain information on existing genetic structure both between and within protected areas. Besides a study of the genetic differentiation among one introduced and three remnant buffalo populations in South Africa (O'Ryan et al. 1998), little is known about the genetic population structure of buffalo within separate ecosystems. The primary aim of this study was therefore to examine the genetic structure of the African buffalo within the Serengeti ecosystem with special emphasis on the potential differentiation between areas with different management practices.

Materials and methods

Study area

The Serengeti ecosystem is located in the north-east of Tanzania between 34°45′–35°50′ E and 2°–3°20′ S (Fig. 1a). The ecosystem covers different conservation administrations. The main are the Serengeti National Park (SNP), the Ngorongoro conservation area (NCA) and the Maswa game reserve (MGR) (Fig. 1b). In addition there is Ikorongo/Grumeti game reserve (IGGR) and Loliondo game controlled area (LGCA). In 2006, estimates from repeated field counting suggested census population sizes of 1,887 buffaloes in NCA, 70,526 in SNP and 47,668 in MGR (TAWIRI 2008). SNP is a totally protected area where no human activity other than photographic tourism is allowed. The MGR lies along the western boundary of SNP and abuts the south-western corner of NCA (Fig. 1b). Here, photographic tourism and game harvesting including trophy hunting is permitted but human habitation is not. From 1976 to 2003, MGR area has twice been reduced in size due to increasing human activities (Kurji 1985; Meertens et al. 1995; Songorwa 2004). By comparison, the NCA is a multiple land-use area where photographic tourism, human habitation and livestock keeping are allowed but harvesting of wildlife is forbidden. In this area human population growth and development has been rapid (Kurji 1981; Kijazi et al. 1997; Estes et al. 2006). Most buffaloes in NCA are found in the Ngorongoro Crater (Fig. 1b) which is particularly famous for its wildlife and photographic safaris. No human settlement is allowed in the crater, although livestock keepers are allowed to make a seasonal use of the crater floor for grazing, watering and salt licking (Fyumagwa et al. 2007). It has been suggested that human impacts and habitat reductions around the crater have affected buffalo migration to nearby areas (Estes et al. 2006).

Samples and DNA extraction

From 2007 to 2009, buffalo blood samples were obtained from within the crater in NCA ($n = 19$) and SNP ($n = 24$),

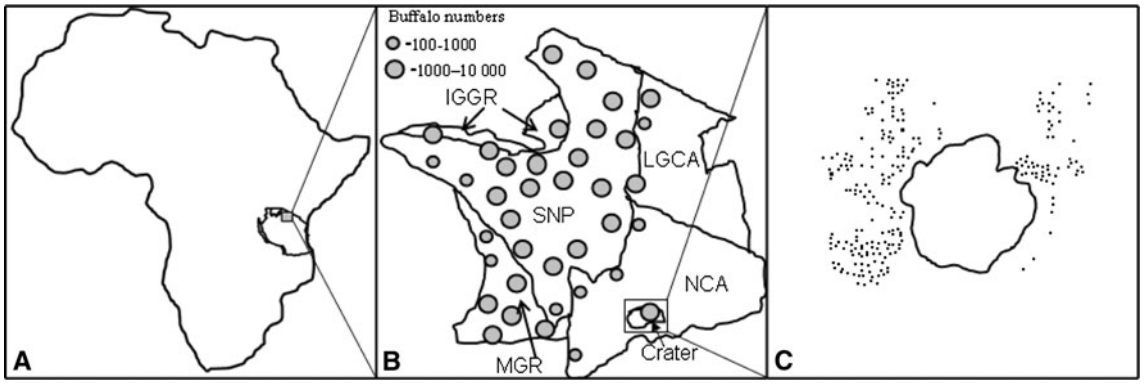


Fig. 1 Map showing Tanzania and location of Serengeti ecosystem (**a**), the buffalo distribution in the Tanzanian part of the ecosystem together with geographical location of the different conservation administration areas including Serengeti National Park (SNP), Ngorongoro Conservation Area (NCA), Maswa Game Reserve (MGR), Loliondo Game Controlled Area (LGCA), Ikorongo/Grumeti Game Reserves (IGGR) (**b**), and the distribution of the human settlement in NCA adjacent to Crater highlands (**c**)

while muscle tissue was sampled in MGR ($n = 25$). In NCA all samples were obtained from the crater floor only, while in SNP and MGR the sampling were more spread out across the respective areas. Whole blood was obtained from immobilized animals during routine disease surveillance. Etorphine hydrochloride (M99® 9.8 mg/ml, Novartis South Africa Ltd) was used for immobilization. Darting was done from a vehicle using a gas powered dart gun (DAN-INJECT® MOD JM, Denmark). Whole blood was collected from any accessible vein in EDTA vacutainers and stored in a cool box for a maximum of 6 h prior to freezing (−20°C). After sampling, immobilized animals were given the antidote Diprenorphine Hydrochloride (M5050® 5 mg/ml, Norvartis South Africa Ltd) and monitored until full recovery and re–joining with the herd. From MGR samples were obtained from hunters and from lion kills. Muscle samples were stored in 5 ml bottles containing absolute ethanol and kept at room temperature. Genomic DNA was isolated from samples using QIAGEN®, DNeasy Blood and Tissue kit.

