POPULATION GENETICS

OF THE AFRICAN BUFFALO

FROM ECOLOGY TO EVOLUTION

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Pim van Hooft

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Population genetics of the African buffalo: from ecology to evolution

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Cover photo: female African buffalo in Lake Nakuru National Park, Kenya (photo by Pim van Hooft, 1998)

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Abstract: The African buffalo (*Syncerus caffer*) is one of the most numerous mammals of sub-Saharan Africa. Since the end of the 19th it has been affected by rinderpest epidemics and by habitat fragmentation due to increasing urbanisation and cultivation. In this thesis the genetic diversity of the African buffalo is investigated. A better knowledge of the population genetics of the African buffalo is necessary for an effective management and protection of this species. The goals of this thesis were to study the effects of rinderpest and habitat fragmentation on the genetic diversity of buffalo populations, as well as the evolutionary history and herding behaviour of the African buffalo. Three types of genetic markers are used: autosomal microsatellites, Y-chromosomal microsatellites and mitochondrial DNA (mtDNA) D-loop sequences. Compared to other mammals buffalo populations show a high genetic diversity and little genetic differentiation for autosomal microsatellites and mtDNA. From the high genetic differentiation it is concluded that rinderpest epidemics and habitat fragmentation had little effect on genetic diversity. The little population differentiation is attributed to a Pleistocene population expansion. Among herds significant differentiation is observed at mtDNA, while among neighbouring populations there are indications that there is relatively little genetic substructuring at autosomal microsatellites. These data support the field observations of male biased dispersal between herds. Buffalo from central and south-western Africa form a separate genetic lineage from those in eastern and southern Africa. Genetic diversity of the first lineage is relatively high for all three types of markers. It is suggested that this is due to fragmentation of wet habitats as a result of climatic changes in the evolutionary past. Finally, it is discussed how the results of this thesis can be used for a more effective management and protection of the African buffalo.

Contents

General introduction

The African buffalo

Historically, the African buffalo (*Syncerus caffer*) inhabits nearly the whole of sub-Saharan Africa (Prins 1996 p. 240). It is primarily found on savannas, but can also be observed in many other habitats, including forest and dry bush, in fact wherever there is permanent water available. Buffalo comprises up to 35% of the large herbivore biomass, which makes it one of the most dominant species in Africa together with elephant (*Loxodonta africana*) and wildebeest (*Connochaetes taurinus*) (Drent and Prins 1987, Prins and Reitsma 1989). In undisturbed sites African buffalo can reach a population density of up to 20 individuals per km^2 (Prins and Douglas-Hamilton 1990, Prins 1996 p. 1-4). Three subspecies of African buffalo are recognised (Prins 1996 p. 242-243): Cape buffalo on the savannas of eastern and southern Africa (*S. c. caffer*), Forest buffalo in the rain forests of western and central Africa (*S .c. nanus*) and West African buffalo from the Sahel-Sudan savannas (*S. c. brachyceros*) (Figure 1.1).

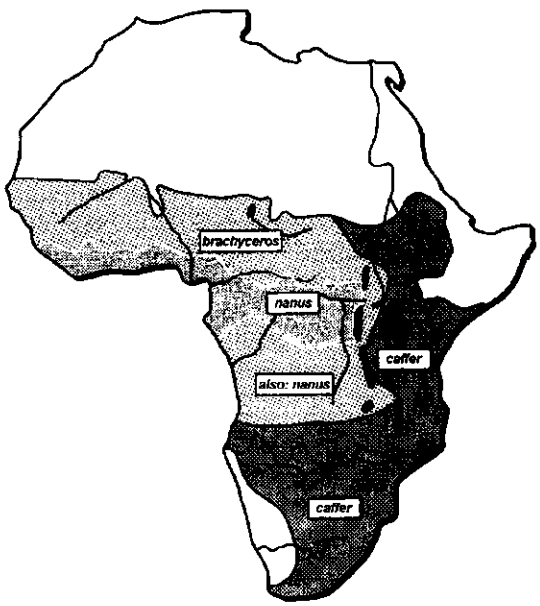

Figure 1.1: Map of Africa showing the distribution of the presently recognised (Haltenorth and Diller 1979) subspecies of African buffalo. The taxonomic position of buffalo from northern Angola and southern Zaire is unclear: they are often considered members of the subspecies nanus (figure from Prins 1996).

Especially Forest buffalo and Cape buffalo are morphologically quite different. Forest buffalo are small animals with a shoulder height of 100-110 cm that weigh maximally some 200 kg, while Cape buffalo have a shoulder height of 150-160 cm and bulls weigh on average 700 kg (Figure 1.2 and 1.3).

Figure 1.2: *Female Forest buffalo.*

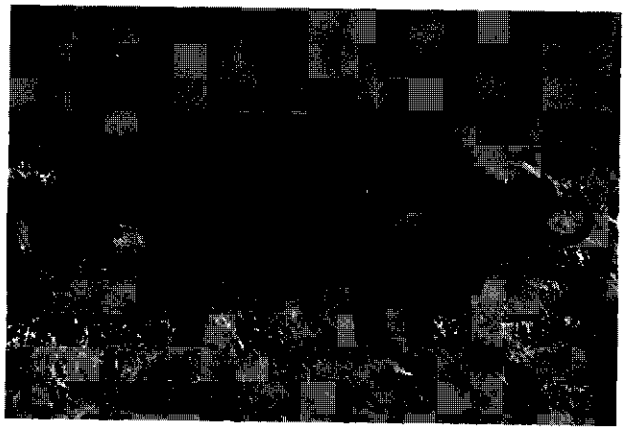

Figure 1.3: Male Cape buffalo.

Various ecological studies have been conducted on Cape buffalo (Grimsdell 1969, Sinclair 1977, Prins 1996). They live in herds that vary in size between 12 and 1600 individuals (Sinclair 1977 p. 119, Prins 1996 p. 72-77). Herds have a fixed home range, which varies in size between 5 and 100 km^2 depending on herd size (Sinclair 1977 p. 98, Prins 1996 p. 219). Herds can be identified over many years

as discrete entities (Prins 1996 p. 77). Migration between herds seems to be predominantly by adult males, as females and subadults have never been observed to leave their native herd (philopatric behaviour, Sinclair 1977 p. 132-142, Kingdon 1982, Prins 1996 p. 72). The generation time of Cape buffalo is 7.5 years (O'Ryan et al. 1998).

Rinderpest epidemics and habitat fragmentation

In Africa at the end of the 19th century, cattle and many wild ungulates were victim of a continent wide rinderpest epidemic, causing catastrophic declines in population numbers. Rinderpest is caused by a morbillivirus, a member of a group of enveloped viruses forming a separate genus within the family *Paramyxoviridae* (Barrett and Rossiter 2000). This genus further includes peste des petits ruminants virus, canine distemper virus and human measles virus. Rinderpest was introduced into Africa probably by the importation of Zebu cattle from India for the Italian armies in 1889 (Plowright 1982). It spread rapidly through the continent, only to be temporarily stopped by the Zambezi River in 1893. In 1896 it jumped the river and reached the Cape in 1897 (Figure 1.4).

Rinderpest is the most lethal and potentially dangerous infectious disease which affects ungulates (Plowright 1982). During the epidemic, mortality in wild ungulates was so high that the tsetse fly was severely reduced or even died out in some areas (Ford 1971). In Kruger National Park (South Africa) in 1902 only 20 buffalo (Stevenson-Hamilton 1911) were observed and in the Umfolozi-Hluhluwe Complex (South Africa) in 1929 only 75 buffalo (O'Ryan et al. 1998) were observed. It has been estimated that between 90 and 95% of all buffalo in Africa died (estimates from Kenya, Zambia and South Africa; Plowright 1982, Shigesada and Kawasaki 1997, O'Ryan et al. 1998, Barrett and Rossiter 2000). In general, buffalo were again numerous within only 30 years. However, in some areas recurrent outbreaks of rinderpest, though less severe than the first one, kept buffalo at low numbers until the 1960s (Sinclair 1977 p. 5-7, Plowright 1982, Prins 1996 p. 122-127, Simonsen et al. 1998).

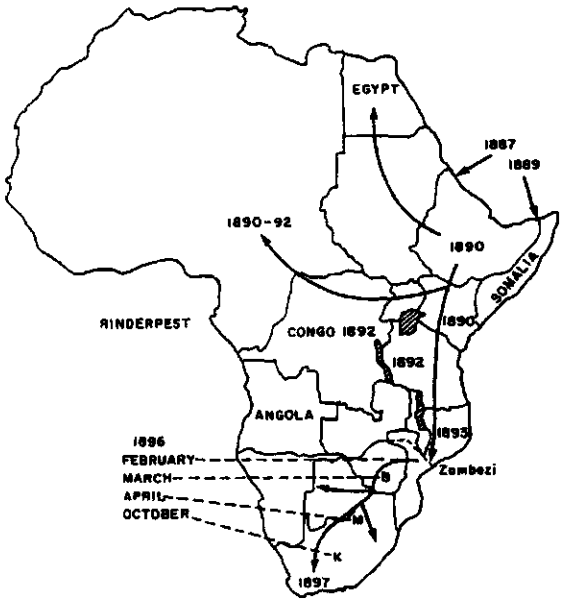

Figure 1.4: Course of the great rinderpest epidemic in Africa at the end of the 19th century (figure from Plowright 1982).

Especially in the last few decades, increasing urbanisation and cultivation has caused a severe decrease and fragmentation of the buffalo's range (Figure 1.5). As a consequence, nowadays most populations are restricted to protected areas and only about 15% of the original habitat is still inhabited. Of the more than 3 million buffalo that roamed the continent in the 19th century only some 400,000 were left at the end of last century (Lessard et al. 1990).

Research goals

The combined effects of rinderpest epidemics and habitat fragmentation on the genetic diversity of African buffalo populations might threaten their continued existence. Both disturbances can result in significant decreases of the effective size and genetic diversity of these populations. A decreased genetic diversity can in the long run limit the ability of buffalo populations to adapt to future environmental changes and cause inbreeding depression. Furthermore, fragmented populations are more prone to stochastic problems. First, there is demographic stochasticity, which is a zero population size for one or both sexes by chance fluctuations in population size. Second, fluctuations in population size due

to fluctuating local environments are larger in small than in large populations (Van Noordwijk 1994), as in the latter case only part of the population is affected. This reduces the effective population size of fragmented populations even more.

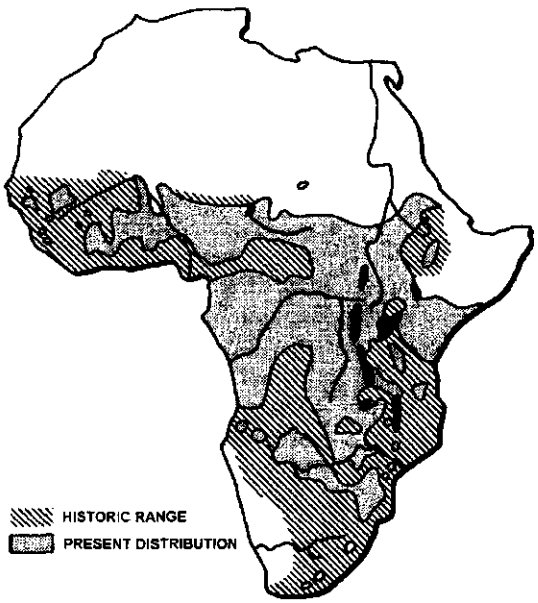

Figure 1.5: Map of Africa showing the present and historical generalised distribution of African buffalo (figure from Prins 1996).

A more intensive management of buffalo populations might become necessary to guarantee their continued existence. Exploring the genetic diversity of buffalo populations can reveal demographic parameters that are essential for an effective management. In that respect population genetics can provide relevant information about the appropriate geographic scale for management, variation in population size and migration both in space and time, mating system, herding behaviour and risk of inbreeding. Furthermore, evolutionary distinct populations, subspecies or Evolutionary Significant Units (Moritz 1994, Crozier 1997), may be identified that require separate management and protection efforts.

In this thesis the genetic diversity of various buffalo populations throughout Africa is analysed, with emphasis on Cape buffalo. The initial goal was to study the effects of rinderpest epidemics and habitat fragmentation on genetic diversity. Later on the

objective shifted towards a comprehensive analysis of the genetic structure of buffalo populations, in order to get more insight into their evolutionary history and to study the effects of herding behaviour on genetic diversity.

Population genetics

This project took place in the field of population genetics, which is the study of how Mendel's laws and other genetic principles apply to entire populations. Population genetics can reveal information about various demographic parameters, both on an ecological and evolutionary timescale. The genetic diversity of populations is generally quantified by allele frequencies at one or more loci. More recently since the invention of the PCR amplification technique, genetic diversity is also increasingly being described by the DNA sequences of the different alleles, which in this context are also known as haplotypes. The different nucleotide sites of a haplotype can be treated as individual loci with their own allelic variation (the four nucleotides A, C, T and G) (Hudson et al. 1992). Allele frequencies are influenced by four factors: genetic drift, mutation, selection and gene flow (Hartl and Clark 1989, ch. 2, 3, 4 and 6). Genetic drift is the random variation in allele frequencies over the course of generations. In the long run genetic drift results in the loss of alleles, as sometimes by chance an allele is not passed on to the next generation. The amount of genetic drift is inversely related to the effective population size, which is the number of individuals in a theoretically ideal population having the same magnitude of genetic drift as the actual population (Hartl and Clark 1989 p. 82). By introducing new alleles into populations, mutation works in the opposite direction as genetic drift. The number of alleles in a population is in equilibrium when genetic drift and mutation cancel each other out (mutation-drift equilibrium). Allele frequencies can also change directionally under the influence of selection. However, in population genetics one generally chooses genetic markers that are assumed not to be under strong selection pressure. When populations get separated their allele frequencies will differentiate over time by the combined action of population specific mutation, genetic drift and selection. This is counteracted by the exchange of genetic material between populations by migrants, a process known as gene flow (Neigel 1997, Bossart and Prowell 19918). Over the course of generations an equilibrium between gene flow and local genetic drift and mutation (and selection) can develop, which is characterised by the fraction of the total genetic variation that is between populations (Wright's F-statistics; Hartl and Clark 1989 p. 293-301). When there is no gene flow after population divergence the

amount of genetic differentiation between populations is proportional to the divergence time.

Since the initial publication of the PCR amplification technique in 1988 DNA analyses seem to be rapidly gaining ground in the field of population biology (Cavalli-Sforza 1998, Silva and Russo 2000). Before that time, analysis of the genotype was usually indirect and limited to a small number of markers. These were based on the study of gene products, almost all protein polymorphisms (allozymes), and only a few hundred were known. Analysing genetic polymorphism at the level of DNA has many advantages. These are, among others, a higher information content, availability of a greater number of genetic markers, availability of different types of polymorphisms (replacement, loss or addition of one nucleotide and insertions/deletions of longer segments), possibility to analyse male and female genetic diversity separately (Y-chromosomal markers and mitochondrial DNA) and relative ease of automation. New statistical methods have been developed to take advantage of the high information content of DNA polymorphisms (Luikart and England 1999, Sunnucks 2000). For example, methods are now available to derive both recent and ancient population bottlenecks and expansions from current genetic diversity (Slatkin and Hudson 1991, Rogers and Harpending 1992, Rogers 1995, Cornuet and Luikart 1996, Luikart et al. 1998). Another big advantage of DNA techniques is that very little sample material is needed that may even be degraded to some extent, as DNA can be amplified by PCR. Non-invasive samples may be used like shed hairs and feathers, faeces, urine and eggshells (Kohn and Wayne 1997, Taberlet et al. 1999), without the need to capture or even observe the animal under study. DNA may also be extracted from historical samples like mounted animals and fossils, opening up the possibility to study population and species history directly, both on an ecological and evolutionary time scale. Recently, the analysis of Neanderthal (*Homo sapiens neanderthalensis*) DNA (Krings et al. 1997, 1999, Ovchinnikov et al. 2000), for example, has been a major breakthrough in the study of fossil DNA.

Microsatellites and mitochondrial DNA

In this research project three types of genetic markers were used: autosomal microsatellites, Y-chromosomal microsatellites and mitochondrial D-loop hypervariable region I (HVI) sequences. Analysis of these markers was automated to a large extent by the use of fluorescent labelling. With this technique nucleotides

with fluorescent dye are added during the PCR reaction and subsequently incorporated in the amplified DNA fragments, whose signal can than directly be read by a computer.

Microsatellites are tandem repeats of sequence units distributed across the whole eukaryotic genome (Bruford and Wayne 1993). The repeat units can vary from one to five nucleotides in length. Microsatellites usually mutate by gaining or losing one or two repeat units, the so-called stepwise model (Goldstein and Pollock 1997). Microsatellites are often highly polymorphic due to their high mutation rate (on average 10^{-4}/generation for dinucleotide repeats, Crawford and Cuthbertson 1996, Schug et al. 1997, Lehmann et al. 1998). Their short length makes them very suitable for PCR amplification. Polymorphism is detected by separating the amplified fragments by length on high resolution polyacrylamide gels. A big advantage of microsatellites is that the repeat-flanking sequences are often conserved between related species, allowing cross-species PCR amplification without the need to develop species specific markers (Schlötterer et al. 1991, Kemp et al. 1995, Moore et al. 1995, Pépin et al. 1995, Primmer et al. 1996). Because of their high polymorphism and relative ease of analysis, microsatellites are used widely in genetic mapping, DNA forensic studies, parentage and relatedness testing, population genetic studies, and in a variety of other applications. Currently, about 8% of the scientific papers published in population genetics use microsatellites (Silva and Russo 2000).

Mitochondrial DNA (mtDNA) plays an important role as genetic marker in population genetic studies (Moritz 1994, Cavalli-Sforza 1998). MtDNA is a circular molecule of some 17,000 bp in length, which is present in many copies in the cytoplasm of cells. The complete nucleotide sequence was first determined in humans (*Homo sapiens*) (Anderson et al. 1981). Simple sequence organisation, maternal inheritance and absence of recombination make mtDNA an ideal marker for tracing maternal lineages. The relatively rapid mutation rate of 1 to 4% substitutions per million years (Wilson et al. 1985, Hoelzer et al. 1998) allows discrimination of recently diverged lineages and populations. The most variable part of mtDNA is the D-loop hypervariable region I (HVI) with a mutation rate in mammals of 3% (horse) to 39% (European bison) substitutions per million years (Bradley et al. 1996, Mannen et al. 1998, Parsons and Holland 1998, Slade et al. 1998, Burzynska et al. 1999). Because recombination between mtDNA molecules occurs rarely or not at all (Wallis 1999 2000, Hey 2000), mtDNA is in essence a set

of completely linked markers (one locus or haplotype) which permits clear definition of maternal genealogies and excellent discrimination between common ancestry and convergence (Harrison 1989). On the other hand however, because the whole mtDNA is only one locus stochastic events and selection will influence the frequency of entire mtDNA genotypes.

Outline of the thesis

This thesis investigates several aspects of the genetic diversity of African buffalo populations. **Chapter 2** describes the testing of a large number of bovine autosomal microsatellites for PCR amplification in African buffalo. It is shown that a large fraction of these microsatellites can be amplified, which also show a high level of polymorphism. In **Chapter 3** the genetic diversity of different African buffalo populations throughout Africa is analysed by using a small subset of microsatellites. Conclusions are made not only about the effects of rinderpest epidemics and habitat fragmentation, but also about historical population sizes and gene flow, and the relationship between different subspecies of buffalo. Most surprisingly, genetic diversity in African buffalo appears to be quite high. In **Chapter 4** a population genetic and phylogeographic analysis of African buffalo is performed using mtDNA HVI sequences and an Y-chromosomal microsatellite. Many insights can be inferred from the data, the most important being the evolutionary history of the subspecies Cape buffalo. There are indications that this subspecies only recently colonised the savannas of eastern and southern Africa. **Chapter 5** investigates the genetic structure of buffalo herds in two populations, using both mtDNA HVI sequences and autosomal microsatellites. The analyses indicate differences in female and male migration behaviour, that were not detected in earlier genetic studies performed at the population level. Finally, in **Chapter 6** results from this thesis and possible implications for the management of buffalo populations are summarised and discussed. It is shown that population genetic information can give a valuable contribution to the management of buffalo populations.

Applicability of bovine microsatellite markers for population genetic studies on African buffalo (*Syncerus caffer*)

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Abstract

The applicability of bovine autosomal microsatellite markers for population genetic studies on African buffalo was investigated. A total of 168 microsatellite markers were tested for PCR amplification on a test panel of seven African buffalo. Amplification was observed for 139 markers (83%), and 101 markers were studied further with 91 (90%) being polymorphic. The mean number of alleles per marker was 5.0 (SE = 0.2) and the mean heterozygosity per marker was 0.61 (SE = 0.03). Considering the overall high level of polymorphism, it was concluded that most bovine microsatellite markers are applicable in African buffalo.

Introduction

African buffalo (*Syncerus caffer*) has a wide geographical distribution, historically inhabiting nearly the whole of sub-Saharan Africa. In many localities it is present at high population densities. Since 1889 buffalo has suffered from rinderpest epidemics and habitat fragmentation (Sinclair 1977, Plowright 1982, Scott 1990, Prins 1996, Shigesada and Kawasaki 1997). As a consequence population numbers have declined and the distribution of most populations is at present restricted to protected areas.

Studying the genetic status of African buffalo populations can make a major contribution to an effective long-term management of this species, by giving new insights into the population dynamics and the effects of population bottlenecks and habitat fragmentation on genetic diversity.