Table 1 Summary information of 15 microsatellite loci analyzed with observed size range, PCR conditions and reference

Locus	Size range (bp)	PCR cycles	Annealing temp (°C)	Reference
BM1009	272–300	25	56	Bishop et al. (1994)
ETH3	92–110	25	56	Bishop et al. (1994)
ETH10	200–208	25	56	Solinas and Fries (1993)
BM4208	145–164	30	56	Bishop et al. (1994)
BM6506	184–204	30	56	Kappes et al. (1997)
SPS115	222–250	25	58	Kemp et al. (1993)
BM2113	108–124	25	56	Kappes et al. (1997)
BM4107	145–167	25	56	Bishop et al. (1994)
BMC3224	179–204	30	56	Kappes et al. (1997)
BM804	134–201	30	56	Bishop et al. (1994)
BM1818	260–280	25	56	Bishop et al. (1994)
OarFCB48	136–158	30	56	Buchanan et al. (1993)
BM1824	167–195	28	56	Bishop et al. (1994)
TGLA53	139–171	28	56	Crawford et al. (1995)
BM757	164–186	30	56	Bishop et al. (1994)

DNA amplification

Samples were genotyped using 15 di-nucleotide microsatellites of bovine and ovine origin (Table 1). The microsatellites were distributed on 13 different chromosomal locations on the cattle genome. The PCR was carried out on approximately 20–40 ng of genomic DNA, 2 pmol of each primer, 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris–HCl, 1 µl dNTP and 0.5 U Taq polymerase (Amplicon®), in a total volume of 10 µl. Amplification was done using Gene Amp® PCR system 9700 (Applied Biosystems). Thermocycling parameters after denaturation at 95°C in 2 min were: 95°C for 30 s, annealing for 30 s and extension at 72°C for 45 s followed by 10 min at 72°C. The number of PCR cycles and annealing temperatures for the different loci are given in Table 1. Forward primers were fluorescently labeled for electrophoresis on an ABI3100 automated sequencer (Applied Biosystems). Commercially prepared size standards (ROX GENESCAN® 400HD) were run with every sample. Alleles were scored using GeneMapper 3.7 (ABI 3100, Applied Biosystems), and new PCR's were performed for samples where genotypes were unclear. Furthermore, 10% of all the samples were selected at random and new PCR and genotyping were performed.

All samples were also amplified and sequenced for a 493 bp region of the mitochondrial control region adjacent to the *tRNA^{pro}* gene, using the primers 5′-AATAGCCCCAC TATCAGCACCC-3′ (Flagstad et al. 2000) paired with 5′-G TGAGATGGCCCTGAAGAAA-3′. Amplification was done

in Gene Amp® PCR system 9700 (Applied Biosystems) using an initial denaturation at 94°C for 4 min followed by 35 cycles of 40 s at 94°C, 40 s at 60°C and 40 s at 72°C and ended by an additional 7 min extension step at 72°C. Amplifications were performed in 25 µl volumes containing 1.35 mM $MgCl_2$, 200 µM of each dNTP, 5 pmol of each primer and 0.5 units of AmpliTaq DNA polymerase (Applied Biosystems). PCR amplicons were purified with ExoSap-IT (GE Healthcare) and sequenced using BigDye terminator chemistry version 1.1 on an ABI3100 automated sequencer (Applied Biosystems) following the manufactures' protocol. Sequences were aligned manually in Proseq 2.9 (Filatov 2002). Sequences were deposited in the GenBank database under accession numbers JN849157-JN849224.

Statistical analysis

For the mitochondrial data Arlequein 3.11 (Excoffier et al. 2005) was used to estimate nucleotide and gene diversity and to obtain pair-wise F_{ST} estimates, taking haplotype frequencies as well as nucleotide variation among haplotypes into account. Statistical significance was evaluated by 1,000 permutations.