Due to their high polymorphism, microsatellites which consist of tandemly repeated short DNA sequences, have proved to be sensitive markers for population genetics studies (Bruford and Wayne 1993, Avise and Hamrick 1996, Smith and Wayne 1996). Several studies have shown that repeat-flanking sequences of microsatellite markers are often conserved between related species, allowing cross-species amplification (Schlötterer et al. 1991, Moore et al. 1994, Kemp et al. 1995, Levin et al. 1995, Moore et al. 1995, Pépin et al. 1995, Rubinsztein et al. 1995, Liu et al. 1996, Primmer et al. 1996). However, it has also been shown that homologous markers tend to be shorter in related species, are less polymorphic and have a higher fraction of null alleles (Ellegren et al. 1995, Pemberton et al. 1995, Pépin et al. 1995, Rubinsztein et al. 1995, Brookfield 1996, Primmer et al. 1996, De Gortari et al. 1997).

Specific microsatellite markers for African buffalo have not been developed. However, a large number of microsatellite loci have now been characterised in domestic cattle (Barendse et al. 1997, Kappes et al. 1997). Two recent population genetic studies have used bovine microsatellite markers on African buffalo (O'Ryan et al. 1998, Simonsen et al. 1998). However, the number of markers studied was small and no data are available on the general applicability of cattle microsatellite markers for population genetic studies on African buffalo.

In this study, bovine autosomal microsatellite markers were tested for Polymerase Chain Reaction (PCR) amplification in African buffalo. In subsequent studies, a subset of these markers will be used to study the genetic diversity of African buffalo populations.

Materials and methods

Seven African buffalo from Serengeti National Park, Tanzania (two females, one male, one of unknown sex) and Kruger National Park, South Africa (two females, one male) were used as a test panel. A male F1 crossbreed between a male N'Dama (*Bos taurus*) and a female Kenyan Boran (*Bos indicus*) was used as a positive control. DNA from African buffalo was isolated from skin using Gene Pure Kit (Gentra Systems).

PCR was carried out on 20 ng of genomic DNA in a 10 µl reaction. All microsatellite markers were polymorphic and autosomal in cattle (references detailed in Table 2.1). Amplification was achieved over 30 cycles, with an annealing temperature of 55 ºC, unless otherwise indicated. PCR's were carried out using a PTC-200 PCR machine (MJ Research). One of each primer pair was fluorescently labelled.

Three marker sets were used.

(i) A set (n=87, (M)ILSTS markers) developed at ILRI. PCR amplification was carried out in 125 µM of dATP, dTTP, dCTP and dGTP, 10 mM TrisHCl pH 8.3, 50 mM KCl, 0.001% Gelatine, 0.025% Tween-20, 0.025% Nonidet-P40, 1.5 mM $MgCl_2$, 0.5 units Taq polymerase and 1.6 ng/µl of each primer (Kemp et al. 1995).

(ii) A set of commercially available markers (n=11, StockMarks™ Kit, Applied Biosystem, see Table 2.1). PCR amplification was carried out in 400 µM dATP, dTTP, dCTP and dGTP, 15 mM TrisHCl pH 8.3, 75 mM KCl, 0.0015% Gelatine, 0.038% Tween-20, 0.038% Nonidet-P40, 2.25 mM $MgCl_2$, 0.8 units Taq polymerase and 2 ng/µl of each primer. The annealing temperature was 53 ºC.

(iii) A set of markers (n=70) that was originally developed in different laboratories, but is currently used at ILRI and the Shirikawa Institute of Animal Genetics for QTL mapping projects. PCR amplification was carried out in 200 µM dATP, dTTP, dCTP

and dGTP, 10 mM Tris HCl pH 9.0, 50 mM KCl, 0.1% Triton X-100, 1.7 mM $MgCl_2$, 0.5 units Taq polymerase and 36 ng/µl of each primer.

The (M)ILSTS and StockMarks™ Kit markers were first analysed on an ethidium bromide stained 1.5% agarose gel. Markers that generated a specific product between 75 and 340 bp were selected for genotyping. The other markers were genotyped directly. Genotyping was performed on a 6% denaturing polyacrylamide gel (1 X TBE, pH 8.3) using a 373 automatic DNA sequencer (Applied Biosystems) with GENESCAN™ 350-TAMRA as internal standard. Data were collected, analysed and genotyped using ABI PRISM™ 373 collection (v. 1.1), GENESCAN™ analysis (v. 1.2.2) and GENOTYPER™ (v. 1.1) software (Applied Biosystems).

Results and discussion

A total of 168 autosomal bovine microsatellite markers were tested for amplification (Table 2.1). Twenty-nine markers (17%) failed to amplify in African buffalo. A total of 101 markers were further analysed for polymorphism. Ten markers (10%) were monomorphic. The mean number of alleles was 5.0 (SE = 0.2) for all markers, and 5.5 (SE = 0.2) for the polymorphic markers. The mean observed heterozygosity per marker was 0.61 (SE = 0.03) for all markers, and 0.67 (SE = 0.03) for the polymorphic markers. Amplification results and allelic diversity are detailed in Table 2.1.

Table 2.1: Characteristics of bovine autosomal microsatellite markers tested on a panel of seven African buffalo.

Marker	No. of alleles	Allelic range	Chrom. no. cattle[a]	Marker	No. of alleles	Allelic range	Chrom. no. cattle[a]
ABS10[1]	5	172-182	13	BM2113[6]	4	114-133	2
ABS13[1]	6[b]	194-210	18	BM3205[4]	5	205-216	1
ABS14[1]	4[b]	103-118	25	BM3517[4]	4	91-101	20
AF5[2]	4	140-148	25	BM4025[4]	10	125-155	16
AGLA293[3,c]	11	220-244	5	BM4028[4]	5	127-137	29
BM0304[4]	7	116-136	11	BM4307[4]	8	173-208	1
BM0719[4]	7	143-157	16	BM6026[4]	-		
BM0737[5]	8	114-142	25	BM7160[5]	2	182-184	7
BM1225[4]	9	237-274	20	BMS130[5]	-		
BM1819[4]	-			CSSM05[7]	6	112-128	23

Table 2.1: Continued.

Marker	No. of alleles	Allelic range	Chrom. no. cattle[a]	Marker	No. of alleles	Allelic range	Chrom. no. cattle[a]
CSSM18[7]	-			ILSTS019[16]	+		29
CSSM19[7]	8	141-155	1	ILSTS020[16]	-		
CSSM23[7]	9	222-246	24	ILSTS021[16]	6	107-127	18
CSSM43[7]	2[b]	235-245	27	ILSTS023[16]	1[b]	154	17
CYP21[8]	7	180-203	23	ILSTS024[16]	-		
DIK020[9]	11	166-205	10	ILSTS025[16]	6	114-126	2
DIK024[9]	4	229-241	1	ILSTS026[16]	9	142-163	2
DIK028[9,e]	5	251-263	11	ILSTS027[16]	4	189-202	15
DIK039[9,e]	2	201-203	19	ILSTS028[16]	10	142-200	11
DIK050[9]	6	116-133	7	ILSTS029[16]	3	144-154	3
DIK079[9]	5	232-242	7	ILSTS030[16]	3	157-163	2
DIK089[9,e]	-			ILSTS031[16]	5	250-260	24
DIK094[9,e]	7	235-248	25	ILSTS032[d]	+		?
DIK102[9,e]	5	224-243	15	ILSTS033[16]	6	139-167	12
DIK104[9,e]	4	172-178	1	ILSTS034[16]	5	156-174	5
ETH11[10]	5	197-203	16	ILSTS035[16]	1[b]	186	6
HAUT24[11]	1[b]	98	22	ILSTS036[16]	1	156	11
HEL05[12]	-			ILSTS037[16]	6	259-285	9
HEL09[12]	-			ILSTS038[16]	+		29
HEL10[12]	6	102-121	19	ILSTS039[16]	+		14
HUJ175[13]	7	137-156	22	ILSTS041[16]	4[b]	127-145	9
HUJ673[14]	-			ILSTS042[16]	-		
HUJII077[13]	5	190-212	3	ILSTS043[16]	2	145-153	29
IDVGA31[15]	7	208-224	18	ILSTS044[16]	5	144-163	3
IDVGA43[15]	5	164-174	28	ILSTS046[16]	+		25
ILSTS001[16]	-			ILSTS047[16]	+		?
ILSTS002[16]	-			ILSTS049[16]	3	135-151	11
ILSTS005[16]	7	173-195	10	ILSTS050[16]	9	120-160	2
ILSTS006[16]	-			ILSTS051[16]	3	164-168	?
ILSTS008[16]	3	169-178	14	ILSTS052[16]	5	146-160	?
ILSTS010[16]	+		12	ILSTS053[16]	4	132-138	10
ILSTS011[16]	3	254-264	14	ILSTS054[16]	1	128	21
ILSTS012[16]	-			ILSTS055[16]	+		?
ILSTS013[16]	3	102-106	9	ILSTS056[16]	-		
ILSTS014[16]	1	126	19	ILSTS057[16]	6	226-262	29
ILSTS015[16]	-			ILSTS058[16]	+		?
ILSTS018[17]	+		6	ILSTS061[16]	+		15

Table 2.1: Continued.

Marker	No. of alleles	Allelic range	Chrom. no. cattle[a]	Marker	No. of alleles	Allelic range	Chrom. no. cattle[a]
ILSTS062[16]	+		4	INRA084[19]	4[b]	98-104	9
ILSTS063[16]	+		29	INRA088[19]	1	105	3
ILSTS064[16]	+		3	INRA121[19]	2	110-112	18
ILSTS065[16]	+		24	INRA123[19]	4[b]	95-106	3
ILSTS066[16]	-			INRA128[19]	6	167-178	1
ILSTS067[16]	-			INRA162[20]	1[b]	161	11
ILSTS068[16]	+		20	INRA209[21]	6	177-193	12
ILSTS069[d]	+		?	IOBT250[22]	+	182-189	3
ILSTS070[16]	6	79-99	10	MGTG04B[3c]	8	117-144	4
ILSTS071[16]	+		11	MGTG07[3,c]	7[b]	279-305	23
ILSTS072[16]	+		20	MGTG13B[3]	-		
ILSTS073[16]	+		?	MILSTS76[16]	4	120-129	9
ILSTS074[16]	+		?	MILSTS77[16]	+		13
ILSTS081[16]	-			MILSTS78[16]	+		28
ILSTS082[16]	6	110-124	2	MILSTS80[16]	+		1
ILSTS084[16]	+		9	MILSTS83[16]	+		1
ILSTS085[16]	+		20	NCAM[23]	9	131-157	15
ILSTS086[16]	+		13	RM041[24]	1	79	2
ILSTS087[16]	4	116-131	6	RM095[23]	5	140-149	1
ILSTS088[16]	+		9	RM162[25]	9	111-144	12
ILSTS089[16]	+		29	TGLA010[3]	2	146-150	8
ILSTS091[16]	6	245-266	26	TGLA048[3,c]	7	76-107	7
ILSTS092[16]	+		21	TGLA049[3]	7	113-125	1
ILSTS093[16]	1	166	6	TGLA053[3,c]	+		16
ILSTS094[16]	+		10	TGLA057[3,c]	7	79-111	1
ILSTS095[16]	+		21	TGLA073[3,c]	6	115-139	9
ILSTS096[16]	-			TGLA086[3]	-		
ILSTS098[16]	3	84-96	2	TGLA122[3,c]	-		
ILSTS099[16]	-			TGLA126[3,c]	4	105-113	20
ILSTS100[16]	+		11	TGLA159[3]	6	226-238	4
ILSTS101[16]	+		24	TGLA227[3,c]	2	74-78	18
ILSTS103[16]	-			TGLA263[3,c]	4	115-127	3
ILSTS105[16]	+		?	TGLA327[3]	-		
INRA006[18]	4	117-125	3	TGLA381[3]	9	166-184	13
INRA026[19]	6	181-201	22	URB7[26]	7	178-192	13
INRA050[19]	-			UWCA33[27]	2	150-152	19
INRA063[19]	-			UWCA47[27]	3[b]	101-117	8

-: no amplification or no specific amplification in African buffalo
+: amplification in African buffalo, marker not tested for polymorphism or number of alleles difficult to determine because of multiple allelic peaks, overloading or failure of PCR
a: Chromosomal assignments were found in Bishop et al. 1994, Kemp et al. 1995, Kossarek et al. 1996, Barendse et al. 1997, Kappes et al. 1997, Perkin Elmer product information, Cattle Genome Mapping Project database (USMARC, Nebraska, USA), BOVMAP database (INRA, Jouy-en-Josas, France), Cattle Genome Database (CSIRO, Brisbane, Australia)
b: Number of alleles based on six African buffalo
c: Primers StockMarks™ Kit, Applied Biosystem
d: Unpublished primer pair sequences
ILSTS032: F: cac agg tca aac act cag gg, R: act gtg tcc tca agc aaa gg
ILSTS069: F: aga gtt gga cat gac tga gg, R: tct gac tgg ttt caa gtt gg
e: One of the primers redesigned since original publication
DIK028: r: cga tgc agg aga cca tgg
DIK039: R: tga tca aaa aag caa ggg ag
DIK089: R: att taa ata cct ttt cta att tgg
DIK094: f: cct gga gaa tcc cag gga t
DIK102: R: aca tag aac ctc aga tcc tag
DIK104: r: aaa tga ata cct taa atg agc c
References for primer pair sequences:
1: Pfister-Genskow et al. 1995; 2: Konfortov et al. 1996; 3: Georges and Massey 1992; 4: Bishop et al. 1994; 5: Stone et al. 1995; 6: Sunden et al. 1993; 7: Moore et al. 1994; 8: Fries et al. 1993; 9: Hirano et al. 1996; 10: Solinas-Toldo et al. 1993; 11: Thieven et al. 1997; 12: Kaukinen and Varvio 1993;13: Shalom et al. 1994; 14: Shalom et al. 1995; 15: Mezzaline et al. 1995; 16: Kemp et al. 1995; 17: Guerin et al. 1994;18: Vaiman et al. 1992; 19: Vaiman et al. 1994b, 20: Vaiman et al. 1994a, 21: Bahri-Darwich et al. 1994; 22: Olsaker et al. 1996; 23: Barendse et al. 1994; 24: Kossarek et al. 1995; 25: Kossarek et al. 1996; 26: Ma et al. 1996; 27: Kirkpatrick et al. 1995.

A comparison was made between amplification characteristics of microsatellite markers in different species of Bovidae with primers originally developed in cattle (*Bos taurus*). The results are shown in Table 2.2. A higher fraction of the bovine markers amplified in African buffalo, Bali cattle (*Bos banteng*) and water buffalo (*Bubalus bubalis*), which belong to the same subfamily Bovinae, compared to goat (*Capra hircus*) and sheep (*Ovis aries*), which belong to the subfamily Caprinae. In addition, more markers were polymorphic in cross-species amplification of Bovinae compared to Caprinae, with the African buffalo showing the highest proportion of polymorphic markers.

The mean allele size per marker in buffalo and cattle was estimated averaging the largest and smallest observed allele size. In cattle, data were derived from 20 individuals of *Bos taurus* (N'Dama and Friesian) and *Bos indicus* (Boran and Zebu) for the (M)ILSTS markers (Kemp et al. 1995, ILRI unpublished data), 17 individuals of *Bos taurus* (Holstein) for the markers from the StockMarks™ Kit (Perkin Elmer,

product information) and 68 individuals of *Bos taurus* (Japanese Black cattle Wagyu) for the other markers (unpublished data Shirakawa Institute of Animal Genetics). The mean allele size per marker was strongly correlated between cattle and buffalo (r = 0.97), which is a strong indication that homologous loci were amplified in African buffalo. The mean allele size per marker in cattle was 165.0 bp (SE = 4.9) while in African buffalo it was 160.8 bp (SE = 4.9). The difference of 4.2 bp was highly significant (p= 0.001, t-test for dependent samples) and varied with the level of polymorphism in African buffalo. Markers with fewer than six observed alleles in buffalo were 8.3 bp (SE of per locus difference = 1.8, n = 57) shorter than in cattle whereas loci with six or more observed alleles were 1.0 bp (SE of per locus difference = 1.4, n = 44) longer. The difference between these two groups of markers in buffalo was highly significant (p = 0.0001, two tailed t-test with separate variance estimates). The difference in marker size between buffalo and cattle was of the same order as observed between sheep and cattle (2.3 bp, SE = 1.3) using cattle markers (De Gortari et al. 1997) and between chimpanzees and humans (8 bp) using human markers (Rubinsztein et al. 1995).

Table 2.2: Comparison between amplification characteristics of microsatellite markers in different species of Bovidae with primers originally developed for cattle (*Bos taurus*).

Species	**% of loci amplified (number of markers, number of animals tested)**	**% polymorphic markers (number of markers, number of animals tested)**
Bali cattle (*Bos banteng*)	94[1] (52, 2)	75[1] (49, 2)
Goat (*Capra hircus*)	57[2] (97, 5), 61[3] (70, 60)	33[2] (55, 5)
Sheep (*Ovis aries*)	58[4] (1036, 8), 61[2] (97, 5), 79[5] (58, 5)	67[4] (605, 8), 41[2] (59, 5), 65[5] (46, 5)
Water buffalo (*Bubalus bubalis*)	70[6] (80, 5), 85[1] (52, 4)	57[1] (44, 4), 82[6] (56, 5)
African buffalo (*Syncerus caffer*)	83[7] (168, 7)	90[7] (102, 7)

1: Hishida et al. 1996; 2: Kemp et al. 1995; 3: Pepin et al. 1995; 4: De Gortari et al. 1997; 5: Moore et al. 1994; 6: Moore et al. 1995; 7: this study.

Ninety percent of the microsatellite markers were polymorphic in African buffalo. This is higher than in other Bovidae when using cattle primers (Table 2.2), with the possible exception of Bali cattle where observed percentage was negatively biased due to small sample size. Furthermore, in cattle populations from Africa, Europe

and Asia the average observed heterozygosity for polymorphic markers was 0.551 (SE = 0.004) for twenty randomly chosen markers (MacHugh et al. 1997), whereas in African buffalo it was 0.67 (SE = 0.03). Our data are therefore compatible with a larger effective population size in African buffalo compared to cattle. Moreover, the difference in heterozygosity between buffalo and cattle could even be underestimated. This is due to the fact that with cloning procedures microsatellite markers are selected for a particularly large number of repeats, which is positively related to polymorphism, whereas such selection is absent when these markers are used in related species (Ellegren et al. 1995). In different studies microsatellite markers have been observed to be smaller and less polymorphic in related species, than in the species from which they were originally cloned (Ellegren et al. 1995, Pépin et al. 1995, Rubinsztein et al. 1995, Primmer et al. 1996, De Gortari et al. 1997). In agreement with this, average allele size in buffalo was significantly smaller than in cattle. Another indication that average allele size is related to polymorphism is the observation that the less polymorphic markers in buffalo had the biggest difference in allele size between buffalo and cattle. This relationship between allele size and polymorphism has also been shown in cattle and humans (Weber 1990, Moore et al. 1994, Vaiman et al. 1994b).

In conclusion, this study shows that a large fraction of bovine microsatellite markers can be amplified and is polymorphic in African buffalo, and that these markers are applicable for population genetic studies on African buffalo.

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Microsatellite analysis of genetic diversity in African buffalo (*Syncerus caffer*) populations throughout Africa

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Abstract

Genetic diversity in nine African buffalo (*Syncerus caffer*) populations throughout Africa was analysed with fourteen microsatellites to study the effects of rinderpest epidemics and habitat fragmentation during the 20th century. A gradient of declining expected heterozygosity was observed among populations in Save Valley Conservancy (Zimbabwe), northern and southern Kruger National Park (South Africa). This was explained by a high mortality in northern Kruger National Park during the rinderpest pandemic at the end of the 19th century followed by recolonisation from neighbouring populations, resulting in intermediate heterozygosity levels in northern Kruger National Park. In other populations expected heterozygosity was very high, indicating that rinderpest and recent habitat fragmentation had a limited effect on genetic diversity. From expected heterozygosity estimates of long-term effective population size were derived. Migration rates among populations in eastern and southern Africa were very high as shown by a weak isolation by distance and significant correlation in allele frequencies between populations. However, there were indications that dry habitats could limit migration. Genetic distances with buffalo in central Africa were relatively large, supporting their status as distinct subspecies. Finally, it was observed that the higher polymorphic microsatellites were less sensitive at detecting isolation by distance and differences in N_e, which may be a result of the high mutation pressure at these loci.

Introduction

Historically, African buffalo (*Syncerus caffer*) inhabits nearly the whole of sub-Saharan Africa. Buffalo live in herds that can vary between 20 and 1600 individuals (Sinclair 1977 p. 120, Prins 1996 p. 72-77). According to field observations, females and subadults stay in their native herd (Sinclair 1977 p. 132-141, Prins 1996 ch. 3). The herding behaviour of adult males is less clear. Both frequent migration between herds (Prins 1996 ch. 4) and an absence of migration (Sinclair 1977 p. 152) is mentioned.

African buffalo have been affected by habitat fragmentation caused by increasing human habitation and cultivation and by rinderpest epidemics. As a consequence, the distribution of most African buffalo populations is restricted to protected areas. Of the more than 3 million buffalo that roamed the continent in the 19th century only some 400,000 are left (Lessard et al. 1990).

Rinderpest was introduced into Africa by humans in 1889, causing a continent wide pandemic with overall mortality estimated between 90 and 95% (Plowright 1982, Shigesada and Kawasaki 1997, O'Ryan et al. 1998). However, by some estimates certain areas were affected more severely. In Kruger National Park (South Africa) only 20 individuals were observed in 1902 (Stevenson-Hamilton 1911) and in the Umfolozi-Hluhluwe Complex (South Africa) only 75 individuals were observed in 1929 (O'Ryan et al. 1998). In general, buffalo populations were able to recover within 30 years but in some areas populations were affected until the 1960s (Sinclair 1977 p. 5-7, Plowright 1982, Prins 1996 p. 122-127, Simonsen et al. 1998). There were subsequent rinderpest epidemics during the remainder of the 20th century, but they were less severe with mortality not exceeding 50% (Plowright 1982).