The microsatellite data were checked for null alleles, stutter errors or short allele dominance by use of the Micro-Checker 2.2.3 (van Oosterhout et al. 2004). Genetic diversity was estimated from the mean number of alleles per locus, allele frequencies, allelic richness (El Mousadik and Petit 1996), observed and expected heterozygosity using both FSTAT 2.9.3 (Goudet 2001) and CERVUS 3.0.3 (Marshall et al. 1998). Deviations from Hardy–Weinberg equilibrium (HWE) were assessed and Fisher's exact tests of pairwise genetic differentiation among the sampled individuals performed, using default settings in GENEPOP 4.0.7 (Rousset 2008). The significance level was sequentially Bonferroni adjusted for repeated tests (Rice 1989). Population genetic differentiation was further addressed through traditional F_{ST} statistics (Weir and Cockerham 1984), analysed in GENEPOP and through the more recently proposed estimator D_{est} (Jost 2008), using the web based application SMOGD (Crawford 2010). F_{ST} has been suggested to be well suited when both sample size and the numbers of applied loci are relatively low (Gaggiotti et al. 1999), while D_{est} appears to more accurately account for differences in allelic diversity, especially for highly polymorphic markers such as microsatellites (Jost 2008).

Genetic structuring was also assessed through Bayesian clustering of the microsatellite genotypes as implemented in STRUCTURE 2.3 (Pritchard et al. 2000; Hubisz et al. 2009). The log likelihood of our data was estimated given different numbers of genetic clusters (ln Pr $(X \mid K)$, $K \in [1, 6]$), using an admixture model with correlated allele frequencies and ten runs of each K. Each run consisted of 500,000 MCMC repetitions and 100,000 burnin cycles. For increasing values of K, the variation between runs of each K value, was used to assess the main genetic structure of the data set (Evanno et al. 2005). The STRUCTURE analyses were done both with and without prior knowledge of sampling locations (with and without LOCPRIOR) (Pritchard et al. 2000; Hubisz et al. 2009). The amount of information provided by the sampling location is expressed by the value r, calculated across all runs. Values equal to or greater than 1 imply that information on the sampling location of individuals is uninformative about ancestry, while smaller r values imply the opposite. To assess first-generation dispersal, STRUCTURE was run in a second analysis using POPINFO at default setting.

The microsatellite data were further analysed in BOTTLENECK 1.2 (Cornuet and Luikart 1996) to test the probability of recent bottlenecks. We applied a two-phase model of stepwise mutation (SMM; Kimura and Ohta 1978) with 20 and 10% variation from the infinite allele model (Kimura and Crow 1964), as this fits most microsatellites better than a strict one-step model (Di Rienzo et al. 1994).

The time of divergence for observed microsatellite differentiation was estimated through the coalescent-based approximate Bayesian computation (ABC) algorithm of DIY ABC (Cornuet et al. 2008). Alternative historic scenarios' of divergence and admixture that may explain today's observed populations were specified and explored with and without demographic events (Fig. 3, Table S1 (supplementary)), some involving un-sampled populations (outside the study area). Conditions were set for 1) the sequence order of historic events and 2) for demographic events (Var*Ne*) according to historic records. From each scenario 500,000 data sets were simulated by drawing from a specified set of prior distributions of the parameters. The 5,000 of the simulated data sets most similar to the observed data were identified through logistic regression and a set of four within and six among populations default summary statistics (means across loci for; the number of alleles, gene diversity (Nei 1987), allele size variance, M-ratio (Garza and Williamson 2001) per sample and between each pair of samples, the F_{ST} (Weir and Cockerham 1984), $(\delta\mu)^2$ (Goldstein et al. 1995), number of alleles, gene diversity, allele size variance and shared allele distance (Chakraborty and Jin 1993)). These 5,000 simulated data sets were then used to estimate posterior probabilities and distributions, for each alternative scenario, respectively. The generalized stepwise model of mutation was applied with default values (GSM; Fu and Chakraborty 1998; Estoup et al. 2002). The 2006 field counting (TAWIRI 2008) was used as basis for the census population sizes. SNP and MGR are geographically continuous

and, as our analysis indicate, genetically non-differentiated, and were in the analysis combined to 118,194 individuals. Generally the effective size of populations, *Ne*, is much smaller than the census size because of age structure, uneven sex-ratios, and variation in family size and temporal fluctuations in population size (Grant and Grant 1992; Frankham 1995). In Buffalo, *Ne* has been reported to be from 10 to 30% of the census size (O'Ryan et al. 1998; Van Hooft et al. 2003). Priors with normal distributions and means of 10 and 30% of the census size were therefore used for present effective sizes. Priors for ancient effective sizes were set large and wide (10,000–100,000).