Recently, different population genetic studies on African buffalo have been conducted (Grobler and Van der Bank 1996, Templeton and Georgiadis 1996, O'Ryan et al. 1998, Simonsen et al. 1998, Wenink et al. 1998, Van Hooft et al. 1999). Low population differentiation and high levels of genetic variability were observed for the MHC DRB3 gene, microsatellites and mitochondrial DNA. These data indicate large effective population sizes (N_e) and high migration rates between populations as well as between herds. Results of mitochondrial DNA and microsatellites data were congruent, contradicting field observations of male

dominated dispersal. Except for one population in South Africa (Umfolozi-Hluhluwe Complex, O'Ryan et al. 1998), population bottlenecks caused by rinderpest epidemics appeared to have had only a limited impact on genetic diversity. The herding behaviour of buffalo could lead to inbreeding resulting in positive deviations from Hardy-Weinberg equilibrium (HWE), but results from different population genetic studies do not show a consistent pattern.

Because of their high polymorphism microsatellites have proved to be very useful for population genetic studies (Bruford and Wayne 1993, Avise and Hamrick 1996, Smith and Wayne 1996, O'Ryan et al. 1998, Simonsen et al. 1998). In this study, we used fourteen microsatellites to analyse the effects of bottlenecks, caused by rinderpest epidemics and habitat fragmentation since the end of the 19th century, on the genetic diversity of African buffalo populations. Not only did we try to verify conclusions from other studies, but we also wanted to find out if using a larger number of microsatellites than in previous studies could lead to new insights.

Materials and methods

Samples and DNA extraction

In the period 1991-1996 a total of 162 samples were collected from different parts of Africa (Figure 3.1). In eastern Africa samples were collected from Amboseli National Park (NP) (population size 1200, DRSRS in Nairobi), Lake Nakuru NP (population size 1500, animal count by Shem Mwasi working at our department), Masai Mara Game Reserve (GR) (population size 11000, DRSRS) and Tsavo NP (population size 12000, DRSRS) in Kenya and Serengeti NP (population size 21000, Tanzania Wildlife Conservation Monitoring 1994) in Tanzania. Amboseli NP and Tsavo NP, and Masai Mara GR and Serengeti NP are adjoining. The maximum distance between sampling localities in Amboseli NP and Tsavo NP is 200 km. In southern Africa samples were collected from northern Kruger NP (Shingwedzi) and southern Kruger NP (Lower Sabie, population size whole Kruger NP 35000, O'Ryan et al. 1998) in South Africa and from the Save Valley Conservancy (C) (population size 600, animal count by the conservancy) in south-east Zimbabwe. Prior to the collection of samples, buffalo in the Save Valley C were restocked in 1993 from Gonarezhou NP (38 animals, population size 5000, Winterbach 1998) in south-east Zimbabwe and Hwange NP (360 animals, population size 4500 in 1989, Winterbach 1998) in west Zimbabwe. The distance

between the sampling localities in northern and southern Kruger NP is 240 km. In central Africa samples were collected from Gabon (two from Lopé Reserve) and Cameroon (one from Zone de Rhinoceros south-west of Bouba Ndjida NP and two from Elephant Camp at Garoua). Buffalo in western and central Africa are morphologically very different from those in eastern and southern Africa and are generally regarded as a distinct subspecies (*S. c. nanus*, Dwarf or Forest buffalo, Prins 1996). Four samples, which on basis of their morphology were identified as Forest buffalo, were obtained from different zoos in Europe: Safari Park Beekse Bergen (The Netherlands), Antwerpen Zoo (Belgium), Dresden Zoo (Germany) and Berlin Zoo (Germany). The precise origin of these samples was not known. The samples from central Africa/Forest buffalo were treated as one population.

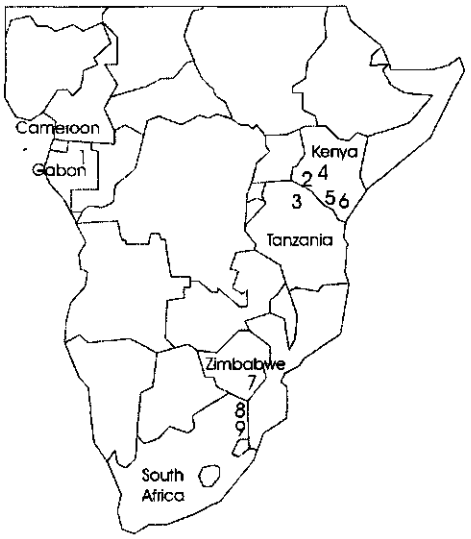

Figure 3.1: Map of sub-Saharan Africa showing the sampling localities. 1: central Africa, various localities in different countries, 2: Masai Mara GR, 3: Serengeti NP, 4: Lake Nakuru NP, 5: Amboseli NP, 6: Tsavo NP, 7: Save Valley C, 8: northern Kruger NP, 9: southern Kruger NP.

Samples were collected from tranquillised animals as whole blood, from darted animals as skin-muscle biopsy, or from culled animals as muscle biopsy. Biopsies were kept in 80% ethanol, 1mM EDTA (pH 8.0) and stored at room temperature. Blood samples were heparinized and kept frozen. DNA was isolated from the samples using Gene Pure Kit (Gentra Systems), following manufacturer's instructions.

Microsatellite amplification

Fourteen microsatellites were selected from a set of 91 polymorphic loci (Van Hooft et al. 1999): ABS010, AGLA293, BM0719, BM3205, BM3517, BM4028, CSSM19, DIK020, ILSTS026, INRA006, INRA128, TGLA057, TGLA159 and TGLA263. These microsatellites were selected for quality of signal on an automatic DNA sequencer, possibility of loading different markers into one lane on an automatic DNA sequencer and level of polymorphism. BM3205, CSSM19, INRA128 and TGLA057 lie on one chromosome in cattle as well as INRA006 and TGLA263. All other loci lie on different chromosomes (Van Hooft et al. 1999). CSSM19 and BM3205 are closely linked in cattle (distance 5 cM, Kappes et al. 1997).

Polymerase chain reaction (PCR) was carried out on 30 ng of genomic DNA in a 12 µl reaction of 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 200 µM dNTP, 1.5 mM $MgCl_2$, 1 mM tetra-methyl-ammonium-chloride, 0.1% Triton x-100, 0.01% gelatine, 4.5 pmol of each primer and 0.25 U Taq polymerase (Goldstar). Amplification was realised, using a PTC-100 machine (MJ Research), in 35 cycles with an annealing temperature of 55 °C, except in the case of INRA006 for which an annealing temperature of 50 °C was used. Of each primer pair one was end-labelled with the fluorescent dye TET, 6-FAM or HEX. After PCR samples were genotyped on a 6% sequencing gel (Ultrapure Sequagel-6) with Genescan™ 350-Tamra as internal standard using a 373 automatic DNA sequencer (Applied Biosystems). Eleven or more microsatellites could be amplified in all samples and in only 2% of the cases no PCR product was obtained. Data were collected, analysed and genotyped using ABI PRISM™ 373 collection (version 1.1), GENESCAN™ analysis (version 1.2.2) and GENOTYPER™ (version 1.1) software (Applied Biosystems).

Statistical analysis

To test for interpopulation differentiation a Fisher's RxC test (Sokal and Rohlf 1995) was performed on allele frequencies of each locus between all pairs of populations and all populations simultaneously (Raymond and Rousset 1995b). Additionally, Fisher's Combined Probability test was employed as a global test over loci to determine the overall significance (Sokal and Rohlf 1995). Both tests were performed with TFPGA 1.3 (Miller 1997).

Genotypic linkage disequilibrium was estimated between all locus pairs with GENEPOP 3.1 (update of version 2.1 described in Raymond and Rousset 1995a). The program performed a probability test using a Markov chain (dememorization 3000, batches 150, iterations per batch 3000). Weir and Cockerham's analogue of Wright's F_{IS} (Weir and Cockerham 1984) was estimated using TFPGA 1.3. Ninety-five percent confidence intervals (CI) were estimated by bootstrapping (10,000X). Significance levels of deviations from HWE were estimated with GENEPOP 3.1. The program performed the score test (Rousset and Raymond 1995) using a Markov chain (dememorization 5000, batches 50, iterations per batch 5000). Significant levels were calculated per locus, per population and over all loci and populations combined.

Genetic diversity within populations was measured as the mean number of alleles per locus (NA), mean observed heterozygosity per locus (H_o) and mean expected heterozygosity per locus (H_e) under HWE (Nei 1987, Hartl and Clark 1989) with GDA 1.0 (Lewis and Zaykin 1999).

Variance of H_o, assuming linkage equilibrium, was calculated as (Weir 1996):

$$\frac{1}{m^2}\sum_{l}\frac{H_l(1-H_l)}{n_l} \qquad (1)$$

where, n_l is the number of genotyped individuals at locus l; m is the number of loci and H_l is the observed heterozygosity at locus l.

Variance of H_e, assuming linkage equilibrium, was calculated as (Weir 1996):

$$\frac{2}{m^2}\sum_{l}\frac{(1+f_l)\left[\sum_{iu} p_{iu}^3 - \left(\sum_{u} p_{lu}^2\right)^2\right]}{n_l} \qquad (2)$$

where, n_l is the number of genotyped individuals at locus l; m is the number of loci; p_{lu} is the frequency of allele u at locus l and f_l is the deviation from HWE at locus l (Weir and Cockerham's analogue of Wright's F_{IS}).

Weir and Cockerham's analogue of Wright's F_{ST} was estimated using TFPGA 1.3 (Cockerham 1973, Nei 1973, Weir and Cockerham 1984). Ninety-five percent CIs were estimated by bootstrapping (10,000X). Rho, which is an estimate of R_{ST} unbiased with regard to sample size, was calculated with RSTCALC (Goodman

1997). Ninety-five percent CIs were estimated by bootstrapping (2500X). R_{ST} is an analogue of F_{ST}. It is specifically adapted to microsatellite data by assuming a stepwise mutation model. R_{ST} is the fraction of the total variance of allele size that is between populations (Slatkin 1995). To test for correlation between genetic and geographical distances (isolation by distance) a Mantel g-test (Mantel 1967) was performed using GENEPOP 3.1 (10,000 permutations).

Two tests were performed to detect effects of recent population bottlenecks on genetic diversity: one based on the presence of heterozygosity excess and the other on a mode shift of the allele frequency distribution. Both tests were performed with BOTTLENECK 1.2.02 (Piry et al. 1999). The first test makes use of the observation that in populations that go through a bottleneck the number of alleles is reduced faster than H_e. Therefore, in a recently bottlenecked population H_e is higher than the expected equilibrium gene diversity (H_{eq}), which is computed from the observed number of alleles (Cornuet and Luikart 1996). In calculating H_{eq} the stepwise mutation model (SMM) was assumed. To determine whether a population exhibited significant H_e excess the Wilcoxon matched pairs test (Sokal and Rholf 1995, test included in software program) was performed. The second bottleneck test makes use of the observation that in a recently bottlenecked population there is a relative decrease in the number of alleles with frequencies lower than 0.1 compared to intermediate frequency classes (e.g. 0.1-0.2). This test can only be used with sample sizes of at least 30 individuals and a minimum of eight polymorphic loci (Luikart et al. 1998).

Nonparametric statistics were applied because for most variables not all the assumptions for parametric tests may have been met. Significance levels of differences in mean H_e between populations were calculated by Friedman ANOVA when more than two populations were compared and Wilcoxon matched pairs test in the case of two populations (Sokal and Rohlf 1995). In both cases single locus H_es were used for the calculations. When analysing correlation among variables Spearman's coefficient, r_s, was used (Sokal and Rohlf 1995).

Results

There was significant population differentiation ($p < 0.0001$) and most population pairs were significantly differentiated ($p < 0.0001$). No significant differentiation was observed between Masai Mara GR and Serengeti NP ($p = 0.42$), Masai Mara GR

and Tsavo NP ($p = 0.1$), Save Valley C and northern Kruger NP ($p = 0.31$), and northern and southern Kruger NP ($p = 0.07$).

No significant linkage disequilibrium was observed ($p > 0.05$). There was a significant deviation from HWE when all populations and all loci were analysed ($p < 0.0001$) but the lower margin of the 95% CI of F_{IS} was very close to zero (95% CI: 0.003- 0.069). To test for consistent departures from HWE across loci, F_{IS} was also analysed per locus. Only loci AGLA293, ABS010 and TGLA159 showed p-values < 0.004. All other loci showed p-values > 0.04. With these three loci excluded, no significant deviation from HWE was observed when data from all populations and all loci were combined ($p > 0.2$, $F_{IS} = 0.006$, 95% CI: -0.014 - 0.022).

Mean NA varied between 5.6 (Tsavo NP) and 9.1 (Serengeti NP), and between 4.6 (southern Kruger NP) and 6.6 (central Africa) when a random sample of eight individuals for each population was used (Table 3.1). H_o varied between 0.647 (southern Kruger NP) and 0.770 (Serengeti NP) with an overall mean of 0.729. H_e varied between 0.664 (southern Kruger NP) and 0.811 (central Africa) with an overall mean of 0.759 (Table 3.1). There were significant differences in H_e between populations ($p < 0.05$). No significant differences were observed in H_o ($p > 0.6$) because of a larger within population variance of H_o than H_e. A gradient of declining H_e was observed comparing the populations in Save Valley C, northern and southern Kruger NP, with southern Kruger NP showing the lowest H_e. When Kruger NP was excluded from the calculations no significant differences in H_e were found ($p > 0.5$).

Differences in H_e were significant between Save Valley C and northern Kruger NP ($p = 0.008$) and nearly significant between northern and southern Kruger NP ($p = 0.06$). The differences in H_e were caused mainly by seven loci with intermediate H_e values (loci b-g and k in Table 3.1), varying between 0.49 and 0.77 averaged across the three populations ($p < 0.005$). Mean H_e of these loci for Save Valley C, northern and southern Kruger NP was 0.734, 0.677 and 0.570 respectively. For the other seven loci with more extreme H_e values, either < 0.18 or > 0.82 averaged across the three populations, no significant differences in H_e were observed ($p > 0.3$, differences in mean $H_e \leq 0.032$). In the latter case only one locus (BM4028) had a mean $H_e < 0.18$, and also with this locus excluded no significant differences in H_e were observed ($p > 0.1$).

Table 3.1: Summary of genetic diversity in African buffalo populations.

Population	SS	NA full	NA un.	H_o ± SE	H_e ± SE	H_e loci													
						a	b	c	d	e	f	g	h	i	j	k	l	m	n
Nakuru	32	7.9	5.1	0.736 ± 0.020	0.781 ± 0.008	0.64	0.60	0.72	0.78	0.77	0.69	0.82	0.78	0.83	0.77	0.87	0.85	0.88	0.93
M. Mara	10	6.8	6.5	0.753 ± 0.035	0.779 ± 0.016	0.60	0.51	0.66	0.70	0.77	0.71	0.85	0.76	0.86	0.84	0.90	0.94	0.86	0.95
Serengeti	33	9.1	5.9	0.770 ± 0.019	0.769 ± 0.009	0.57	0.58	0.69	0.58	0.69	0.73	0.83	0.79	0.87	0.85	0.87	0.89	0.89	0.93
Amboseli	20	7.1	5.5	0.721 ± 0.025	0.753 ± 0.013	0.68	0.50	0.58	0.60	0.79	0.73	0.79	0.83	0.81	0.74	0.87	0.89	0.85	0.88
Tsavo	8	5.6	5.6	0.740 ± 0.036	0.790 ± 0.015	0.59	0.59	0.68	0.65	0.77	0.87	0.87	0.83	0.83	0.82	0.88	0.85	0.89	0.95
S. Valley	10	6.6	6.3	0.735 ± 0.034	0.762 ± 0.013	0.10	0.72	0.58	0.74	0.68	0.76	0.82	0.91	0.88	0.91	0.83	0.87	0.95	0.91
n. Kruger	22	7.2	5.6	0.724 ± 0.023	0.724 ± 0.011	0.17	0.59	0.55	0.66	0.66	0.73	0.75	0.87	0.86	0.84	0.80	0.86	0.90	0.90
s. Kruger	16	6.2	4.6	0.647 ± 0.029	0.664 ± 0.017	0.23	0.47	0.33	0.70	0.54	0.53	0.76	0.83	0.90	0.83	0.66	0.75	0.86	0.91
c. Africa	9	7.1	6.6	0.735 ± 0.035	0.811 ± 0.018	0.82	0.75	0.93	0.82	0.59	0.86	0.86	0.77	0.61	0.90	0.85	0.79	0.87	0.93
Mean	18	7.1	5.7	0.729	0.759	0.49	0.59	0.63	0.69	0.70	0.73	0.82	0.82	0.83	0.83	0.84	0.86	0.88	0.92

SS: sample size, NA: mean number of alleles per locus, full.: full sample size, un.: random sample of eight individuals for each population, H_e: mean expected heterozygosity per locus, H_o: mean observed heterozygosity per locus, a: BM4028, b: INRA128, c: BM3517, d: ABS010, e: TGLA263, f:INRA006, g: TGLA057, h: BM3205, i: ILSTS026, j: TGLA159, k: CSSM19, l: BM719, m: DIK020, n: AGLA293.

Between most population pairs single locus H_e values were strongly correlated ($p < 0.02$, $r_s > 0.6$) except when the population from central Africa was included ($p > 0.3$, $r_s < 0.3$). The latter was mainly due to a strong increase of H_e in central Africa for the six least polymorphic loci (0.80 vs. 0.62 for the other populations). Between most population pairs allele frequencies were significantly correlated ($p < 0.05$, $r_s > 0.18$,) except when central Africa was included ($p \geq 0.05$, $r_s < 0.17$). With each of the three regions eastern Africa (Kenya and Tanzania), southern Africa (Zimbabwe and South Africa) and central Africa treated as one population, central Africa contained the highest percentage of private alleles (Table 3.2).

Table 3.2: Private alleles.

Region	Eastern Africa	Southern Africa	Central Africa
Eastern Africa	x	3.3	10.0
Southern Africa	12.6	x	25.5
Central Africa	27.4	26.3	x

Figures represent total percentage of alleles that are unique in the region in the upper row compared to the region in the left column. Pairwise comparisons within rows are expected to be uninfluenced by sample size differences. eastern Africa: pooled sample of Masai Mara GR, Serengeti NP, Lake Nakuru NP, Amboseli NP and Tsavo NP, southern Africa: pooled sample of Save Valley C, northern Kruger NP and southern Kruger NP.

The two bottleneck tests did not show a significant effect of recent population bottlenecks, except for Tsavo NP where a significant heterozygosity excess was observed under the SMM ($p = 0.0004$, other populations $p > 0.18$). It must be noted that, because most samples contained fewer than 30 individuals, the test for mode shift of allele frequency distribution could only be performed on samples from Lake Nakuru NP, Serengeti NP and a pooled sample from northern and southern Kruger NP.

F_{ST} and Rho calculated across all populations were 0.059 and 0.065 respectively, while mean values of F_{ST} and Rho between population pairs were 0.059 and 0.073 respectively (Table 3.3). Between most population pairs 95% CIs for F_{ST} and Rho were largely overlapping, except between Lake Nakuru NP and Masai Mara GR and for four pairs that included central Africa. Significant isolation by distance was observed for both F_{ST} ($p < 0.00001$) and Rho ($p < 0.02$) (Figure 3.2). Rho values between central and eastern Africa were much larger than between eastern and southern Africa at comparable geographical distances. When central Africa was

treated as a distinct group there was still significant isolation by distance with F_{ST} ($p < 0.0005$) but not with Rho ($p = 0.16$).

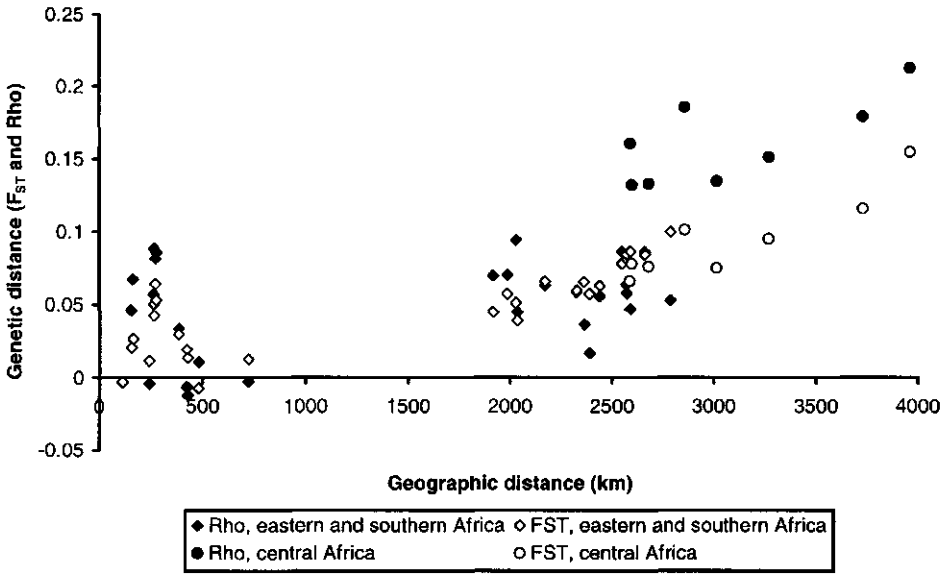

Figure 3.2: Isolation by distance - relationship between F_{ST} and Rho and geographic distance. F_{ST} and Rho were calculated between all possible population pairs. Diamonds refer to genetic distances among populations in eastern and southern Africa and circles to genetic distances between central Africa and all other populations.

Discussion

Kruger NP

The population in Kruger NP showed the lowest genetic diversity, while mortality during the rinderpest pandemic was estimated to have been especially high in this park. In principal, the low H_e in Kruger NP compared to the nearby Save Valley C could also be a result of an artificially high H_e in the latter due to restocking from different localities. However, pooling of samples from eastern Africa indicated that this could lead to an increase of only 0.02, whereas a difference of 0.04 was observed. The gradient of decreasing H_e along the transect between Save Valley C and southern Kruger NP combined with the absence of significant differentiation between neighbouring populations may be due to gene flow from the north after the rinderpest pandemic. However, loci with H_e values close to 0 or 1 did not show

Table 3.3: Genetic distance matrix.

	Nak.	M. M.	Ser.	Amb.	Tsa.	Sav.	n. Kru.	s. Kru.	c. Afr.
Nak.	X	0.067	0.057	0.081	0.034	0.063	0.064	0.053	0.133
		[0.045|0.153]	[0.038|0.112]	[0.057|0.153]	[0.013|0.144]	[0.050|0.139]	[0.039|0.130]	[0.035|0.123]	[0.098|0.237]
M. M.	0.026	X	-0.004	0.085	-0.012	0.094	0.056	0.086	0.161
	[0.009|0.045]		[-0.004|0.069]	[0.059|0.181]	[-0.016|0.110]	[0.080|0.210]	[0.047|0.147]	[0.071|0.194]	[0.133|0.278]
Ser.	0.042	-0.003	X	0.088	-0.007	0.070	0.058	0.086	0.132
	[0.021|0.071]	[-0.011|0.005]		[0.066|0.156]	[-0.014|0.095]	[0.048|0.157]	[0.044|0.117]	[0.069|0.153]	[0.106|0.239]
Amb.	0.064	0.053	0.050	X	0.046	0.070	0.036	0.058	0.186
	[0.037|0.095]	[0.021|0.089]	[0.029|0.074]		[0.030|0.153]	[0.051|0.174]	[0.019|0.111]	[0.034|0.150]	[0.146|0.294]
Tsa.	0.030	0.011	0.019	0.021	X	0.045	0.017	0.047	0.135
	[0.012|0.049]	[-0.005|0.031]	[0.007|0.030]	[0.002|0.044]		[0.030|0.179]	[0.009|0.123]	[0.040|0.162]	[0.089|0.274]
Sav.	0.066	0.051	0.045	0.057	0.039	X	0.010	-0.003	0.151
	[0.032|0.115]	[0.014|0.114]	[0.023|0.073]	[0.034|0.082]	[0.008|0.083]		[0.000|0.123]	[-0.011|0.126]	[0.119|0.289]
n. Kru.	0.081	0.063	0.060	0.066	0.057	-0.007	X	-0.004	0.179
	[0.053|0.122]	[0.031|0.114]	[0.042|0.080]	[0.041|0.095]	[0.025|0.097]	[-0.017|0.003]		[-0.006|0.068]	[0.144|0.294]
s. Kru.	0.100	0.084	0.078	0.085	0.087	0.013	0.011	X	0.213
	[0.073|0.137]	[0.043|0.139]	[0.052|0.107]	[0.051|0.127]	[0.047|0.132]	[-0.006|0.031]	[-0.002|0.027]		[0.173|0.329]
c. Afr.	0.076	0.066	0.078	0.102	0.075	0.095	0.116	0.155	X
	[0.051|0.103]	[0.039|0.096]	[0.055|0.101]	[0.072|0.132]	[0.044|0.108]	[0.050|0.147]	[0.079|0.161]	[0.106|0.208]	

Figures above diagonal represent Rho, figures below diagonal represent F_{ST} between localities. 95% CI between brackets. Nak.: Lake Nakuru NP , M.M.: Masai Mara G.R., Ser.: Serengeti NP, Amb: Amboseli NP, Tsa.: Tsavo NP , Sav.: Save Valley C, n. Kru.: northern Kruger NP, s. Kru.: southern Kruger NP, c. Afr.: central Africa.

a significant gradient of decreasing H_e in contrast to loci with intermediate H_e values. According to the formula for the rate of change in H_e from genetic drift, the decrease in H_e during a bottleneck is • $H_e/2N_e$ per generation (Nei et al. 1975, Hartl and Clark 1989 p. 76-79). Therefore the strongest gradient would be expected at loci with high pre-bottleneck H_e values. Furthermore, two tests for population bottlenecks gave a negative outcome. In agreement with the latter observation, in a recent study on African buffalo, by doing a likelihood analysis, it was concluded that the population in Kruger NP has retained most of its original genetic variation (O'Ryan et al. 1998).

The seemingly contradictory observations for the population in Kruger NP can be explained by historical data about the rinderpest pandemic (Kruger National Park, Department of Scientific Services). According to these data mortality was especially high in northern Kruger NP where only few buffalo were reported until the 1930s, whereas buffalo recovered very quickly in southern Kruger NP. Since then, it has been observed that northern Kruger NP was recolonised from southern Kruger NP and possibly also from Mozambique and Zimbabwe in the north. Recolonisation from both south and north explains the intermediate H_e in northern Kruger NP, as well as the absence of significant population differentiation between neighbouring populations. The absence of significant differences for extreme H_e values can be explained if one assumes ancient population differentiation between Zimbabwe and southern Kruger NP, and a relatively small long-term (time to reach mutation-drift equilibrium) N_e for the latter. According to both the SMM and infinite alleles model of mutation (IAM) variation in long-term N_e has relatively little effect on H_e at values close to 0 or 1, as is shown in figure 3.3. A small long-term N_e in southern Kruger NP is not unlikely as south-east South Africa is relatively isolated from the north of Africa by the arid Limpopo Valley at the north-eastern border. The Limpopo Valley is a known ecological barrier for various vertebrates (Van Bruggen 1964, Pienaar 1970). In support of this explanation buffalo in the Umfolozi-Hluhluwe Complex, located south of Kruger NP, show an even lower H_e (O'Ryan et al. 1998). However, in this population rinderpest could have had an effect as well. The negative outcome of the bottleneck tests for northern Kruger NP can be explained by assuming immigration of rare alleles. Particularly rare alleles are lost during a bottleneck, and it is this loss of rare alleles to which the two bottleneck tests used in this study are sensitive (Cornuet and Luikart 1996, Luikart et al. 1998). As it is unlikely that different populations loose exactly the same set of

alleles during a bottleneck, gene flow can reintroduce many of the lost alleles in each population.

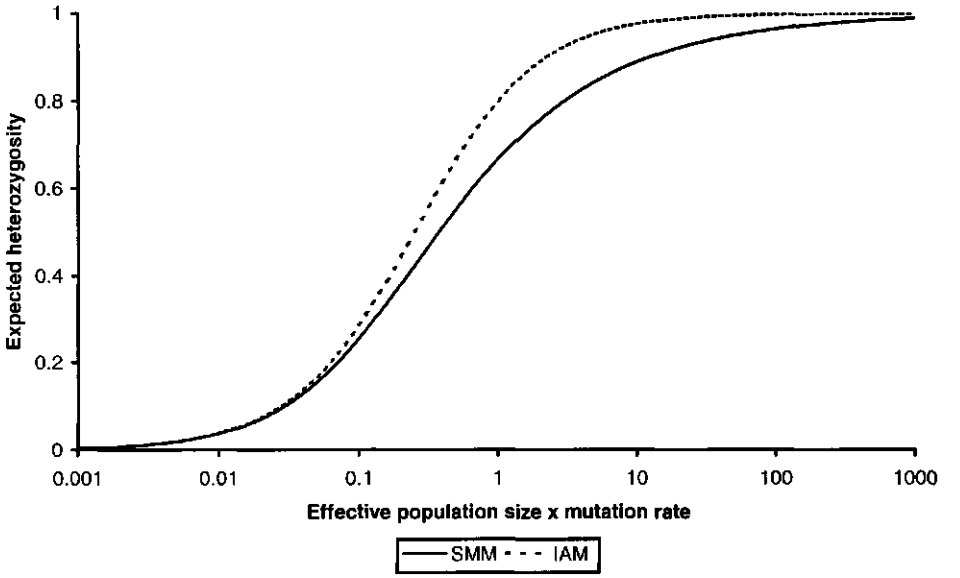

Figure 3.3: Relationship between expected heterozygosity (H_e) and effective population size x mutation rate ($N_e\mu$) for populations in mutation-drift equilibrium. This graph was derived from the infinite alleles model of mutation (IAM, $H_e = 1/(1+1/4N_e\mu)$ formula 13.30 in Nei 1987) and stepwise mutation model (SMM, $H_e = 1-(1+8N_e\mu)^{-1/2}$ formula 13.48 in Nei 1987).

Estimate of N_e

Estimates of long-term N_e can be derived from H_e and mutation rate using either the IAM or the SMM (formulas 13.30 and 13.48 in Nei 1987). The mean mutation rate of microsatellites in a number of mammals is known, which could be applied to buffalo. Unfortunately, mean H_e per population was biased since the microsatellites used in this study were selected for polymorphism. However, reasonable minimum and maximum estimates of overall H_e could be made. Of 101 bovine microsatellites that could be amplified on a test panel of seven buffalo 10% were monomorphic (Van Hooft et al. 1999). By assuming a mean H_e for the polymorphic markers equal to that observed in this study a maximum estimate of overall H_e of 0.68 was obtained. The H_o of the test panel was 0.61, which could be used as a minimum estimate. This resulted in an estimate of long-term N_e between 2,600 and 24000, assuming a mean mutation rate of $4.5*10^{-5}$ - $15*10^{-5}$/generation (Crawford and

Cuthbertson 1996, Schug et al. 1997, Lehmann et al. 1998). This estimate of N_e is large compared with current census sizes, which often are < 2,600. Our estimate of long-term N_e is between 7.5% and 70% of current census sizes in the Masai Mara-Serengeti and Kruger ecosystems. This is comparable to the short-term (two generations) N_e estimate of 10 - 30% made by O'Ryan et al. (1998).

Rinderpest and habitat fragmentation

The high mean H_e and the negative outcomes of the bottleneck tests for all but one population indicate that, in general, rinderpest epidemics and recent habitat fragmentation had a small effect on genetic diversity. The positive outcome of one bottleneck test for Tsavo NP was probably because of the small sample size, as this population showed a relatively high H_e. The absence of a significant effect of rinderpest is probably due to the fact that most buffalo populations were able to recover within only a few generations after major epidemics. In addition, immigration from neighbouring populations could have restored part of the lost genetic diversity, as postulated for northern Kruger NP. The absence of a significant effect of habitat fragmentation is probably due to the fact that there have been only a few generations since the strong increase of human habitation and cultivation in Africa.

Isolation by distance was observed both for F_{ST} and Rho. Because H_e has probably decreased very little during this century, it seems reasonable to assume that positive F_{ST} and Rho values reflect ancient habitat fragmentation. Correlation in allele frequencies among all populations in eastern and southern Africa and small F_{ST} and Rho values (< 0.1) indicate high historical migration rates. The observation of significant population differentiation between Amboseli NP and Tsavo NP was surprising as these are adjoining populations. As with southern Kruger NP, limited migration between Amboseli NP and Tsavo NP may be explained by dryness of the habitat. The low H_e in Amboseli NP ($p = 0.05$ for the difference with Tsavo NP) suggests a small, isolated population. Population differentiation between Amboseli NP and Tsavo NP has also been observed for Grant's gazelle (mitochondrial D-loop and two microsatellites, Arctander et al. 1996) and elephant (mitochondrial genes ND5 and ND6, Siegismund and Arctander 1995).

Pairwise Rho values between eastern and southern Africa were much smaller than those observed by Simonsen et al. (1998) using six microsatellite loci (0.06 vs.

0.15). One possible explanation for this difference is that we used higher polymorphic loci (mean H_e 0.76 vs. 0.58). When only the five lowest polymorphic loci were selected, Rho values were twice as high (0.11 vs. 0.06). Furthermore, with only these loci significant isolation by distance between eastern and southern Africa ($p = 0.05$) was observed in agreement with the study by Simonsen et al. (1998) ($p = 0.01$), in contrast to the more polymorphic loci ($p = 0.16$). These results indicate that microsatellites can be too polymorphic for detecting isolation by distance. This could be caused by a high mutation pressure which, because of the limited number of possible alleles at microsatellite loci, tends to shift different populations to the same distribution of alleles (Nauta and Weissing 1996).

Central Africa

Rho values between central and eastern Africa were much larger than those between eastern and southern Africa at comparable geographical distances. Furthermore, central Africa had the highest frequency of private alleles. Both observations indicate that the population in central Africa was relatively isolated due to reduced gene flow, which support their classification as a distinct subspecies. Reduced gene flow may be a result of stronger habitat fragmentation in central Africa due to the presence of rainforest. Since the samples came from various localities, the increase of H_e from 0.62 to 0.80 in central Africa at the lower polymorphic markers may be attributed to a Wahlund effect caused by this habitat fragmentation (Hartl and Clark 1989, p. 282-293). In comparison, when samples from eastern and southern Africa were pooled H_e only increased from 0.62 to 0.67. A larger N_e seems an unlikely explanation for the increase in H_e because of the relatively low population density in this part of Africa (Prins and Reitsma 1989, Prins 1996 p. 249).

In contrast to Rho, F_{ST} values between eastern and southern Africa were similar to those between eastern and central Africa. This may be due to the fact that F_{ST} becomes negatively biased at lower gene flow levels (Slatkin 1995). Although overall F_{ST} and Rho were not significantly different, four out of eight pairwise population comparisons with central Africa showed no or little overlap in the 95% CIs.

Conclusions

This study focussed on the effects of rinderpest epidemics and habitat fragmentation since the end of the 19th century on the genetic diversity of African buffalo populations. We conclude that rinderpest epidemics did not lead to significant loss of genetic diversity. However in the past, genetic diversity could have been decreased temporarily. Habitat fragmentation did not appear to have influenced genetic diversity to a great extent, although it may still have profound effects in the near future. Current census sizes of many buffalo populations are smaller than the long-term N_e, which implies that without migration they will not be able to maintain historical levels of genetic diversity at microsatellite loci. Genetically, buffalo in central Africa were relatively isolated supporting their status as separate subspecies. Finally, by using twice as much microsatellites as in most other population genetic studies on wild animals we could study the influence of mutation rate on the ability to detect isolation by distance and differences in N_e.

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Phylogeography of the African buffalo based on mitochondrial and Y-chromosomal loci: indications for a Pleistocene origin and population expansion of the subspecies Cape buffalo

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Abstract

Population genetics and phylogeography of the African buffalo (*Syncerus caffer*) are inferred from genetic diversity at mitochondrial D-loop hypervariable region I sequences and an Y-chromosomal microsatellite. Included are subspecies Cape buffalo from eastern and southern Africa and buffalo from central and south-western Africa. Nucleotide diversity of Cape buffalo at the hypervariable region I is high with little differentiation between populations. In buffalo from central and south-western Africa both mitochondrial and Y-chromosomal diversity are considerable higher than in Cape buffalo, for which several explanations are hypothesised. There are several indications that there was a Pleistocene population expansion in Cape buffalo. Furthermore, in the same period buffalo in eastern and southern Africa seem to have developed as a separate subspecies. These two observations are in agreement with the hypothesis of a rapid evolution of Cape buffalo based on fossil data. However, as alternative explanations are possible, further analyses with autosomal loci are needed.

Introduction

Historically, African buffalo (*Syncerus caffer*) inhabits nearly the whole of sub-Saharan Africa. With elephant (*Loxodonta africana*) and wildebeest (*Connochaetes taurinus*) it forms the bulk of large herbivore biomass in Africa. Three subspecies of buffalo are recognised (group boundaries: Blancou 1935; group names: Haltenorth and Diller 1979, see also Prins 1996 p. 241-243): Cape buffalo on the savannas of eastern and southern Africa (*S. c. caffer*), Forest buffalo in the rain forests of western and central Africa (*S. c. nanus*) and West African buffalo from the Sahel-Sudan savannas (*S. c. brachyceros*) (Figure 4.1). Of only Cape buffalo have the population genetics (Templeton and Georgeadis 1996, O'Ryan et al. 1998, Simonsen et al. 1998, Wenink et al. 1998, Van Hooft et al. 1999, Van Hooft et al. 2000) and behaviour (Sinclair 1977, Prins 1996) been studied in detail.

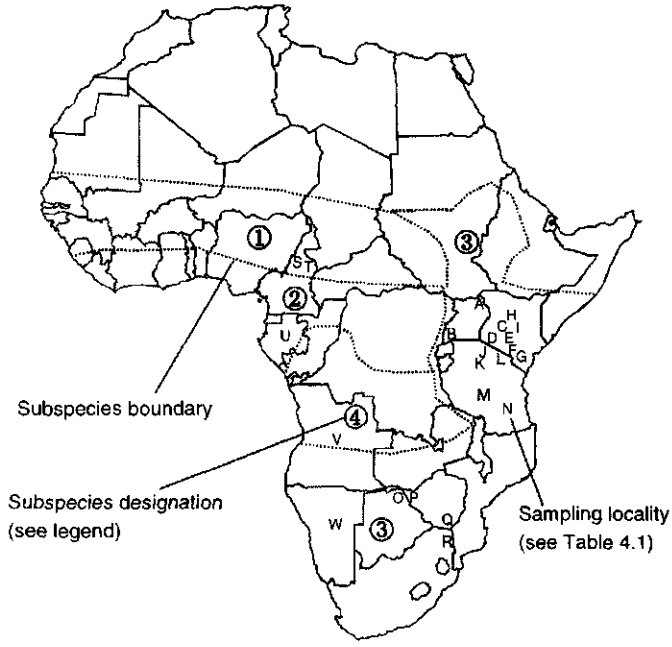

Figure 4.1: Map of Africa with sampling localities and distribution of the three subspecies of African buffalo. 1: *S. c. brachyceros*, 2: *S. c. nanus*, 3: *S. c. caffer*, 4: unclear taxonomic position, either *S. c. nanus* or *S. c. caffer*.

Since the end of the 19th century African buffalo has been affected by rinderpest epidemics and by habitat fragmentation due to increasing human habitation and

cultivation. According to different population genetic studies (O'Ryan et al. 1998, Simonsen et al. 1998, Wenink et al. 1998, Van Hooft et al. 2000) rinderpest epidemics and habitat fragmentation have not resulted in a significant decrease of genetic diversity. High levels of genetic diversity and low levels of population differentiation were observed for most populations, both for mitochondrial DNA (mtDNA) and microsatellites.

To derive its recent evolutionary history, no phylogeographic studies on African buffalo have been performed yet. This history may have been strongly influenced by climatic changes. In Africa climates and habitats have fluctuated widely, especially since the last million years (DeMenocal 1995). This period is known as the late Quaternary and is characterised by 9 major and 12 minor glacial periods (last glacial maximum 23,000 - 18,000 years ago). The climate varied between cold and dry during glacial periods and warm and wet during interglacial periods. The repeated drying out of habitats followed by their renewal and expansion has led to repeated isolations and has driven entire ecosystems to adapt to the changes (Kingdon 1989 p. 14).

MtDNA has been used in many intraspecific phylogeographic studies, because its high mutation rate allows discrimination of recently diverged lineages. The most variable part of mtDNA is the D-loop hypervariable region I (HVI) with a mutation rate in mammals of 3% (horse) to 39% (European bison) substitutions per million years (Slade et al. 1998, Burzynska et al. 1999). As mtDNA is maternally inherited, analysis of mtDNA diversity gives a female biased view on population structure and history. However, additional use of Y-chromosomal microsatellites allows for a direct comparison between male and female specific contributions.

In this study we analysed mtDNA HVI and Y-chromosomal microsatellite diversity in Cape buffalo from eastern and southern Africa to find out to what extent its evolutionary history has been influenced by late Quaternary environmental and climatic changes (review on this subject: Hewitt 2000). In contrast to most other genetic studies on African buffalo we also included samples from central and south-western Africa belonging to various subspecies of buffalo.

Materials and methods

Samples

Details and approximate locations of the samples are shown in Table 4.1 and Figure 4.1. A total of 195 samples were collected. A further 186 buffalo HVI sequences were obtained from Genbank (accession nos. AF028843 - AF029038, sequences AF028863, AF028871, AF028876 - AF028882, and AF028891 were excluded because they contained too much unresolved sites). These sequences have been published in Simonsen et al. 1998. An additional 33 samples from Amboseli and Lake Nakuru were used for analysing Y-chromosomal microsatellite diversity. These samples are identical to the ones used in Simonsen et al. 1998. This resulted in a total of 66 male Cape buffalo and 7 male samples from central and south-western Africa being used for Y-chromosomal analyses.

DNA extraction

Samples were collected as blood or tissue biopsy. In addition, dung samples were obtained from Arusha (48), Tsavo West (8), Tsavo East (8) and Elephant Camp (4). Dung samples were kept in ethanol and stored at room temperature. DNA was extracted from dung following the protocol in Gerloff et al. 1995. As positive sample controls tissue samples of five individuals were used, from who also dung was available. As negative sample controls sample storage medium was used to which no dung was added.

Microsatellite amplification

Primers for five Y-chromosomal microsatellites in cattle, INRA008, INRA057, INRA062, INRA124 and INRA126 (Vaiman et al. 1994), were tested for PCR amplification. PCR's and subsequent genotyping on the ABI 373 automatic DNA sequencer (Applied Biosystems) were conducted as in Van Hooft et al. 2000. The annealing temperature was 55 °C and the forward primers were end-labelled with fluorescent dye for automatic genotyping. Only INRA008 could be amplified in male African buffalo. No amplification was obtained in nine female buffalo, confirming its Y-chromosomal location. Multiple DNA fragments were observed after PCR. However, different alleles could still be discerned. Due to the low quality and low amounts of DNA in dung, PCR was performed on tissue and blood samples only.

Table 4.1: List of samples used in this study.