Results

Among the 493 sequenced mtDNA base pairs (bp), insertions and deletions were identified at four sites which were omitted in further analysis. Among the remaining 489 bp, a total number of 28 haplotypes were identified through substitutions at 75 positions. At only two positions more than two different nucleotides were observed. The level of genetic variability showed a similar gene and nucleotide diversity among the three sampling areas (Table 2), while the number of haplotypes appeared to be lower in NCA. Pair-wise comparisons of F_{ST} (Table 3) indicated low and non-significant differences between the SNP and MGR but significant differences between NCA and each of the two other populations (both $P < 0.001$). The haplotype sharing was generally low. Among the 39 haplotype observed, eight were shared between SNP and MGR while only one and two haplotype were shared between NCA and each of SNP and MGR, respectively. Most haplotype were rare, and as much as 18 haplotype occurred in only one or two copies while only two haplotype occurred in five or more copies. With such a high level of variation, haplotype frequency estimates become very inaccurate and are thus of limited value in analysing population structure.

All microsatellites were polymorphic in the investigated areas, with allele numbers ranging from four to 14 (Table 4). Micro-Checker gave no evidence of scoring errors, large allele dropout or null alleles in any locus/population combinations except for BM4208 in MGR where a homozygote excess was indicated. Neither of the areas showed any deviations from HWE after sequential Bonferroni corrections. Overall, the level of genetic variation was high in all sampled areas with the mean number of alleles across loci ranging from 6.0 in NCA to 7.9 in SNP and MGR (Table 2). The mean expected heterozygosity (H_E) ranged from 0.68 in NCA to 0.71 and 0.73 in SNP and MGR, respectively.

The number of private alleles ranged from four in NCA to 14 in SNP and 17 in MGR. No single individual had a pronounced number of private alleles (three at most), nor did any markers (Table 4). Among a total of 142 observed alleles, 78 were observed in all three samples and 82 alleles had frequencies >0.05 when pooled across the three samples. Both the average number of alleles, allelic richness, and heterozygosity were similar in SNP and MGR but relatively lower in NCA (Table 2). A Student *t*-test revealed that the mean number of alleles was significantly lower in NCA than in MGR ($t = 2.160$, $P = 0.040$). A similar trend was also seen when NCA was compared with SNP, although the difference was not significant ($t = 1.63$, $P = 0.114$). Furthermore, the mean number of alleles and allelic richness were both lower in NCA when compared with SNP and MGR pooled ($t = 2.917$, $P = 0.007$; $t = 3.335$, $P = 0.002$). Similarly, a χ^2-test of the total numbers of heterozygote's and homozygote's across all loci revealed a significant reduction of genetic diversity in NCA

Table 3 Genetic differentiation (F_{ST}) between African buffalo populations from three regions within the Serengeti Ecosystem

	SNP	NCA	MGR
SNP		0.090	0.005
NCA	0.039		0.152
MGR	0.005	0.035	

Figures below and above the diagonal are based on microsatellite and mtDNA variation, respectively

Table 2 Levels of genetic variability across fifteen microsatellite loci and a 489 bp mtDNA fragment in African buffalo in Serengeti Ecosystem regions

Region	*N*	MtDNA			Microsatellites		
		N_h	*h*	π	N_a	A_r	H_E
SNP	24	16	0.96 (0.03)	0.03 (0.02)	7.9 (2.8)	7.6 (2.5)	0.71 (0.21)
NCA	19	8	0.88 (0.05)	0.03 (0.02)	6.0 (3.0)	5.8 (2.6)	0.68 (0.14)
MGR	25	15	0.93 (0.03)	0.02 (0.01)	7.9 (2.7)	7.6 (2.4)	0.73 (0.15)

N gives number of individuals analysed. Included for the mtDNA are number of haplotypes (N_h) and haplotype (*h*) and nucleotide diversity (π), and for microsatellites are mean values for number of alleles (N_a), allelic richness (A_r) and expected heterozygosity (H_E). Estimates of standard deviations (SD) given in brackets

Table 4 Genetic diversity of microsatellites in African buffalo in Serengeti National Park (SNP), Ngorongoro Conservation Area (NCA) and Maswa game reserve (MGR), including number of alleles (N_a), allelic richness (A_r), number of private alleles (P_a), observed (H_o) and expected (H_E) heterozygosity for each microsatellite locus