Region/Country	Locality	Subsp.	SS	SS GB
E. Africa (285)*				
Uganda	Kidepo NP (A)	a	-	1
	Q. Elisabeth NP M.[1] (B1)	a/b	-	23
	Q. Elisabeth NP I.[2] (B2)	a/b	-	17
Kenya	Lake Nakuru NP (C)	a	19	16
	Masai Mara GR (D)	a	9	19
	Nairobi NP (E)	a	-	10
	Amboseli NP (F)	a	-	20
	Tsavo West NP (G1)	a	8	-
	Tsavo East NP (G2)	a	14	-
	Tsavo NP (G)	a	1	-
	Laikipia Plateau (H)	a	-	10
	Mount Kenya[3] (I)	a	1	-
Tanzania	Serengeti NP (J)	a	37	-
	Maswa GR (K)	a	2	20
	Arusha NP (L)	a	48	-
	Kizigo NP (M)	a	-	9
	Selous GR (N)	a	1	-
S. Africa (82)*				
Botswana	Chobe NP (O)	a	-	11
Zimbabwe	Hwange NP (P)	a	-	16
	Gonarezhou NP (Q)	a	-	14
South Africa	northern Kruger NP[4] (R1)	a	23	-
	southern Kruger NP[5] (R2)	a	18	-
central and S.W. Africa (14)*				
Cameroon	Elephant Camp[6] (S)	c	6	-
	Zone de Rhinoceros[7] (T)	c	1	-
Gabon	Lope Reserve (U)	b	2	-
Angola[8]	unknown (V)	d	1	-
Namibia[9]	Okahandja-Windhoek (W)	d	2	-
Forest buffalo[10]	unknown	b	2	-

SS: sample size, GB: genbank, NP: National Park, GR: Game Reserve, Subsp.: Subspecies, capitals in parentheses refer to Figure 4.1. *: sample size per region, 1: Mweya sector, 2: Ishasha sector, 3: Lewa Downs, 4: Shingwedzi, 5: Lower Sabie, 6: Garoua, 7: close to Bouba Ndjida NP, 8: Dresden Zoo, great-grandmother from Angola (mother's side only), 9: Antwerpen Zoo, one with father from Windhoek and one with mother from Okahandja, 10: Berlin Zoo and Safari Park Beekse Bergen, unknown origin but morphologically identified as Forest buffalo.

a: *S. c. caffer*, a/b: Region at the boundary between the ranges of *S. c. caffer* and *S. c. nanus*. In this region both subspecies are observed. However, microsatellite and mtDNA diversity (Simonsen et al. 1998) show that the samples in this study are genetically more closely related to Cape buffalo.. b: *S. c. nanus*, c: *S. c. brachyceros*, d: unclear taxonomic position, either *S. c. nanus* or *S. c. caffer*.

MtDNA HVI amplification and sequencing

For tissue and blood samples PCR conditions were the same as those for the Y-chromosomal microsatellites. Forward primer M13.CRL-7 (M13.AAC TAC TCC CTG AAC ACC GC, M13-sequence: TGT AAA ACG ACG GCC AGT) and reverse primer A.dlo.H (ATG TAT GAC AGC ACA GTT ATG T) were used (Anderson et al. 1982). In the case of dung samples a hot start PCR was performed (Chou et al. 1992). A 5 µl lower layer of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 8 mM $MgCl_2$, 800 µM dNTP and 1.8 µM of each primer (M13.CRL-7 and A.dlo.H), and a 15 µl upper layer of 13.3 mM Tris-HCl (pH 8.3), 66.7 mM KCl, 1.33 mg/ml Rabbit Serum Albumin (RSA, Sigma, fraction V), 0.05 U/µl Taq polymerase (Goldstar) and 1/3 volume (5 µl) DNA sample was used with Ampliwax (PCR Gem 50, Perkin Elmer) as intervening wax layer. Amplification was realised in 40 cycles, with the first seven cycles at an annealing temperature of 50 °C instead of 55 °C. A positive and negative PCR control and a negative sample control was used for each PCR. All sequences from the positive sample controls (5) were identical to those from dung. All negative PCR (5) and sample controls (7) failed to amplify any PCR product. The observation that the dung samples were adequate for mtDNA HVI amplification and sequencing but not microsatellite amplification, is probably a result of the high copy number of the mitochondrial genome in mammalian cells (Taberlet et al. 1999).

PCR products were purified on Millipore Ultrafree-DA columns. Subsequent sequencing of PCR product was done with ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit and a M13 forward primer. Half of the terminator ready reaction mix was replaced with Halfterm DNA Sequencing Reagent (Genpak).

The sequence PCR product was run on a 6% sequencing gel (Long Ranger gel solution) using a ABI 373 automatic DNA sequencer (Applied Biosystems). Data were collected and analysed using Applied Biosystems software. Sequences were deposited in the Genbank database under accession nos. AF313151 - AF313345.

Statistical analysis

HVI sequences were aligned with the program DAMBE version 3.7 (Xia 2000) and some correction by eye. All calculations were performed after exclusion of sites

with alignment gaps or unresolved nucleotides, except when calculating nucleotide diversity (π). In that case such sites were determined and excluded for each population separately, as otherwise π would be underestimated due to unresolved nucleotides in the Genbank sequences.

Phylogenetic relationships

Maximum-likelihood distances between haplotypes were calculated using DAMBE assuming the substitution model TN93 (Tamura and Nei 1993) and the among-site heterogeneity to be γ distributed. With the program TREE-PUZZLE version 4.0.2 (Strimmer and Von Haeseler 1996) α was estimated, the shape parameter of the γ-distribution. Maximum-likelihood distances were used to calculate a minimum spanning network (MSN) with the program MINSPNET (L. Excoffier, Dept. of Anthropology, University of Geneva). MSN's are preferable above neighbour joining trees for intraspecific studies because haplotypes are not forced to occupy tip positions and multifurcations are allowed (Crandell and Templeton 1996).

Population structure

To test for interpopulation differentiation a Fisher's RxC test (Sokal and Rohlf 1995) on haplotype frequencies between all population pairs (Raymond and Rousset 1995a) was performed with the program ARLEQUIN version 2.000 (Schneider et al. 2000). Population HVI haplotype diversity (H) and π as well as their standard deviations were estimated with the program DNASP version 3.14 (Rozas and Rozas 1999), after pooling of adjoining populations that showed no significant differentiation. Significance levels of differences in π between populations were calculated by ANOVA and student's t-tests, with the population variance estimated with DNASP, and between groups of populations by Mann-Whitney U tests. The genetic structure of populations was investigated by analysis of (molecular) variance (AMOVA or F-statistics) (Excoffier et al. 1992) with ARLEQUIN, using as a genetic distance the number of pairwise differences between haplotypes. This resulted in F-values (F_{ST}, F_{SC} and F_{CT}), which are the fraction of the genetic variation that is between groups of buffalo at different geographic levels. F_{ST} was also calculated between population pairs, which values were used to test for isolation by distance using a Mantel g-test (Mantel 1967), included in the program GENEPOP version 3.1 (Raymond and Rousset 1995b). In calculating F_{ST} between

population pairs, Kizigo-Selous and Cameroon-Gabon were treated as one population.

The female effective population size (N_{ef}) was estimated by:
$N_{ef} = \theta/2v$ (Tajima 1996)
where v is the mutation rate per nucleotide site per generation and θ the expected nucleotide diversity taking into account rate heterogeneity.
θ can be estimated from π and α by:
$\theta = \pi \exp(\pi(4/3)(1+1/\alpha))$ (Tajima et al. 1998)
The number of net nucleotide substitutions between populations (D_A) (Nei 1987, eqn. 10.21) was calculated with DNASP. The latter equation was used for correcting D_A for rate heterogeneity by replacing π with D_A.
Gene diversity of the Y-chromosomal microsatellite and its standard deviation were calculated with ARLEQUIN.

Population demography

Different tests were performed for past population expansions. In the first test, performed with ARLEQUIN, the distribution of the observed pairwise nucleotide site differences (mismatch distribution) is compared with the expected distribution in growing populations (expansion model) (Rogers and Harpending 1992). In an exponentially growing population the mismatch distribution will be nearly a (unimodal) Poisson distribution (Slatkin and Hudson 1991). However, it must be mentioned that a similar distribution may be obtained by selection of a favourable mutant that sweeps the whole population (Marjoram and Donelly 1994) or mutation rate heterogeneity (Aris-Brosou and Excoffier 1996). The test is based on three parameters (Rogers and Harpending 1992): θ_0, θ_1 (θ before and after the population growth), and τ (date of the growth in units of mutational time; $\tau = 2\mu t$, where μ is the mutation rate for the whole sequence and t is the time). By letting θ_1 as infinite it is possible to estimate both θ_0 and τ (Rogers 1995). Confidence intervals for τ were estimated by a parametric bootstrap approach (Schneider and Excoffier 1999). The validity of the expansion model was tested by the same parametric bootstrap approach, which compares the fit to the expected mismatch distribution of the observed and 100 simulated mismatch distributions. When 95% or more of the simulated mismatch distributions show a better fit than the observed one, the expansion model is rejected. The fit to the expected mismatch distribution was quantified by the sum of squared deviations (SSD) between the observed,

simulated and expected mismatch distribution. SSD was calculated after dividing the number of observations per mismatch number by the total number of observations.

Other test that were performed for past population expansions are Tajima's D (Tajima 1989a,b), and Fu's Fs (Fu 1997). Tajima's D was calculated with DNASP and Fu's Fs with an online program available on the webpage of Fu (reference in Fu 1997). These statistical tests analyse whether a population evolves according to the Wright-Fischer model and all mutations are selectively neutral (Fu 1997). The purpose of these tests is to detect departures characterised by an excess of rare alleles and a reduction of common alleles, which tends to give negative values (nucleotide sites treated as separate loci) (Fu 1997). Such departures may be a result of a population expansion or, alternatively, selection.

To test for a population expansion in Cape buffalo at the subspecies level, samples from different populations were pooled. This has also been done for the mismatch distributions and Tajima's D in greenfinch (*Carduelis chloris*), Fennoscandian willow tit (*Parus montanus borealis*) and humans (*Homo sapiens*) (Rogers 1995, Merila et al. 1997, Kvist et al. 1998, Hawks et al. 2000). When combining data from eastern and southern Africa, 8 samples per population were chosen at random from eastern Africa (populations with no significant differentiation were treated as one) plus the single samples from Kidepo Valley, Selous GR and Mount Kenya. This resulted in a sample size of 83 for eastern Africa and 82 for southern Africa.

Results

MtDNA

The analysis of HVI was based on 351 bp, including insertions and deletions. Two haplotypes were observed in one individual from Angola and two from Forest buffalo with unknown origin, that had a 74 bp deletion. Because this deletion decreased the number of polymorphic sites by 38%, these haplotypes were not included in further calculations. In a MSN these haplotypes grouped together with haplotypes from central and south-western Africa. A total number of 124 haplotypes were observed. Insertions and deletions were identified at 35 sites (10%), 140 sites (40%) were polymorphic and 176 sites (50%) monomorphic. At only 8 sites were three different nucleotides detected. No sites with four different

nucleotides were observed. The expected transition:tranversion ratio was 25:1 and α was 0.34. Excluding sites with alignment gaps or unresolved nucleotides left 286 bp for analysis of which 120 sites were polymorphic and resulted in 109 haplotypes. The 286 bp sequence showed an α of 0.29.

Between most populations pairs there was significant differentiation ($p < 0.05$, many pairs $p < 0.00001$). No significant differentiation was observed between adjoining populations Queen Elisabeth Mweya - Ishasha, Chobe - Hwange, Gonarezhou - northern Kruger and between populations in the Masai Mara - Serengeti - Maswa ecosystem ($p > 0.05$). In contrast, significant differentiation was observed between adjoining populations Amboseli - Tsavo West ($p = 0.017$), Tsavo West - Tsavo East ($p = 0.046$) and northern Kruger - southern Kruger ($p = 0.014$).

Among Cape buffalo populations from eastern and southern Africa H varied between 0.800 and 0.937 and π between 0.0282 and 0.0539. Among these populations significant differences were observed in π ($p = 0.03$). Populations from southern Africa showed a significant higher π than those from eastern Africa ($p = 0.04$, Table 4.2). Genetic diversity was compared at the regional level between Cape buffalo and buffalo from central and south-western Africa. This was done after selecting six samples at random from six randomly chosen Cape buffalo populations (Queen Elisabeth NP I., Laikipia Plateau, Selous GR, Hwange NP, Gonarezhou NP, southern Kruger NP) and six samples from central and south-western Africa (Elephant Camp (1, randomly chosen), Zone de Rhinoceros, Lope Reserve (2), Okahandja, Forest buffalo with unknown origin). With this sampling scheme, buffalo from central and south-western Africa showed a π of 0.075 (SD = 0.009), which is significantly higher than the value of 0.042 (SD = 0.009) observed for Cape buffalo ($p = 0.025$).

Table 4.2: Summary of mtDNA HVI diversity in African buffalo populations.

Region	π (SD)	H (SD)	θ
E. Africa	0.038 (0.002)	0.89 (0.01)	0.047
S. Africa	0.049 (0.003)	0.92 (0.02)	0.063
central Africa	0.065* (-†)	0.94* (-†)	0.091

*: calculated as (15 * π-H Elephant Camp + 1 * π-H Lope Reserve)/16. Numbers in the denominator refer to the number of pairwise comparisons between haplotypes. †: two populations only.

Significant isolation by distance was observed among Cape buffalo populations from eastern and southern Africa ($p < 0.0005$, Figure 4.2). F_{ST}, (Figure 4.2), F_{CT} and D_A (Table 4.3) values between Cameroon-Gabon and Cape buffalo were at least three times as large as those observed among Cape buffalo populations.

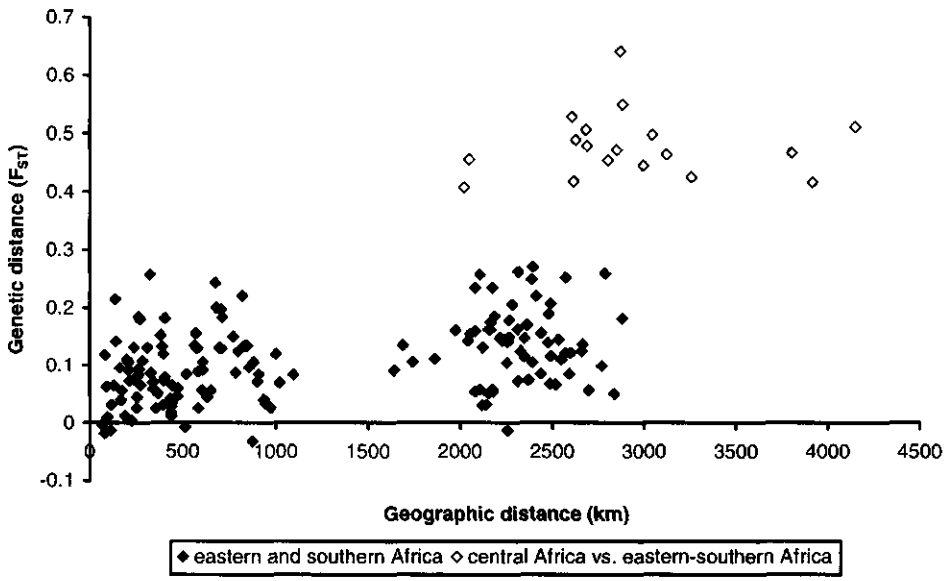

Figure 4.2: Correlation between geographic and genetic distance (F_{ST}). F_{ST} was based on 286-bp HVI haplotypes and calculated between all possible population pairs.

Table 4.3: Genetic distances between African buffalo populations.

Region	F-statistics	D_A (%)
between populations in E. and S. Africa*	F_{SC}: 0.099	0.32† (0.33‡)
between E. and S. Africa	F_{CT}: 0.071	0.34§ (0.35‡)
between all populations in E. - S. Africa	F_{ST}: 0.163	0.44† (0.45‡)
between central Africa and E. - S. Africa	F_{CT}: 0.481**	3.82§ (4.67‡)

*: no comparisons between eastern and southern Africa, †: mean of all pairwise population comparisons, ‡: correction for rate heterogeneity, §: pooling of populations, ** In this case F_{ST} and F_{SC} are not appropriate as central Africa was treated as one population only.

In the MSN (Figure 4.3) two clades of haplotypes could be discerned. One consisted of haplotypes from Cape buffalo and the other of those from buffalo in central and south-western Africa. The latter clade included three haplotypes from

six Cape buffalo as well as the two haplotypes with a 74 bp deletion. Haplotypes from central and south-western Africa did not group according to geographical origin and were separated by relatively large genetic distances. Among the haplotypes from Cape buffalo, those from southern Africa could be divided into two subgroups: one that grouped with haplotypes from Uganda (11 haplotypes) and another that was found at tip positions of haplotypes from Kenya and Tanzania (20 haplotypes).

The mismatch distribution among pooled Cape buffalo populations from eastern and southern Africa was bimodal with the height and the place of the highest peak similar to that expected for an expanding population (Figure 4.4). However, when the haplotypes were divided into three parts with equal numbers of polymorphic sites, the mismatch distribution for each part was unimodal and showed a better fit to the expected distribution than the whole sequence (Figure 4.5-4.7). These partial sequences showed a higher α (0.44, 0.47 and 0.86 respectively) than the whole sequence (0.29), indicative of less mutation rate heterogeneity. The mismatch distributions of the whole and partial sequences showed a similar fit to the expected distributions as the simulated data sets (55-95% of the latter showed a worse fit than the actual data set). The parameters of the 'expansion model' were derived from the whole sequences and were estimated as follow: $\theta_0 = 0.023$, $\theta_1 = 0.236$, $\tau = 7.1$ (95% confidence interval: 3.8-20.1). Among the pooled Cape buffalo populations the value of Fu's Fs was -19.78, which is significant ($p < 0.05$), in contrast to the value of -0.944 for Tajima's D ($p > 0.1$). However, the latter showed a significant value of -1.832 ($p < 0.05$) for the first part of the sequence.

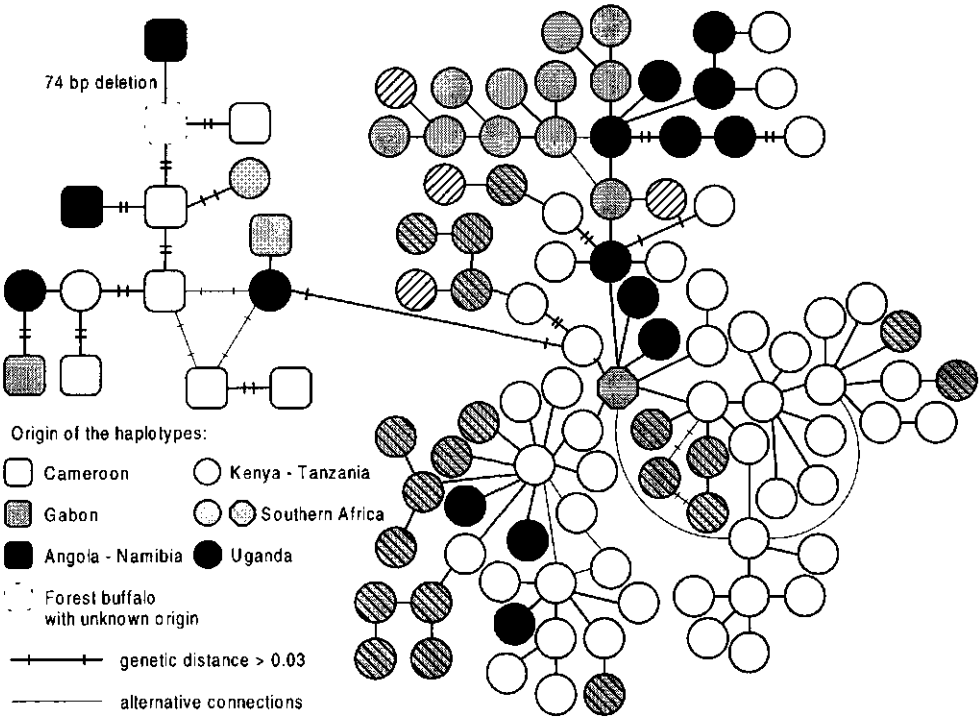

Figure 4.3: Minimum spanning network of 110 286 bp HVI haplotypes using TN93 genetic distances. The haplotypes from central and south-western Africa (Cameroon, Gabon, Angola, Namibia, forest buffalo with unknown origin) and those from Cape buffalo (Uganda, Kenya, Tanzania, southern Africa) form two clades. These two clades seem to converge at the octangular haplotype in the centre. The two haplotypes connecting the two clades are separated by 6.6% sequence divergence. Haplotypes from southern Africa on tip positions of those from Kenya-Tanzania and vice versa are hatched.

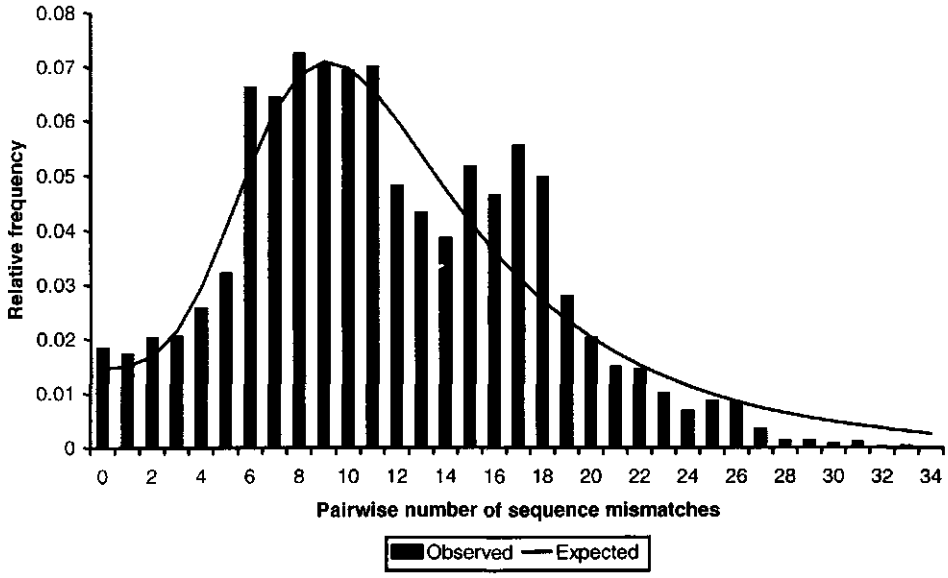

Figure 4.4: Mismatch distribution between HVI haplotypes of Cape buffalo, whole sequence (286 bp). This figure is based on the number of point mutations between all possible haplotype pairs after pooling of populations from eastern and southern Africa. Polymorphic sites 91, SSD = $21.8*10^{-4}$.

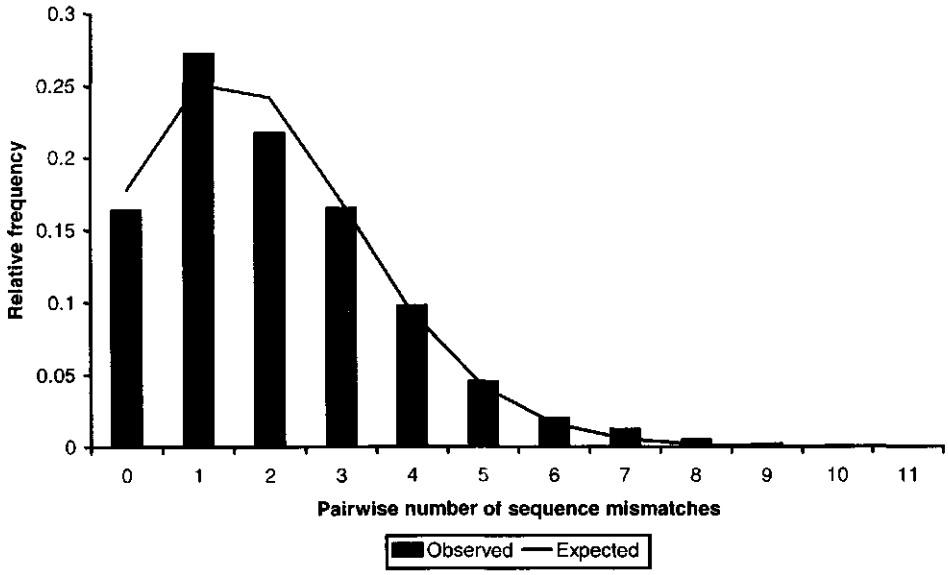

Figure 4.5: Mismatch distribution between HVI haplotypes of Cape buffalo, nucleotide sites 1-150. This figure is based on the number of point mutations between all possible haplotype pairs after pooling of populations from eastern and southern Africa. Polymorphic sites = 31, SSD = $13.8*10^{-4}$.

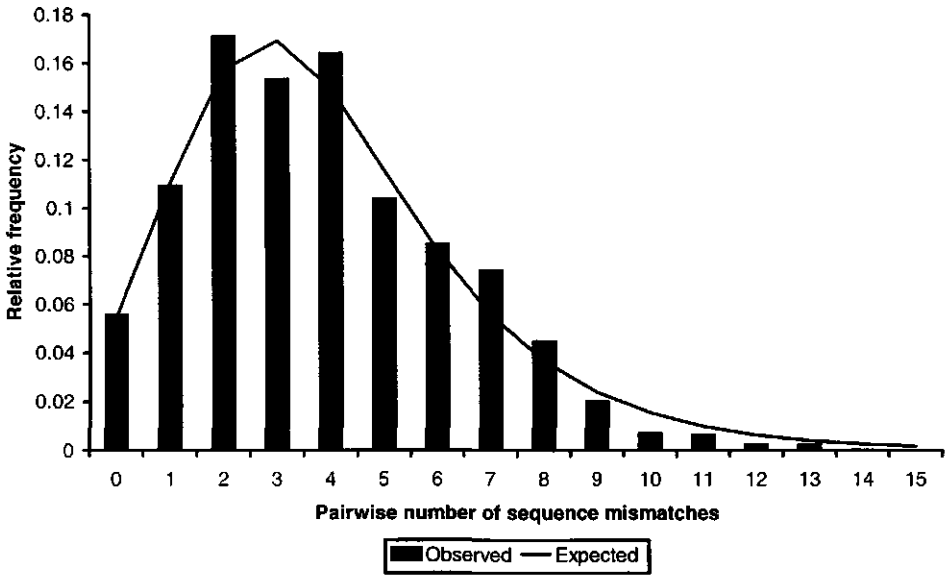

Figure 4.6: Mismatch distribution between HVI haplotypes of Cape buffalo, nucleotide sites 151-233. This figure is based on the number of point mutations between all possible haplotype pairs after pooling of populations from eastern and southern Africa. Polymorphic sites = 30, SSD: $13.2*10^{-4}$.

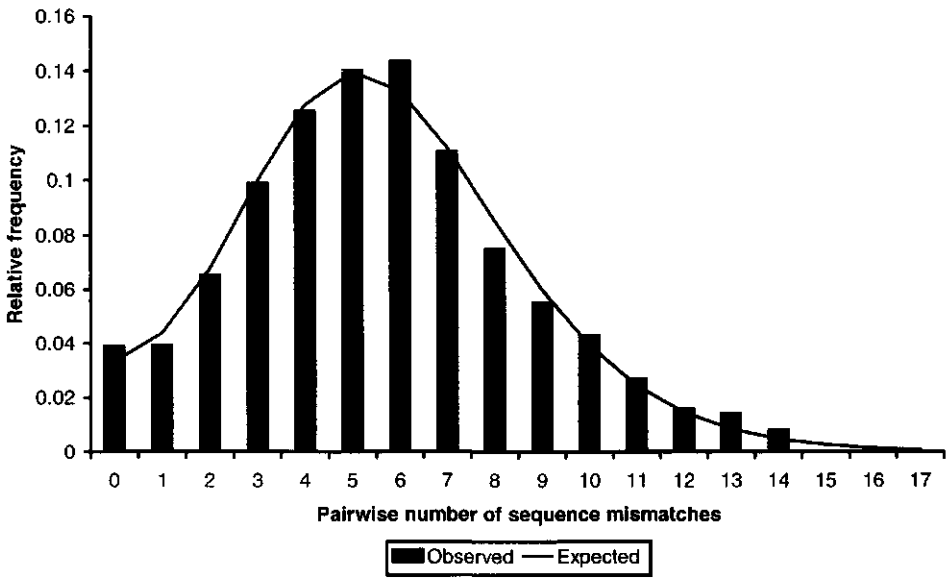

Figure 4.7: Mismatch distribution between HVI haplotypes of Cape buffalo, nucleotide sites 234-286. This figure is based on the number of point mutations between all possible haplotype pairs after pooling of populations from eastern and southern Africa. Polymorphic sites 30, SSD $3.9*10^{-4}$.

Y-chromosomal microsatellite INRA008

A total of 66 males from eastern and southern Africa and 7 males from central and south-western Africa were genotyped at INRA008. This microsatellite was monomorphic in eastern and southern Africa but polymorphic in central and south-western Africa where four alleles were observed (Table 4.4), all different from the allele in eastern and southern Africa. Gene diversity of the pooled central and south-western Africa samples was 0.857 +/- 0.102. Due to multiple DNA fragments after PCR and the fact that this microsatellite was monomorphic in eastern and southern Africa, no genetic distances could be calculated. The fact that the Y-chromosomal microsatellite did not amplify in any female and was subspecies and country specific (Table 4.4) make us feel confident of the results, despite the multiple DNA fragments that made determination of allele length impossible.

Table 4.4: Summary of Y-chromosomal microsatellite INRA008 diversity.

Region	Alleles (sample size)
Kenya	a (37)
Tanzania	a (14)
South Africa	a (15)
Cameroon	b (2)
Gabon	c (2)
Namibia	d (1)
Forest buffalo (unknown origin)	d (1), e (1)

Size of DNA fragments in bp (multiple peaks on ABI sequencer):
a: 115, 127, b: 115, 151, c: 115, 135, 144, 148, d: 115, 121, 131, 135, 141, 146, e: 115, 129, 137, 146, 153.

Discussion

Cape buffalo from eastern and southern Africa

As in previous mtDNA studies on Cape buffalo within population mtDNA diversity was high with little differentiation between populations. However, weak but significant isolation by distance was observed. Average pairwise F_{ST} values between populations from eastern Africa on one hand and southern Africa on the other (0.14, Figure 4.2) are comparable to the mean pairwise Rho (a F_{ST} analogue) for microsatellites (0.11-0.15) between these regions (Simonsen et al. 1998, Van Hooft et al. 2000). Mean π in Cape buffalo is the highest while

population differentiation is the lowest among African mammals (π: 0.014 (Elephant) - 0.04 (Impala), F_{ST}: 0.17 (Waterbuck) - 0.80 (Sable Antelope)) (Simonsen et al. 1998, Matthee and Robinson 1999, Nyakaana and Arctander 1999). In agreement with earlier studies using microsatellites (Simonsen et al. 1998, Van Hooft et al. 2000), significant differentiation between adjoining populations was observed only in the Amboseli - Tsavo ecosystem and between northern and southern Kruger. These results show that on an evolutionary time scale female buffalo have a high migration rate, which seems to contradict their observed philopatric behaviour (Sinclair 1977, Prins 1996). However, the small genetic distances might be a result of specific demographic events in evolutionary history.

Mutation rate was estimated from the number of transversions between cattle (*Bos taurus*, Genbank accession number U87905) and buffalo, assuming a divergence time of 4.0-7.7 million years (Modi et al. 1996, Hassanin and Douzery 1999). Transitions were observed at 6.4% of the sites, which after correcting for rate heterogeneity resulted in an estimate of 9.0%. With a transition:tranversion ratio of 25:1, this led to an estimate of 15-29% substitutions per million years. Assuming a generation time of 7.5 years in buffalo (O'Ryan et al. 1998), our estimate of mutation rate indicates a N_{ef} of 10,700-27,500. This is similar to the estimate of overall effective population size of 2,600-24,000 derived from microsatellite diversity (Van Hooft et al. 2000).

The mismatch distributions were close to the theoretically expected unimodal distributions in case of population expansion while Fu's Fs showed a significant negative value. The combination of these two observations is an indication for a recent population expansion. A significant negative Tajima's D only for the first part of the sequence may be due to the high mutation rate heterogeneity at HVI. According to a simulation study (Aris-Brosou and Excoffier 1996), high mutation rate heterogeneity ($\alpha \leq 0.4$) results in less negative or positive Tajima's D values, even in the presence of a population expansion. The relatively high mutation rate heterogeneity of the whole sequence, may also explain the bimodality of its mismatch distribution. Alternatively, it could be that some substructuring developed since the onset of the expansion, too weak to be visible in the mismatch distributions of the partial sequences. From the mismatch distribution and the mutation rate estimate it was derived that the hypothesised expansion started 235,000-23,000 years ago, which coincides with the latter part of the middle

Pleistocene (700,000-130,000 years ago) and the upper Pleistocene (130,000-11,000 years ago). Furthermore, according to their net sequence divergence buffalo in central Africa and Cape buffalo diverged only 156,000-81,000 years ago, which more or less coincides with the estimated date of the expansion. Interestingly, from fossil data it has been hypothesised that during the upper Pleistocene African buffalo expanded its range from the rain forests in central and western Africa to the savannas of eastern and southern Africa (Kingdon 1982, Klein 1988, Vrba 1995). Our data thus seem to support this expansion hypothesis. However, as our data may also be a result of selection on a part of the mitochondrial genome or mutation rate heterogeneity the expansion hypothesis needs to be studied further by analysing additional autosomal loci.

Extra support for the expansion hypothesis was observed in the MSN. The pattern of haplotypes from Cape buffalo in the MSN was starlike, as would be expected for recently expanded populations (Slatkin and Hudson 1991). Furthermore, a large fraction of haplotypes (20/33 = 60.6%) from southern Africa was found at the tips of haplotypes from Kenya and Tanzania, but a considerably smaller fraction the other way round (4/52 = 7.7%). As haplotypes at the tips are relatively young, this pattern indicates a predominant north-south gene flow between Kenya-Tanzania and southern Africa. This directionality may be explained by a past southward expansion of populations in Kenya and Tanzania. This southward expansion might be related to vegetation changes in Africa since the middle Pleistocene. During the last 220,000 years there has been two full glacial cycles. During many glacial periods large parts of Africa had a dry climate and savanna and woodland areas had a more northerly distribution than present (Van Zinderen Bakker 1978). During an interglacial the savanna and woodland areas extended their range southwards together with the mammals they support. This southward expansion might also explain the small genetic distances between eastern and southern Africa for mtDNA and microsatellites. The other fraction of southern African haplotypes (13/33) that is not found at tip positions of Kenyan-Tanzanian haplotypes might be descended from a population that was present before the southward expansion or from a group of early colonisers. Mixing of the two groups of haplotypes would explain the relatively high π in southern Africa. A past geographical barrier between eastern and southern Africa has also been suggested for three other bovids, where mtDNA haplotypes from these regions formed separate clades (Arctander et al. 1999). Interestingly, a population expansion has also been hypothesised for the African elephant (Georgiadis et al. 1994), the only other African mammal that is

found both on savannas and in rain forests. This might suggest a similar response to vegetation changes, which is not unlikely as elephant is the major competitor of African buffalo for food (Prins 1996, p. 174-177).

Buffalo from central and south-western Africa

In the MSN haplotypes from central and south-western Africa formed a separate clade. Furthermore, genetic distances between central Africa and Cape buffalo were considerably larger than those among Cape buffalo populations. This is in agreement with an earlier study on buffalo using autosomal microsatellites, where buffalo from central Africa are shown to have a large fraction of alleles not present in Cape buffalo (Van Hooft et al. 2000). A similar observation has been done in this study with respect to the Y-chromosomal microsatellite. The observation that buffalo from Angola and Namibia, the latter currently classified as Cape buffalo (Figure 4.1), seem to be more closely related to buffalo from central Africa than to Cape buffalo was surprising. Currently no strong geographical barriers are present between south-western and southern Africa, except for the arid Kalahari desert (southern Botswana) and Karoo (western South Africa) in the most southern part of Africa. However, in the past populations in these two areas might have been separated by an arid corridor that during glacial periods run from Namibia in a north-easterly direction to Tanzania (Kingdon 1989 p. 9, Van Zinderen Bakker 1978). More samples are needed to reveal to precise taxonomic position of buffalo from this part of Africa and its potential relationship with past geographical barriers.

Buffalo from central and south-western Africa showed a higher Y-chromosomal microsatellite and nucleotide diversity (π) than Cape buffalo. Furthermore, the number of microsatellite alleles may even be underestimated due to the small sample size in this part of Africa. This high genetic diversity at the regional level may be a result of habitat fragmentation or ancestral isolation in allopatry, followed by secondary contact (Georgiadis et al. 1994). The observation that in the MSN the haplotypes from central and south-western Africa did not group according to geographical origin seem to support the latter explanation. Ancestral isolation in allopatry is not unlikely, as especially this part of Africa is characterised by long periods of aridity (Kingdon 1989, p. 14-17, Stokes et al. 1998, Dupont et al. 2000) resulting in islands of wet habitats. To our knowledge, the only other mammal with comparable levels of HVI diversity is the Chimpanzee (*Pan troglodytes*, π = 0.075 with populations pooled) from the African rain forest (Wise et al. 1997). Also for this

species recurrent fragmentation of its natural habitat has been put forward as an explanation of its high mtDNA diversity (Morin et al. 1994). However, our explanation is necessarily tentative due to the limited number of buffalo samples from central and south-western Africa.

In conclusion, the population genetic part of this study showed that in African buffalo the within population mtDNA diversity is high with little differentiation between populations, in agreement with earlier studies. In addition, estimates of female effective population size could be derived. The evolutionary history of Cape buffalo since the Pleistocene as derived from the phylogeographic analyses, appeared to be in agreement with fossil data. Large genetic differences, both mitochondrial and Y-chromosomal, were observed between Cape buffalo and buffalo in central and south-western Africa. This can have consequences for the conservation policy for African buffalo as it stresses the need for the preservation of different genetic lineages.

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Genetic structure of African buffalo herds based on variation at mitochondrial and microsatellite loci: indications for male biased gene flow

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Abstract

Sexual differences in herding behaviour of buffalo are studied by analysing mitochondrial D-loop hypervariable region I and microsatellite diversity at both the population and herd level. A total of eight herds were sampled from Arusha National Park in Tanzania and Kruger National Park in South Africa. Significant genetic differentiation is observed among populations and herds with maternally inherited mitochondrial DNA but not with fourteen autosomal microsatellites. These results support the ecological observations of philopatric behaviour of female buffalo and male biased migration between herds. However, the results are in contrast to observations in other studies, where mitochondrial DNA and microsatellite data appear to be congruent at the population level. Various explanations are put forward to explain this difference. Finally, differentiation at the mitochondrial genome appears to be stronger between herds than at the population or even regional level (eastern versus southern Africa). This is explained by assuming a Pleistocene population expansion, as has been postulated in an earlier phylogeographic study.

Introduction

The African buffalo, *Syncerus caffer*, historically inhabits nearly the whole of sub-Saharan Africa, where it constitutes up to 35% of the large herbivore biomass. African buffalo live in herds that vary in size between 12 to 1600 individuals (Sinclair 1977 p. 119, Prins 1996 p. 72-77). The average herd size can vary considerably between different areas, depending on vegetation density and food abundance. For example in Tanzania, on the Serengeti plains and in the fertile Lake Manyara National Park (NP), the average herd size is some 350 individuals (Sinclair 1977 p. 120, Prins 1996 p. 72-77), while in the montane forest of Mount Meru it is only 50 individuals (Sinclair 1977 p. 120, own observations 1998). Herds have a fixed home range, which varies in size between 5 and 100 km^2 depending on herd size (Sinclair 1977 p. 98, Prins 1996 p. 19). The period a herd can survive as a separate entity is unknown, but for some herds it is at least 36 years (Prins 1996 p. 72-77).

Migration between herds seems to be male biased. Female and subadult buffalo appear to remain in their native herd (philopatric behaviour, Sinclair 1977 p. 132-142, Kingdon 1982, Prins 1996 p. 72). The migratory behaviour of male buffalo may vary between different areas. In Lake Manyara NP adult males have been observed to frequently migrate between herds and travel distances up to 50 km (Prins 1996 p. 87-89), whereas in the Serengeti most adult males appear to stay in their native herd (Sinclair 1977 p. 132, 142). However, in the latter case it must be mentioned that the mentioned study was not very clear on that point. In Lake Manyara NP male buffalo preferentially associate in small groups when they are outside a herd. Also in the Serengeti male buffalo form groups on their own, but these groups tend to remain associated with their native herd.

Different population genetic studies on African buffalo have recently been conducted, mostly focussing on the subspecies Cape buffalo from eastern and southern Africa (Grobler and Van der Bank 1996, Templeton and Georgiadis 1996, O'Ryan et al. 1998, Simonsen et al. 1998, Wenink et al. 1998, Van Hooft et al. 1999, Van Hooft et al. 2000, Van Hooft et al. 2001). Considering the different migration behaviour of female and male buffalo, one might expect differences in the population genetic structure for the two sexes. However, this has not been observed. The level of population differentiation as indicated by Wright's F_{ST} (or analogues) was similar for mitochondrial DNA (mtDNA) and autosomal

microsatellites. Actually, the F_{ST} values for mtDNA were low compared to those observed in other African mammals (Simonsen et al. 1998, Matthee and Robinson 1999, Nyakaana and Arctander 1999, Van Hooft et al. 2001), indicating high levels of female gene flow. Furthermore, between some neighbouring populations no significant differentiation was observed for mtDNA (Simonsen et al. 1998, Van Hooft et al. 2001). The latter two results seem to disagree with the alleged philopatric behaviour of female buffalo. However, data analysis was performed at the population level and it may not be justifiable to extrapolate to the herd level. For example, in a recent phylogeographic study (Van Hooft et al. 2001) there were indications that the low F_{ST} values between populations at the mitochondrial genome could at least partly be a result of a Pleistocene population expansion.

In this study we analysed mtDNA and autosomal microsatellite diversity of African buffalo (subspecies Cape buffalo) at both the population and herd level in order to shed light on their herding behaviour. Comparisons between mtDNA and microsatellites might show differences between the two sexes. Unfortunately, no polymorphic Y-chromosomal microsatellites are known for Cape buffalo (Van Hooft et al. 2001), which in this respect would be optimal.

Materials and methods

The samples in this study have also been used in previous studies (Van Hooft et al. 2000, Van Hooft et al. 2001). However, then statistical analyses were performed only at the population level. In those studies sample collection, DNA extraction, PCR amplification, microsatellite genotyping and sequencing are described in detail. Here additional information is given relevant for the herd analyses.

Sample collection

A total number of 89 samples were used from Kruger National Park (NP) in South Africa and Arusha NP in Tanzania. Kruger NP embraces an area of 19,500 km^2 and contains some 35,000 buffalo (O'Ryan et al. 1998). Arusha NP, situated on the slopes of Mount Meru, embraces an area of 52 km^2 and contains at least 500 buffalo (own counts from 1998). However, the actual number could be much higher, as count were performed in open areas only, while a large fraction of the park consists of montane forest.

In Kruger NP 41 tissue samples were collected from five herds. In earlier studies (Van Hooft et al. 2000, 2001) significant differentiation has been observed between northern Kruger NP (near Shingwedzi) and southern Kruger NP (near Lower Sabie). Therefore, for herd differentiation these localities were analysed separately (northern Kruger NP three herds, southern Kruger NP two herds). Herds varied in size between 140 and 320 individuals. Herds in northern and southern Kruger NP were separated by at least 25 and 8 km distance respectively. The distance between the sampling localities in northern and southern Kruger is 240 km. When analysing herd differentiation, samples from male buffalo older than two years were excluded (5 out of 41), to prevent a possible influence of mtDNA from male migrants. Male buffalo do not migrate out of their native herd or form groups on their own until they are three years old (Sinclair 1977 p. 121, Prins 1996 p. 85). The sample size per herd varied between 5 and 8 individuals (Table 5.1).