Locus	SNP					NCA					MGR				
	N_a	A_r	P_a	H_o	H_E	N_a	A_r	P_a	H_o	H_E	N_a	A_r	P_a	H_o	H_E
BM1009	8	6.9	2	0.88	0.76	6	5.5		0.84	0.67	8	6.7	2	0.76	0.68
ETH3	4	3.6		0.50	0.52	3	2.9		0.37	0.56	7	5.7	3	0.56	0.63
ETH10	4	3.7	1	0.61	0.63	3	3.0		0.42	0.59	4	3.6	1	0.80	0.67
BM4208	2	1.9		0.09	0.09	2	2.0		0.26	0.42	4	3.8	2	0.15	0.31
BM6506	7	6.6	1	0.68	0.78	4	4.0		0.53	0.73	6	5.5		0.68	0.74
SPS115	9	8.9		0.86	0.88	10	9.6	1	0.84	0.84	9	8.8	1	0.88	0.86
BM2113	6	5.4		0.52	0.70	5	4.8		0.44	0.56	4	3.9		0.75	0.64
BM4107	9	7.8	1	0.91	0.81	6	5.8		0.83	0.69	8	7.8		0.96	0.84
BMC3224	11	10.3		0.78	0.88	10	9.7		1.00	0.88	12	10.9	1	1.00	0.90
BM804	12	10.9		0.87	0.89	11	10.6	2	0.84	0.88	11	10.2		0.92	0.88
BM1818	10	8.5	2	0.79	0.83	7	6.7	1	0.74	0.71	8	7.1		0.79	0.77
OarFCB48	10	8.6	1	0.88	0.84	7	6.7		0.79	0.78	11	9.9	1	0.87	0.87
BM1824	8	7.2	1	0.95	0.82	6	5.8		0.89	0.80	11	9.5	3	0.96	0.85
TGLA53	10	8.5	3	0.59	0.59	6	5.8		0.63	0.52	8	6.8	2	0.56	0.60
BM757	8	7.4	2	0.71	0.72	4	4.0		0.63	0.58	8	6.9	1	0.67	0.70

compared with the pooled SNP and MGR ($\chi^2 = 4.496$, $P = 0.034$).

Buffaloes from the three conservation areas were genetically differentiated according to Fisher's exact tests ($P < 0.0001$), with ten out of 15 loci showing significant differentiation. Analysis of each sample pair across loci revealed that NCA was significantly different from both SNP and MGR ($P < 0.001$), while the differences between SNP and MGR were insignificant ($P = 0.197$). The lower level of genetic differentiation between SNP and MGR was further illustrated by their substantially lower pair-wise F_{ST} value ($\theta = 0.005$) compared to that between NCA and each of the two others (SNP: $\theta = 0.039$; MGR $\theta = 0.035$) (Table 3). A similar trend was detected for D_{est}, with values of 0.069 and 0.042 for NCA-SNP and NCA-MGR compared to a value of 0.004 between SNP and MGR. No signals of recent bottlenecks were detected in any of the populations with the use of neither 20 nor 10% variation from the infinity model.

The STRUCTURE analyses provided support for the genetic differentiation indicated by the Fisher exact tests. With prior information on sampling locations (LOCPRIOR) there was a significant increase in mean likelihood when the number of genetic clusters was increased from one to two, after which no further subdivision was indicated (Fig. 2a). The Bayesian assignment revealed for all runs with $K = 2$ that all NCA individuals assign to one cluster while all SNP and MGR individuals assigned to the other, suggesting a low level of admixture (Fig. 2b). An r value of 0.1 suggests that information about sampling locations contributed substantially in inferring the dichotomy population structure. Without using the sample location information the STRUCTURE analyses did not uncover any significant genetic structure. Use of POPINFO with $K = 2$ indicated that no first-generation dispersers were among the sampled individuals, ($q > 0.8$ for all individuals, data not shown).

The most likely scenario according to the DIY ABC posterior probabilities was a demographic model with previous population reduction (*tm2*), subsequent expansion (*td1*) and a very recent split between the NCA and the pooled SNP/MGR (*ta*, scenario 4, Fig. 3, Table S1). In this scenario the divergence and founding of NCA was as recent as around 30 generations ago, while the posterior estimate of previous population reduction and subsequent expansion were around 6,400 and 1,600 generations old, respectively. The results were quite similar regardless of using a *Ne* of 30 or 10% of census population size (Fig. 3 and Fig. S1 (supplementary), respectively). The other less likely scenarios involved either a relatively recent divergence time between NCA and SNP/MGR (scenario 1, Fig. 3) or an origin of the NCA from an even more recent admixture event with an unsampled population (scenario 2, Fig. S1).

Discussion

The buffalo has been reported to harbour high levels of nucleotide diversity compared to the other species of large

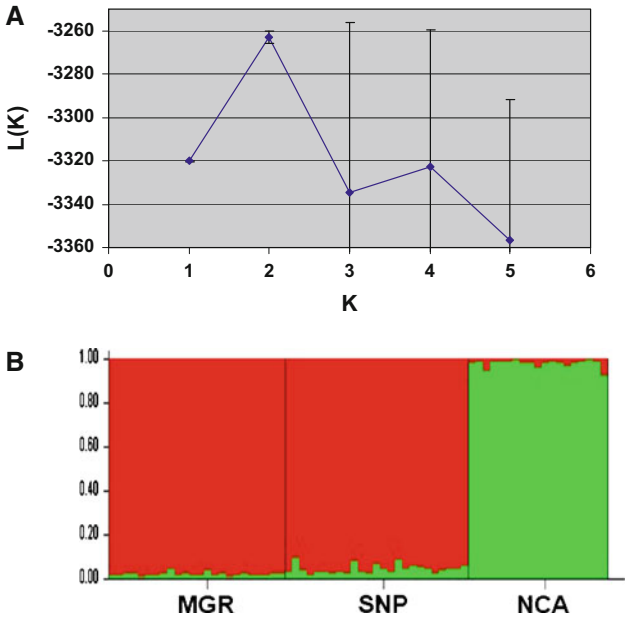


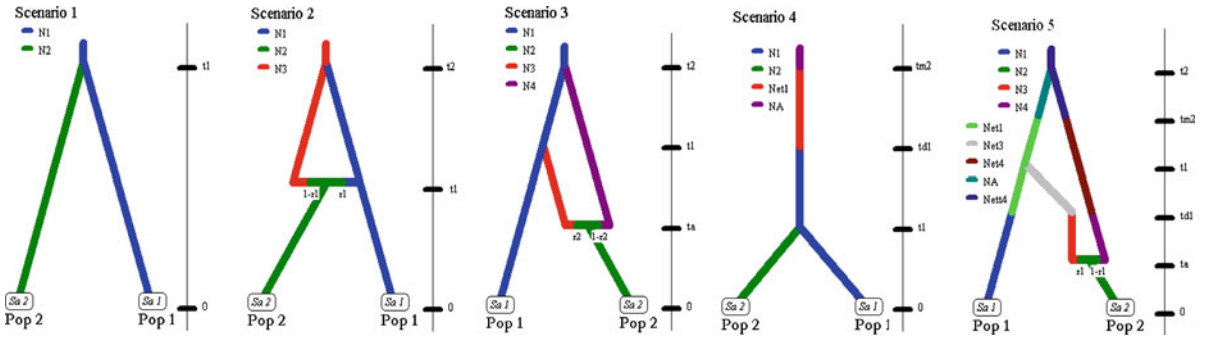
Fig. 2 Bayesian assignment of African buffaloes in the Serengeti Ecosystem. **a** gives mean posterior probability (Ln Pr($X|K$)) and standard deviation (*error bars*) over 10 runs for each of the K genetic clusters ($K \in [1, 6]$). **b** gives individual probability of assignment to each of two clusters ($K = 2$, different colors) among 68 buffalo sampled in the MGR, SNP and NCA

mammals in Africa (Simonsen et al. 1998). Accordingly, both our mtDNA and microsatellite data suggest a high level of within population variation similar to that reported for buffaloes across even larger areas in Africa (Simonsen et al. 1998; Van Hooft et al. 2002; Heller et al. 2008). However, to obtain accurate estimates of the allele frequencies of highly variable markers may be challenging with regard to sample size, particularly within protected areas where all handling of wildlife initially is forbidden. This is exemplified by the 28 observed haplotypes of mtDNA which are distributed across only 68 samples in this study, limiting the accuracy of estimates of frequencies and genetic differentiation. The relatively high numbers of private microsatellite alleles within the sampled populations probably reflect a similar effect. However, most microsatellite alleles were present in all three samples and had frequencies higher than 0.05 across the material, making the present analyses of genetic structure reasonably appropriate and reliable.

In this study, both the applied mtDNA and microsatellites demonstrate genetic differentiation and structure between buffalo in the different management areas of the Serengeti ecosystem, with the NCA being significantly differentiated from the SNP and MGR. The observed structure, particularly for the microsatellites, is not very strong, as illustrated by the absence of any clear structure in the STRUCTURE analyses without priors on sampling locations, although this may have been influenced by the relatively low sample sizes and the highly variable markers. However, the lower level of genetic variation in NCA, the F_{ST} statistics, *Dest* estimator and the STRUCTURE analyses with priors on sampling locations suggested the same dichotomy in genetic structure between buffalo in the Serengeti. The differentiation between the NCA and the two others sampling areas appear substantial higher for mtDNA ($F_{ST} = 0.152$) than for microsatellites ($F_{ST} = 0.039$). On one hand, mtDNA is haploid and maternally inherited, it is thus effectively a quarter of the population size compared to diploid nuclear DNA, and consequently more sensitive to demographic events like bottlenecks and genetic drift. However, if dispersal has been male biased, the reduced gene flow we report may also have contributed to the relatively higher mtDNA differentiation.