In Arusha NP 16 dung samples were collected from each of three herds observed at Serengeti Ndogo (herd a, 200 individuals), West of Momela gate (herd b, 60 individuals) and Momela Lakes (herd c, 65 individuals) Herds were separated by at least 6 km distance. Dung samples were collected as fresh as possible from places recently visited by a herd. By applying this sampling strategy some animals might have been sampled more than once. However, this is not expected to result in biased estimates of haplotype frequencies. Sexing of the individuals by using Y-chromosome specific markers was not successful due to the low quality of the DNA in the dung samples (data not shown). Therefore, some of the samples may be from adult male migrants. Herds can consist of some 5% to 33% adult males (Sinclair 1977 p. 122-123, Prins 1996 p. 72 - 114).

Statistical analysis

In Kruger NP 335 bp and in Arusha NP 333 bp of the mtDNA D-loop hypervariable region I (HVI) were analysed, which are the same subset of sites as in Van Hooft et al. 2001 when calculating nucleotide diversity (π). These sequences are available from Genbank under number AF313243 - 253, 256 - 265, 268 - 274, 276 - 283, 298 - 345. To test for differentiation between herds a Fisher's RxC test (Sokal and Rohlf 1995 p. 738-739) on haplotype frequencies (Raymond and Rousset 1995b) was performed with the program ARLEQUIN version 2.000. Haplotype diversity (H) and nucleotide diversity (π) plus their standard deviations were estimated with the program DNASP version 3.14 (Rozas and Rozas 1999). The genetic structure of

herds was investigated by Wright's F-statistics (analysis of variance) with ARLEQUIN, based both on the number of pairwise site differences between haplotypes as well as on haplotype frequencies. Gene flow was estimated as the number of migrants per generation (M) using the equation $M = \frac{1}{2}(1/F_{ST} - 1)$ (island model of population structure, Wright 1951).

In Kruger NP a set of fourteen microsatellites (Van Hooft et al. 2000) was analysed: ABS010, AGLA293, BM0719, BM3205, BM3517, BM4028, CSSM19, DIK020, ILSTS026, INRA006, INRA128, TGLA057, TGLA159 and TGLA263. PCR amplification of microsatellites in the dung samples from Arusha NP was not possible due to the relative low quality of DNA isolated from dung. Microsatellites were used for analysing both herd and population differentiation. In the latter case a comparison was made between microsatellites and mtDNA for differentiation between northern and southern Kruger NP. In earlier studies (Van Hooft et al. 2000, 2001) significant or near significant differentiation was observed for both genetic markers (microsatellites $p = 0.07$, mtDNA $p = 0.014$). However, in the case of microsatellites about twice as much alleles per locus were sampled (diploid vs. haploid). In order to match the sample sizes, in the case of microsatellites a random sample of twelve individuals from northern Kruger NP and nine individuals from southern Kruger NP was selected (including male buffalo). To test for differentiation between herds and populations a Fisher's RxC test was performed on allele frequencies of each locus between all pairs of herds/populations and all herds/populations simultaneously (Raymond and Rousset 1995b). Additionally, Fisher's Combined Probability test (Sokal and Rohlf 1995 p. 794-797) was employed as a global test over loci to determine the overall significance. Both tests were performed with TFPGA 1.3 (Miller 1997).

Results

Significant differentiation at mtDNA was observed among herds in Arusha NP (global $p < 0.00001$, all pairwise comparisons $p < 0.013$) but not in Kruger NP, neither with mtDNA nor microsatellites (global $p > 0.3$, all pairwise comparisons $p > 0.2$). Furthermore, significant differentiation was observed between northern and southern Kruger NP with mtDNA ($p = 0.014$, Van Hooft et al. 2001) but not with microsatellites ($p = 0.33$, this study).

MtDNA diversity and haplotype composition per herd are shown in Table 5.1 and Figure 5.1 and 5.2. The number of mtDNA haplotypes per herd varied between 4 and 7. The average number of haplotypes per herd in Kruger NP was 5.8 while in Arusha NP it was 6.0. The estimate for Arusha NP decreased to 4.3 when 7 animals per herd were selected at random to match to mean herd size in Kruger NP. In Arusha NP mean π and H of the herds was 8% lower than that of the pooled herds. In Arusha NP F_{ST} among herds was 0.111 (pairwise site differences between haplotypes) - 0.122 (haplotype frequencies) and varied between 0.018 and 0.176 among pairs of herds (Table 5.2). M among herds was estimated as 3.6 - 4.0. Between northern and southern Kruger NP the F_{ST} value for mtDNA was 0.080 while for microsatellites it was only -0.004 (F_{ST} analogue Rho using all available samples, Van Hooft et al. 2000).

Table 5.1: Summary of mitochondrial DNA diversity in herds of African buffalo in northern Kruger NP, southern Kruger NP and Arusha NP.

Herd	Sample size	NH	π (SD)	H (SD)
Arusha herds pooled	48	14	0.0282 (0.0033)	0.895 (0.025)
Arusha herd a	16	6	0.0212 (0.0019)	0.833 (0.056)
Arusha herd b	16	7	0.0306 (0.0038)	0.892 (0.040)
Arusha herd c	16	5	0.0257 (0.0081)	0.733 (0.079)
n. Kruger herds pooled	21	14	0.0531 (0.0038)	0.948 (0.031)
n. Kruger herd a	8	7	0.0541 (0.0082)	0.964 (0.077)
n. Kruger herd b	8	7	0.0583 (0.0073)	0.964 (0.077)
n. Kruger herd c	5	4	0.0460 (0.0133)	0.900 (0.161)
s. Kruger herds pooled	15	9	0.0468 (0.0063)	0.914 (0.047)
s. Kruger herd a	7	4	0.0438 (0.0107)	0.810 (0.130)
s. Kruger herd b	8	7	0.0514 (0.0093)	0.964 (0.077)

NH, number of haplotypes, π: nucleotide diversity, H: haplotype diversity.

Table 5.2: Genetic distance matrix for African buffalo herds in Arusha NP.

	herd a	herd b	herd c
herd a	x	0.068	0.176
herd b	0.018	x	0.119
herd c	0.170	0.135	x

Figures above diagonal represent F_{ST} based on mtDNA haplotype frequencies, figures below diagonal represent F_{ST} based on the number of pairwise site differences between mtDNA haplotypes.

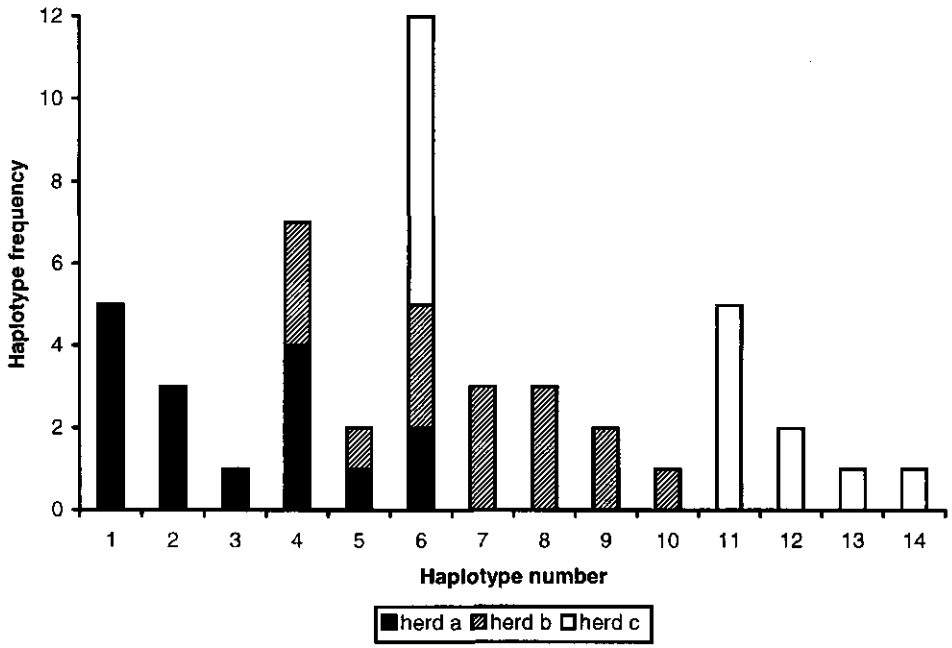

Figure 5.1: Haplotype composition of African buffalo herds in Arusha NP.
Corresponding Genbank numbers (last three digits) of the haplotypes: 1: 302-306, 2: 298-299, 301^, 3: 307, 4: 328-334, 5: 325-326, 6*: 300^ (herd a), 335-345, 7: 317-319, 8: 320-322, 9: 323-324, 10: 327, 11*: 311-315, 12: 309-310, 13#: 308, 14#: 316. *, #: These haplotypes are identical when sites with insertions/deletions and missing data are excluded according to Van Hooft et al. 2001 (resulting in a sequence length of 286 bp). ^: haplotypes -300 and -301 contain unresolved sites at the sequence end.

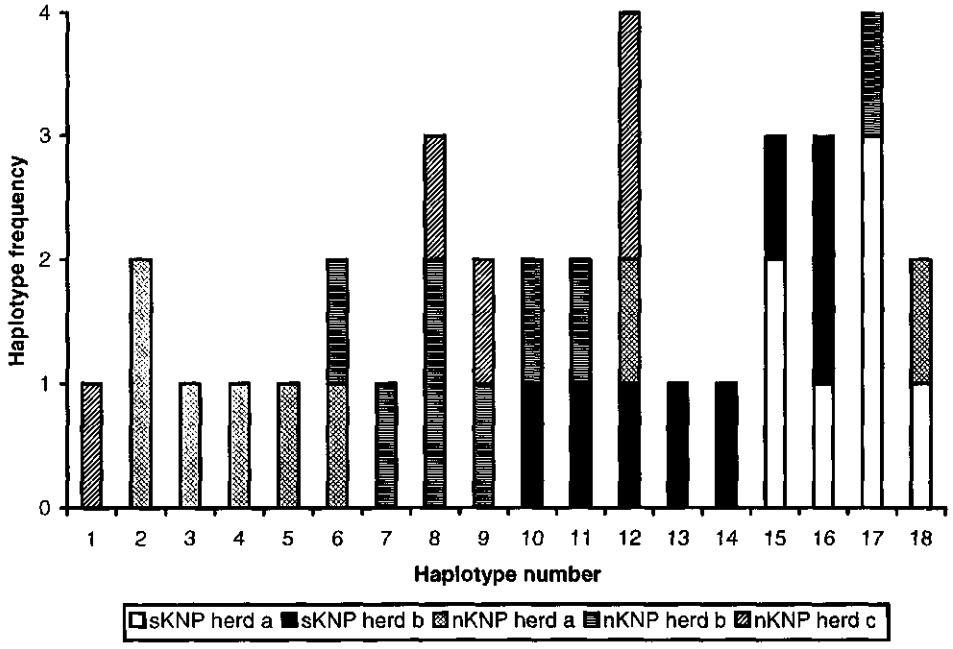

Figure 5.2: Haplotype composition of African buffalo herds in Kruger NP.
Corresponding Genbank numbers (last three digits) of the haplotypes: 1: 258, 2*: 246-247, 3: 243, 4^: 244, 5: 245, 6: 248-249, 7: 250, 8#: 251-253, 9: 256-257, 10: 261,276, 11: 262, 281, 12*: 263-265, 282, 13: 277, 14: 283, 15: 272-274, 16^: 278-280, 17: 259, 268-270, 18#: 260, 271. *, #, ^: These haplotypes are identical when sites with insertions/deletions and missing data are excluded according to Van Hooft et al. 2001 (resulting in a sequence length of 286 bp).

Discussion

Significant differentiation at mtDNA was observed among herds in Arusha NP, despite the possible inclusion of adult male migrants in the sample set, while no significant differentiation was observed among herds in Kruger NP, neither with mtDNA nor microsatellites. However, absence of significant herd differentiation at mtDNA may be due to small sample sizes in combination with a high haplotype diversity. When northern and southern Kruger NP were compared significant differentiation was observed for mtDNA, but still not for microsatellites. In addition, mtDNA showed a considerably larger F_{ST} value than the microsatellites. These observations indicate stronger herd differentiation at mtDNA than at microsatellites, thereby supporting the ecological observations of female philopatric behaviour and male biased migration. The alternative explanation of a smaller effective population size for mtDNA, because it is haploid and maternally inherited, seems unlikely as

ecological data (Prins 1996 p. 114) indicate a considerable larger female than male effective population size (factor 4).

Further support for the philopatric behaviour of female buffalo is given by the observation that significant mtDNA differentiation has been observed between adjoining populations in the Amboseli NP- Tsavo West NP- Tsavo East NP ecosystem, which are separated by only 100 km (Van Hooft et al. 2001). Although this has been attributed to limited migration due dryness of the habitat (Van Hooft et al. 2000), this may explain not all population differentiation. Considering the small geographic distances, herd differentiation might also be involved. It must be mentioned that significant differentiation between Amboseli NP and Tsavo NP (with at least 7 out of 8 samples from Tsavo East NP) has been observed for microsatellites as well (Van Hooft et al. 2000). However with microsatellites, allele frequencies were correlated. In contrast, the frequent (sampled more than once) mtDNA haplotypes in Amboseli NP and Tsavo East NP, with a total frequency of 70%, were unique for their population. Additional support for high levels of male gene flow between herds is given by the fact that in buffalo 21 out of 27 (78%) microsatellites do not show significant deviations (combined significance of individual loci $p = 0.1$) from Hardy-Weinberg equilibrium. This was calculated by a Fischer's combined probability test on data from O'Ryan et al. 1998 (5 loci $p = 0.1$), Simonsen et al. 1998 (5 loci $p = 0.2$) and Van Hooft et al. 2000 (11 loci $p = 0.25$). No significant deviations from Hardy-Weinberg equilibrium indicates absence of strong genetic substructuring in most populations.

Another African mammal where females form matrilineal groups is the elephant (*Loxodonta africana*). In contrast to African buffalo, in this species F_{ST} values for mtDNA were considerably larger than those for microsatellites between all population pairs, indicating male biased gene flow (Nyakaana and Arctander 1999). In addition negative F_{IS} (Weir and Cockerham 1984) values were observed, indicating that males are expelled from their native group. The absence of negative F_{IS} values in buffalo may indicate that male buffalo also mate with females from their native herd.

In both parks the mean number of haplotypes per herd was quite high considering the small size of the average herd and the small sample sizes. If there never had been gene flow between herds, a herd should contain no more than 1 or 2 haplotypes (Hartl and Clark 1989 p. 125, equation for effective number of alleles).

This indicates that the genetic diversity of a herd is primarily determined by gene flow. The amount of gene flow between herds in Arusha NP was estimated as 3.6-4.0 female migrants per generation, which is equal to 0.5 female migrants per year, assuming a generation time of 7.5 years (O'Ryan et al. 1998).

The low level of gene flow in Arusha NP in itself could explain why migration of females between herds has not been observed in ecological studies. Furthermore, gene flow probably is limited to certain periods and not a result of regular migration of individual female buffalo, as the few female buffalo that have been observed to migrate were not accepted by the other herd. However, it must be mentioned that this migration was a direct result of the animals being disoriented by the tranquillisation used in marking experiments (Sinclair 1977 p. 133, 139). More likely migration takes place during rare events when herds are forced to abandon their home range and split up. Such events could for example be droughts, flooding, fires or epidemics. When the conditions get better again buffalo may regroup to form new herds. Alternatively, migration might take place under 'normal' conditions in case a new herd is being formed. The social hierarchy in a recently formed herd might be less strict than normal, and such a herd might easier accept immigrant females. Many herds recurrently split into smaller fragments (fusion-fission society, Prins 1996 p. 77-83), which can ultimately lead to the formation of new herds (Sinclair 1977 p. 141, Prins 1996 p. 80-81).

The observation of population and herd differentiation at mtDNA seems to contradict the absence of significant mtDNA differentiation between neighbouring populations in other studies. One explanation could be that the amount of herd differentiation is related to herd size. The mean herd size in most populations is larger than that in Arusha NP. As a consequence, genetic drift could be less strong resulting in a slower increase of genetic differentiation with time. Furthermore, it could be that herds migrate in the course of generations. This may result in an evenly distribution of genetic variation throughout a population, while at the same time herds are differentiated. Apparently, in Kruger NP and the Amboseli NP-Tsavo NP ecosystem this has not taken place. This could be due to the dryness of the habitat in large part of these parks, which can limit migration (Van Hooft et al. 2000). In support of the 'migrating herd' hypothesis, in Lake Manyara NP immigration of two new herds has been observed in a period of 36 years (± 5 generations). One herd consisted of over 200 individuals, which results in an estimate of 40 immigrants/generation. The migratory potential of herds is further

supported by the observation that home ranges in the wet and dry season can lie apart as far as 40 km (Prins 1996 Figure 3.10).

In an earlier study, the F_{ST} value derived from the pairwise site differences between haplotypes among populations in either eastern or southern Africa was 0.099, while the F_{CT} value between these two regions (as F-statistics are hierarchical, this can be interpreted as F_{ST} with regions treated as populations) was 0.071 (Van Hooft et al. 2001). Both estimates are smaller than the value of 0.111 observed among herds in Arusha NP. The amount of genetic differentiation between different groups of buffalo thus appears to decrease rather than increase with increasing geographic scale, as one would expect in the case of isolation by distance. Furthermore, F_{ST} values in buffalo are low compared to other African mammals, while one would expect relatively strong isolation by distance because of the philopatric behaviour of female buffalo. The observations in African buffalo may be explained by a recent population expansion from a small ancestor population. In such a case the different contemporary populations will genetically be very similar and there will be no apparent or only weak isolation by distance (Slatkin 1993). In agreement with this explanation, an (upper) Pleistocene population expansion in African buffalo has been postulated in other studies based on both mtDNA (Van Hooft et al. 2001) and fossil data (Kingdon 1982, Klein 1988, Vrba 1995).

In conclusion, this study shows that significant mtDNA differentiation between buffalo herds is possible, supporting the field observations of philopatric behaviour of female buffalo. Furthermore, a decrease of the amount of genetic differentiation with increasing geographic scale was observed, which is an additional support for the hypothesis of a Pleistocene population expansion postulated in earlier studies.

Acknowledgements

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Summary and discussion

Introduction

Historically, African buffalo inhabits nearly the whole of sub-Saharan Africa (Prins 1996 p. 240). With elephant (*Loxodonta africana*) and wildebeest (*Connochaetes taurinus*) it comprises the bulk of large herbivore biomass in Africa (Drent and Prins 1987, Prins and Reitsma 1989). They live in herds that vary in size between 12 and 1600 individuals (Sinclair 1977 p. 119, Prins 1996 p. 72-77). Since the end of the 19th century African buffalo has been affected by rinderpest epidemics and by habitat fragmentation due to increasing human habitation and cultivation.

In this thesis the genetic diversity of the African buffalo (*Syncerus caffer*) is investigated. A better knowledge of the population genetics of the African buffalo might be necessary for an effective long-term management and protection of this species.

The initial goal was to study the effects of rinderpest epidemics and habitat fragmentation on the genetic diversity of buffalo populations. It was hypothesised that the local population bottlenecks caused by these two disturbances resulted in a significant decrease of genetic diversity. Later on the objective of this study shifted towards a comprehensive analysis of the genetic structure of buffalo populations, in order to get more insight into the evolutionary history of the African buffalo and to study the effects of its herding behaviour on genetic diversity.

Three types of genetic markers were used: autosomal microsatellites, Y-chromosomal microsatellites and sequences of mitochondrial DNA (D-loop hypervariable region I or HVI), which is maternally inherited. Both microsatellites and mtDNA have a high information content due to their high level of polymorphism.

Population genetics of the African buffalo

Buffalo from eastern and southern Africa (subspecies Cape buffalo)

To obtain microsatellites that could be used in African buffalo, 168 bovine autosomal microsatellites were tested for PCR amplification on a panel of seven Cape buffalo from both eastern and southern Africa (**Chapter 2**). Of the 139 microsatellites that could be amplified 90% was polymorphic, which is the highest

percentage among different species of *Bovidae* (Asian water buffalo, Bali cattle, goat, sheep) (Moore et al. 1994 1995, Kemp et al. 1995, Pépin et al. 1995, Hishida et al. 1996, De Gortari et al. 1997). Furthermore, mean observed heterozygosity of the polymorphic markers was 0.67, which is considerably higher than that observed in cattle (0.55) from Africa, Asia and Europe (*Bos taurus* and *Bos indicus*) (MacHugh et al. 1997). These observations were a first indication that Cape buffalo populations have a high genetic diversity, despite recent rinderpest epidemics and habitat fragmentation. This was confirmed by analyses of a subset of 14 microsatellites and mtDNA sequences on various Cape buffalo populations from eastern (Uganda, Kenya and Tanzania) and southern Africa (Botswana, Zimbabwe and South Africa) (**Chapter 3 and 4**). Both types of genetic markers showed a high genetic diversity, indicative of large long-term (time to reach mutation-drift equilibrium) effective population sizes: 2,600-24,000 individuals when microsatellites were used as marker and 10,700-27,500 females when mtDNA was used. Furthermore, with microsatellites statistical tests for a recent population bottleneck gave a negative outcome.