We did not detect any signals of recent bottlenecks despite records of catastrophic droughts and disease epidemics that resulted in population crashes of the African buffalo in many areas in the late 1800s (Sinclair 1977; O'Ryan et al. 1998; Simonsen et al. 1998; Wenink et al. 1998; Van Hooft et al. 2000). Similar results have been reported for the buffalo in both the nearby Masai Mara population (Kenya) and the Queen Elizabeth population (Uganda) in a microsatellite study by Heller et al. (2008), who suggested that the late 1800s Rinderpest plague had little effect on the genetic variation and structure of African buffalo. Overall, many species in this region display absence of genetic signatures from recent population bottlenecks, as in the black rhinoceros, *Diceros bicornis* (Harley et al. 2005), waterbuck, *Kobus ellipsiprymnus* (Lorenzen et al. 2006a), impala, *Aepycerus melampus* (Lorenzen et al. 2006b) and elephant, *Loxodonta africana* (Okello et al. 2008). Generally, a reduction in effective population size following a bottleneck is correlated with a decrease in allelic diversity and later on heterozygosity (Wright 1931), a phenomenon we did not observe in our study. Rather, the general level of genetic variation in the Serengeti buffalo was high, which may indicate that the population has been large historically. However, the African buffalo is suggested to be capable of maintaining non-critical population sizes, high growth rates and good dispersal capabilities, enabling re-colonisation of available habitat and exchange of genetic material across large distances following adverse conditions (Heller et al. 2010). This could explain why no signals of previous bottlenecks or any old genetic structure have been detected.

Our DIY ABC analyses thus suggests that the most likely scenario to explain the genetic structure of Buffalo in the Serengeti Ecosystem is a demographic model with population decline and subsequent expansion before a very recent divergence of the NCA from SNP/MGA (scenario 4 in Fig 3). The recent estimates of divergence or admixture events explaining the origin of the NCA in the other



PP	0.07 (0.04 - 0.09)	0.02 (0.01 – 0.03)	0.01 (0.01 - 0.01)	**0.90 (0.88 - 0.93)**	0.01 (0.01 - 0.01)
*N*1	22660 (7654 - 44871)	27762 (8928 - 46885)	34839 (14687 - 50126)	**36841 (11877 - 55966)**	38635 (14428 - 56261)
*N*2	617 (291 - 903)	582 (255 - 890)	551 (227 - 880)	**626 (304 - 905)**	626 (299 - 900)
Net1				**3035 (481 – 9143)**	2895 (203 - 9333)
NA				**53896 (10671 - 97441)**	66535 (11237 - 98745)
ta			47 (9 - 133)		75 (18 - 223)
t1	57 (17 - 146)	57 (14 - 142)	443 (105 - 2711)	**62 (20 - 141)**	555 (108 - 3871)
td1				**1617 (104 - 7272)**	3846 (549 - 8664)
tm2				**6435 (1641 - 9849)**	4108 (171 - 8893)
t2		2914 (360 - 9361)	2887 (539 - 9276)		7381 (2764 - 9859)
*r*1		0.82 (0.12 - 0.99)	0.80 (0.10 - 0.99)		0.92 (0.36 - 0.99)
μ	1.4×10^{-4} ($(1.0\text{-}4.0)\times10^{-4}$)	1.2×10^{-4} ($(1.0\text{-}3.5)\times10^{-4}$)	1.1×10^{-4} ($(1.0\text{-}2.4)\times10^{-4}$)	2.5×10^{-4} ($(1.1\text{-}8.3)\times10^{-4}$)	1.8×10^{-4} ($(1.1\text{-}6.1)\times10^{-4}$)

Fig. 3 Scenarios explored with DIY ABC to explain observed genetic structure in the Serengeti buffalo population. Pop 1 consist of SNP & MGA ($n = 49$) and pop 2 is NCA ($n = 19$). Applied present effective population sizes are 30% of estimated census sizes. Posterior probabilities of each scenario (PP (95% quartile)) after logistic regression on the 1% simulated data most similar to the observed data, and median (95% CI) estimated time in numbers of generations since divergence between NCA and SNP/MGA (*t1*), and admixture (*ta*) and divergence (*t2*) with an unsampled population outside the study area

explored scenarios supports this, suggesting that the lower level of genetic variation in the NCA population stems from a founder event rather than an old bottleneck event. The estimated onsets of a 90% decline around 6,400 generations ago and a subsequent tenfold expansion at about 1,600 generations ago (Fig. 3), are in agreement with the previous results of Heller et al. (2008), suggesting a 75–98% reduction 3–7,000 years ago during mid Holocene. However, our estimates could be biased by sample size. Moreover, the DIY ABC algorithm assumes no migration between the events of scenarios (Cornuet et al. 2008). Even though the STRUCTURE analyses with POPINFO support such an assumption, the sample sizes may be too low to detect first-generation dispersers if dispersal rates are low. A reduction of genetic structure through gene flow and introduction of new gene copies may thus have introduced a bias on the divergence estimates, potentially involving estimates that are closer to present than actual divergence time. Nonetheless, the genetic differentiation of NCA is real and most probably of a recent origin. Considering the lack of genetic signatures also in many of the other regional species (Harley et al. 2005; Lorenzen et al. 2006a, b; Okello et al. 2008), this may suggest that it is not until now that fragmentation and isolation have become grave enough to have a genetic impact in the region. This highlights how rapidly fragmentation can lead to isolation and genetic differentiation, even in such a highly genetically variable and numerous key species as the buffalo, illustrating the real risk of fragmentation in the region.

Much has changed over the last decades in NCA, including large increases in the human populations of both Maasai pastoralists and other human immigrants with interests in agriculture (Kijazi et al. 1997; Galvin et al. 2004; Boone et al. 2006). Diseases and drought, among other factors, have resulted in decreasing livestock, which are the sole reliant source of food and income to pastoralists (NCAA 1999; Lynn 2000; Fyumagwa et al. 2007). Therefore, land-use has become intensified in recent years as the Maasai pastoralists are forced to become more sedentary and practice agriculture in response to food scarcity (McCabe et al. 1997b; Neumann 1998; Boone et al. 2006). Unfortunately, much of the present agriculture is taking place on fertile areas that otherwise would have provided a good habitat for buffaloes and that could have represented a corridor important for genetic exchange between the NCA and other Serengeti management areas (Fig. 1c). The relatively recent separation of the buffaloes

in NCA from SNP and MGR could therefore be attributed to human settlements, infrastructure and agriculture around the Crater highlands potentially blocking natural dispersal corridors. Besides anthropogenic factors, Estes et al. (2006) outlined that also the recent climatic changes may have influenced wildlife in the area, and Van Hooft et al. (2000) stated that migration among buffalo populations in East Africa could be limited by the distribution of dry habitats. The progressively drier environment to the eastern terminus of the Serengeti plains may thus have made this area unsuitable for large wild ungulates like the buffaloes and could be acting as a barrier against their dispersal.

In small populations, harvesting may cause significant genetic drift as age structures are altered, sex-ratios skewed and effective sizes decreased, but to assess these effects requires a correct assessment of population genetic structure, which acts as an enforcing factor (Frankham 1996; Coltman 2008). Harvest may in MGR have involved genetic drift and differentiation, as compared to the protected SNP. Absence of any genetic change in MGR, despite its harvesting regime probably reflects a large effective population size. Moreover, the hunting regime in the area is trophy based and targets mainly reproductively senescent individuals that already have undergone most of their successful reproduction with subsequently small effects on effective population sizes. There are no distinct physical barriers between MGR and SNP, except for a small seasonal river in the southern part of the borderline. This, together with the high ability of African buffalo to migrate or switch between herds and populations (Estes 1991; Prins 1996; Halley et al. 2002; Cross et al. 2005; Korte 2009) may explain why MGR and SNP appear as one large population in which genetic effects of hunting are of minor importance. The present study points therefore towards the importance of maintaining buffaloes in large populations or in populations with extensive gene flow to maintain historical levels of genetic diversity, a conservation effort which may become important also in other African savannah ungulates.

Conclusions and conservation implications

We have shown that within the Serengeti ecosystem, buffalo in the NCA are genetically different from the buffalo in the SNP and MGR management areas, and that the differentiation probably is of recent origin. This may indicate that recently onset factors in this area are restricting the gene flow between NCA and the other parts of the Serengeti Ecosystem. It also demonstrates and highlights the importance of genetic studies in actual conservation planning and management. Although African buffalo are highly capable of maintaining non-critical population sizes, dispersal and re-colonization of different habitats, we believe that this may not be the case when anthropogenic developments reach the extent of blocking and depleting potential dispersal corridors and habitats between protected areas. We therefore acknowledge the need for, and encourage, the facilitation of gene flow between protected areas to prevent further genetic differentiation.

In essence, both humans and buffaloes compete for the same land. While humans need land for agriculture and other developments, buffaloes are an indigenous part of the original habitat. However, Tanzania as well as other countries in the region is facing a rapid human population growth and with the anticipated associated increases in anthropogenic activities, wildlife habitat exploitation and fragmentation, both the buffalo and other wildlife may soon be threatened in the region. The potential effects are illustrated by this paper, and as further fragmentation may be critical, this problem will obviously pose a conservation challenge in the future.

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