The apparent absence of a significant effect of rinderpest epidemics on genetic diversity is probably due to the fact that most buffalo populations were able to recover within only a few generations after major epidemics (Sinclair 1977 p. 5-7, Plowright 1982, Prins 1996 p. 122-127, Simonsen et al. 1998). Furthermore, considering the large effective population sizes, even after a severe bottleneck the absolute number of survivors in many populations could have been large enough to prevent a decline in genetic diversity. In addition, considering the high dispersal capabilities of buffalo (Prins 1996 p. 72-77, 87-89), immigration from neighbouring populations could have restored part of the lost genetic diversity. For example, the high genetic diversity in northern Kruger National Park (NP) (South Africa) despite the fact that between 1896 and the 1930s very few buffalo were here observed (Kruger NP, Department of Scientific Services), may be explained by immigration from Zimbabwe and southern Kruger NP. This explanation was supported both by genetic and historical ecological data (Kruger NP, Department of Scientific Services).

The apparent absence of a significant effect of habitat fragmentation on genetic diversity is probably a result of the fact that there have been only some 15 generations since the end of the 19th century and that many populations still have census sizes in the thousands, thereby limiting the effects of genetic drift. In the

cases where significant differentiation was observed between neighbouring populations (northern and southern Kruger NP, South Africa; Amboseli NP and Tsavo West NP, Tsavo West NP and Tsavo East NP, Kenya), it was probably related to the dryness of the habitats rather than human habitation or cultivation.

Although this thesis shows that at the moment buffalo populations are not genetically impoverished, this may change in the near future. Current census sizes of many buffalo populations are close to or smaller than the minimum estimates of long-term effective population size. In light of the ongoing destruction of natural habitats in Africa, this implies that without migration or translocation they will not be able to maintain historical levels of genetic diversity for long.

Genetic distances between buffalo populations were small even on a continental scale, both for microsatellites and mtDNA (**Chapter 3 and 4**). Only 11% of the microsatellite variation was observed among pairs of populations from eastern and southern Africa. Less than 8% of the mtDNA variation was observed between these two regions, which is low compared to various other African mammals (at least 17%) (Simonsen et al. 1998, Matthee and Robinson 1999, Nyakaana and Arctander 1999). The data on mtDNA thus indicate relatively high female gene flow levels on a continental scale, thereby contradicting the ecological observations of female philopatric behaviour (they appear to stay in their native herd throughout lifetime) (Sinclair 1977 p. 132-142, Kingdon 1982, Prins 1996 p. 72). However, genetic distances may not represent equilibrium values. This is supported by the observation that the fraction of mtDNA variation that is between groups of buffalo decreases rather than increases with increasing geographical scale (from herd to continental level) (**Chapter 5**). The small genetic distances may be explained by a Pleistocene population expansion, as indicated by four types of statistical analyses performed on the mtDNA data (mismatch distribution, Tajima's D-values, Fu's Fs values and minimum spanning network). After a recent (in evolutionary terms) population expansion, the different contemporary populations will genetically be very similar and there will be no apparent or only weak isolation by distance (Slatkin 1993). The hypothesised date of the expansion coincided with the estimated divergence time between Cape buffalo and buffalo in central and south-western Africa. Interestingly, from fossil data it has been hypothesised that during the upper Pleistocene African buffalo expanded its range from the rain forests in western and central Africa to the savannas of eastern and southern Africa (Kingdon 1982, Klein 1988, Vrba 1995). Our data can thus be regarded as support for this

expansion hypothesis. Furthermore, the recent ancestral haplotypes of many haplotypes from southern Africa seem to come from eastern Africa, indicating that at least a part of the population expansion went from eastern to southern Africa. This southward expansion might be related to vegetation changes in Africa. During many glacial periods large parts of Africa had a dry climate and savanna and woodland areas had a more northerly distribution than present. During an interglacial the savanna and woodland areas extended their range southwards together with the mammals they supported.

The whole mtDNA genome is in essence only one locus and it is therefore important to try to confirm the expansion hypothesis with autosomal and Y-chromosomal loci. Recently, methods have become available which test for past population expansions and directional migration using microsatellite data (Luikart and England 1999).

In **Chapter 5** the genetic structure of buffalo herds is investigated. MtDNA analysis of three buffalo herds in Arusha NP (Tanzania) showed highly significant herd differentiation with more than 10% of the genetic variation between herds. These data support the field observations of philopatric behaviour of female buffalo. In addition, there were several indications that within populations there was less genetic substructuring at microsatellites than at mtDNA, supporting the field observations of male biased dispersal between herds (Prins 1996 p. 87-89). A total number of 8 herds from Arusha NP and Kruger NP was analysed, each of which contained at least 4 haplotypes. This is quite high considering the small size of the average herd and it indicates that the genetic diversity of a herd is primarily determined by gene flow. On the other hand, migration of female buffalo between herds has never been observed in ecological studies. However, this may be a result of gene flow primarily taking place during natural disasters, like drought, flooding, fire and epidemics, when herds are forced to split up or during the formation of new herds. Between some neighbouring populations there was no significant mtDNA differentiation despite the philopatric behaviour of female buffalo. This might be a result of the migratory potential of herds, especially over the course of various generations.

Buffalo from central and south-western Africa

According to all three types of genetic markers buffalo from central (Cameroon and Gabon) and south-western Africa (Angola and Namibia) form a separate genetic lineage (**Chapter 3 and 4**). In a minimum spanning network the mtDNA haplotypes did not group according to geographical origin or currently recognised subspecies distribution, according to which there are at least two subspecies (Prins 1996 p. 242-243). This suggests that buffalo from this region actually form only one subspecies. It was surprising that the buffalo from south-western Africa were not more closely related to the Cape buffalo in southern Africa (Botswana, Zimbabwe and South Africa). Morphological they are quite similar and currently there are no strong geographical barriers present between these areas, except for the arid Kalahari desert (southern Botswana) and Karoo (western South Africa) in the most southern part of Africa. However, in the past populations in these two areas might have been separated by an arid corridor that during glacial periods runs from Namibia in a north-easterly direction to Tanzania (Kingdon 1989 p. 9, Van Zinderen Bakker 1978).

At the regional level, buffalo from central and south-western Africa showed a considerably higher genetic diversity than Cape buffalo for all three markers. MtDNA diversity was among the highest observed in mammals (Wise et al. 1997, Simonsen et al. 1998, Matthee and Robinson 1999, Nyakaana and Arctander 1999). It is unlikely that this is a result of large effective population sizes, considering the relative low population densities in this part of Africa. More likely, it is a result of long-term isolation in allopatry resulting in the development of different genetic lineages, followed by secondary contact. Secondary contact would explain why the haplotypes did not group according to geographical origin. This scenario is not unlikely, as especially this part of Africa is characterised by long periods of aridity in the past (evolutionary time scale) resulting in islands of wet habitats (rain forest refuge theory, extension of the Kalahari and the Sahara deserts) (Kingdon 1989, Stokes et al. 1998, Dupont et al. 2000). In an earlier study, this hypothesis has also been postulated to explain the comparable high levels of mtDNA diversity and presence of different mtDNA lineages in chimpanzees from the rain forest of central and western Africa (Morin et al. 1994). High levels of mtDNA diversity and significant mtDNA lineages have also been observed in gorilla, which is also found in the African rainforest (Ruvolo et al. 1994). In both species levels of sequence divergence between different mtDNA lineages were as large as those generally

observed between species. In African buffalo sequence divergence between some haplotypes from central Africa was as large as that on average between haplotypes from central Africa and Cape buffalo. Taken together, these observations indicate that many mammals of the African rainforest show a relatively high phylogenetic diversity (Bininda-Emonds et al. 2000, Smith et al. 2000), as a result of recurring habitat fragmentation. However, for a thorough testing of this hypothesis more detailed comparative phylogeographic studies (Moritz and Faith 1998, Arctander et al. 1999, Smith et al. 2000) are needed.

Relevance for the management of buffalo populations

Introduction

In the management of many natural populations and areas often low priority is given to the assessment of genetic diversity (Moritz 1994). It is generally assumed that protection of diversity at or above the species level will automatically protect genetic diversity (Moritz and Faith 1998). Also there is the idea that population genetic information is more relevant for long-term than short-term needs (Moritz 1994). However, information on genetic diversity may give many new insights into the taxonomy, demography and ecology of a species, making a more effective management possible (Milligan et al. 1995). Besides that, an optimal conservation of genetic diversity may be defined as a management goal in itself (Humphries et al. 1995, Crozier 1997, Moritz and Faith 1998).

The ongoing destruction of natural habitat in Africa might in the future require a more active management of wildlife populations, to guarantee their continued existence. There is also a strong self-interest for the African countries to conserve the remaining natural habitats. The tourism industry, one of the foremost earners of foreign revenue in African's economy, is primarily focussed on wildlife safaris, supporting a substantial proportion of the rural population in game areas.

Local populations

According to ecological studies buffalo need drinking water on a daily basis (Prins 1996 p. 7-11), and are therefore very vulnerable to drought. This is supported by the observation in this thesis that dry habitats form a strong barrier against migration. In the cases where significant differentiation was observed between

adjoining populations these are separated by relatively dry habitats. Therefore, in the management of wild populations during drought extra attention might be paid to buffalo. Periods of drought may increase in the near future in frequency and duration due to human induced climate change. Regional scenarios indicate increased aridity in currently dry areas (Magadza 2000), while a decrease in rainfall is expected for southern Africa from the 12 ºS parallel southwards (Meigh et al. 1999).

The large effective population sizes indicate that in many cases buffalo populations should be managed on a geographical scale above the level of national parks and game reserves (GR). For example, this thesis shows that buffalo in Masai Mara GR-Serengeti NP-Maswa GR (Kenya, Tanzania) (see also Simonsen et al. 1998), Chobe NP-Hwange NP (Botswana, Zimbabwe) (Simonsen et al. 1998) and Gonarezhou NP-northern Kruger NP (Zimbabwe, South Africa) genetically are one population, both according to autosomal microsatellites and mtDNA. These results support the observations in an earlier ecological study, where it has been postulated that the movement of adult bulls from Lake Manyara NP (Tanzania) should be studied on a scale equivalent to the size of the Masai Mara GR-Serengeti NP-Maswa GR ecosystem (Prins 1996 p. 254-255). On the other hand, in dry areas populations may genetically be subdivided into subpopulations, as was shown for the Amboseli NP-Tsavo West NP-Tsavo East NP ecosystem and Kruger NP. Populations that are genetically distinct might also be demographically independent, and thus require separate management strategies. In that respect genetic distinct populations are also being referred to as Management Units (Moritz 1994 1995).

Historical demographic patterns

In some population genetic studies it has been suggested to minimise the effects of habitat fragmentation by translocating animals between populations, thereby preventing inbreeding and keeping historical gene flow patterns in tact (Simonsen et al. 1998, O'Ryan et al. 1998, Matthee and Robinson 1999, Storfer 1999). Isolated populations of buffalo run a high risk of inbreeding, as the short-term (few generations) effective population sizes in African buffalo may be as small as 10% of the census size (O'Ryan et al. 1998). Furthermore, the large long-term effective population sizes indicate that most probably no adaptive evolution has taken place to small population sizes with low genetic diversity, making African buffalo extra

vulnerable to habitat fragmentation. Also the argument of preserving historical gene flow patterns is important as it is generally accepted among conservation genetics that the focus should be on maintaining the overall process, rather than the individual populations or Management Units (Smith et al. 1993, Moritz 1995, Crandall et al. 2000).

In African buffalo, the indications for unidirectional southward gene flow (**Chapter 4**) implies that translocations from southern to eastern Africa should be avoided. Furthermore, gene flow estimates may be overestimates of the short term situation due to a Pleistocene population expansion. Therefore, if translocations should take place with buffalo it is advisable to obtain estimates of short-term gene flow. One possible way to achieve this is by the use of assignment tests (Sih et al. 2000). With these tests individuals are assigned to their most probable population of origin on basis of their allelic composition across different loci. In this way migration is measured directly. Another possibility may be the use of relatively rapidly mutating microsatellites (Neigel 1997). At such microsatellites individual alleles generated by mutation will appear and be dispersed only briefly in a population before they are changed by new mutations. In such a case, the spatial distribution of alleles in a population can be interpreted as short-term traces of gene flow. Mutation rates at some microsatellites may be as high as $5*10^{-2}$ per generation (Neigel 1997), which in the case of African buffalo would be one mutation per 150 years. Finally, one should be cautious with translocations as there is the risk that different genetic lineages are mixed and/or local genetic adaptation is being prevented (outbreeding depression), which may threaten the survival of certain populations (Storfer 1999).

The presence of at least two different genetic lineages shows that there are different groups of buffalo characterised by an unique evolutionary history. These lineages therefore deserve separate conservation efforts and no translocations should take place between them. Furthermore, there are indications of a recent colonisation of southern Africa by buffalo from eastern Africa and for many mammals different mtDNA lineages are observed in eastern and southern Africa. It can therefore be argued that separate conservation efforts are required for the ecosystems in these two regions.

It has been estimated that 66% of the mammals are characterised by at least two highly distinctive mtDNA lineages, which in most circumstances coincide with at least part of currently defined subspecies boundaries (Avise and Walker 1999).

This shows that in population genetics the subspecies concept can be meaningful. However, the total number of subspecies is probably less based on genetic information than on morphological characters, as in this thesis has also been suggested for buffalo from central and south-western Africa. Groups of populations with distinctive mtDNA lineages that also differ significantly for the frequency of nuclear alleles are also referred to as Evolutionary Significant Units (ESU; Moritz 1994 1995). The concept of an ESU used in this context is a strict application of the phylogenetic (sub)species concept (Vogler and Desalle 1994, Avise and Walker 2000). However, there is still debate in to what extent also ecological data (life history traits, ecology, morphology, demography) should be valued in the characterisation of ESUs (Beninda-Emonds et al. 2000, Crandall et al. 2000).

Areas that contain different species with a relatively high phylogenetic diversity, have a particularly high conservation value (Moritz and Faith 1998, Bininda-Emonds et al. 2000, Smith et al. 2000). Earlier in this chapter this has been shown for the African rain forest, with respect to buffalo and various other mammals. Moreover, the African rain forest is already renowned for its high biodiversity at the interspecies level (Dupont et al. 2000).

Genetic monitoring

Observation of significant mtDNA differentiation between herds implies they have a unique haplotype composition. This might be monitored over time, which may result in more insight into the stability of buffalo herds as discrete units. Possible effects of natural disasters on the composition and movement of buffalo herds might give us a better view on the viability of buffalo populations. Of course genetic monitoring is also possible at the population level. Until recently genetic monitoring of natural populations was not possible due to the high costs and difficulties involved in obtaining tissue and blood samples. However, in this thesis it is shown that also non-invasive material like dung may be used as a source of mtDNA (**Chapter 4 and 5**). Certain microsatellites and sex-determining markers could be PCR amplified from dung as well, but due to time constraints this part of the project could not be completed. With different types of genetic markers genetic monitoring is possible with a higher resolution. Estimates may be obtained, among others, of short-term (a few generations) effective population size, short term gene flow, census size and sex ratio (Schwartz et al. 1998).

The possibility of using non-invasive sampling techniques may facilitate a more wide-spread use of population genetic information in the management of species. As larger samples sizes are possible, the information content may increase as well. A drawback may be that the full potential of DNA analysis can not be exploited due to degradation and low amounts of DNA in most non-invasive samples. Another disadvantage are the high costs involved in using DNA markers (Schwartz et al. 1998). However, these problems may be solved in the near future with the ongoing advancements in DNA technology.

Concluding remarks

I conclude that with highly polymorphic genetic markers it was possible to study in detail the population and genetic structure of the African buffalo. A better understanding was obtained of its recent and evolutionary history as well as its ecology. The genetic structure could be analysed at different geographic levels and for both sexes separately. The insights gained by me in this thesis are essential for a more effective management and protection of buffalo populations and preservation of their genetic diversity.

The African buffalo is now one of the best studied wild mammals, both genetically and ecologically. As such, it can be considered as a model species for all those other species of the African and other ecosystems. Despite that, a lot of questions with respect to its genetic diversity remain unanswered. These can not be solved by simply increasing genetic and geographic resolution. The continuing development of new statistical analysis techniques shows that a lot of valuable information from genetic markers is still untouched. Furthermore, a lot of additional insights are to be expected by more extensive comparisons with the population genetics of other species. Thus many more studies are required before we can hope to fully understand the population genetics of the African buffalo.

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Samenvatting

De Afrikaanse buffel *(Syncerus caffer)* komt van origine voor in vrijwel heel Afrika ten zuiden van de Sahara. Samen met de olifant (*Loxodonta africana*) en de gnoe (*Connochaetes taurinus*) vormt hij het leeuwedeel van de biomassa aan grote grazers in Afrika. Buffels leven in kuddes die in grootte kunnen variëren tussen 12 en 1600 individuen. Sinds het einde van de 19de eeuw worden buffelpopulaties in hun voortbestaan bedreigd door runderpestepidemieën (een virusziekte) en habitatfragmentatie als gevolg van menselijke invloeden.

In dit proefschrift wordt de populatiegenetica van de Afrikaanse buffel onderzocht, met accent op de ondersoort Kaapse buffel (*S. c. caffer*) uit oostelijk en zuidelijk Afrika. In de populatiegenetica wordt de genetische diversiteit van populaties onderzocht, waarbij men probeert conclusies te trekken over evolutionaire geschiedenis, demografie en ecologie. De genetische diversiteit van een populatie wordt weergegeven aan de hand van frequenties van allelen. Allelen zijn genetische varianten van een locus of gen, wat een gegeven, nauw omschreven gedeelte van het genoom (totale DNA van alle chromosomen) is.

Een betere kennis van de populatiegenetica van de Afrikaanse buffel is wenselijk voor een effectief beheer en bescherming van deze soort, gericht op de lange termijn. De oorspronkelijke doelstelling van dit project was om de effecten te onderzoeken van runderpestepidemieën en habitatfragmentatie op de genetische diversiteit van buffelpopulaties. Als hypothese werd gesteld dat de lokale bottlenecks (sterke aantalsreducties van populaties) die deze twee verstoringen veroorzaakten, hebben geleid tot een significante afname van de genetische diversiteit. Tijdens een bottleneck worden namelijk minder nakomelingen geproduceerd, zodat slechts een deel van de oorspronkelijke genetische diversiteit aan volgende generaties kan worden doorgegeven. Later in het onderzoek verschoof het accent naar een gedetailleerde analyse van de genetische structuur van buffelpopulaties, om zodoende meer inzicht te krijgen in de evolutionaire geschiedenis van de Afrikaanse buffel alsmede de effecten van zijn kuddegedrag op de genetische diversiteit.

Er werden drie soorten loci gebruikt: autosomale microsatellieten (niet gelegen op het X- of Y-chromosoom), Y-chromosomale microsatellieten en sequenties van een gedeelte van het mitochondriaal DNA (mtDNA), dat overgeërfd wordt via de vrouwelijke lijn. Microsatellieten zijn kleine loci met een eenvoudige, repeterende

DNA-sequentie. Het mitochondriale genoom is een enkel, circulair stuk DNA, dat niet voorkomt in de celkern maar in het omringende cytoplasma. Zowel microsatellieten als mitochondriaal DNA worden gekenmerkt door een hoge graad van polymorfisme (met twee of meer verschillende allelen).

Vergeleken met andere leden van de *Bovidae* familie, waartoe onder andere ook rund, schaap en geit behoren, en andere Afrikaanse zoogdieren is de genetische diversiteit erg hoog, zowel voor autosomale microsatellieten als voor mtDNA. De genetische diversiteit duidt op effectieve populatiegroottes (grootte van een 'theoretisch ideale' populatie die zich genetisch hetzelfde gedraagt als de echte populatie) van tussen 2600 en 27500 individuen. Hieruit kan geconcludeerd worden dat runderpestepidemieën en habitatfragmentatie slechts een gering effect hebben gehad op de genetische diversiteit. De afwezigheid van een significant effect van runderpest is waarschijnlijk een gevolg van het feit dat de meeste buffelpopulaties in staat waren zich binnen een paar generaties te herstellen. Ook zou, gelet op de migratie-capaciteiten van buffels, immigratie vanuit naburige populaties een deel van de verloren genetische diversiteit kunnen hebben hersteld. De afwezigheid van een significant effect van habitatfragmentatie is waarschijnlijk te danken aan het feit dat er slechts zo'n 15 buffelgeneraties zijn geweest sinds het einde van de 19de eeuw en dat veel populaties nog steeds een grootte hebben van duizend of meer. Hierdoor is het verlies aan allelen beperkt gebleven.

Alhoewel dit proefschrift dus laat zien dat buffelpopulaties op dit moment genetisch niet verarmd zijn, kan dit in de nabije toekomst wel veranderen. De huidige aantallen van veel buffelpopulaties liggen in de buurt van of zijn kleiner dan de schattingen van de gemiddelde effectieve populatiegrootte. Dit houdt in dat ze bij voortschrijdende migratie niet in staat zullen zijn hun historische niveau aan genetische diversiteit te handhaven. Alleen door de inbreng van nieuwe allelen uit andere populaties, via natuurlijke migratie of translocatie, kan de genetische diversiteit van individuele populaties op peil gehouden worden.

Zelfs op continentale schaal waren de genetische verschillen tussen buffelpopulaties klein, zowel voor autosomale microsatellieten als mtDNA. Maximaal 11% van de totale genetische variatie werd waargenomen tussen de populaties. In het geval van mtDNA is dit aanzienlijk lager dan de waarnemingen in andere Afrikaanse zoogdieren. Dit laatste duidt erop dat er een relatief hoge mate van genetische uitwisseling en dus migratie is onder vrouwelijke buffels. Dit lijkt in

tegenspraak te zijn met ecologische waarnemingen, volgens welke vrouwelijke buffels gedurende hun hele leven in de kudde blijven waarin ze geboren zijn. Het is echter ook mogelijk dat de genetische verschillen zijn onderschat omdat ze nog niet hun evenwichtswaarde hebben bereikt. Hiervoor werden verschillende aanwijzingen gevonden. Als alternatieve verklaring voor de kleine genetische verschillen werd een middel tot laat Pleistocene (235.000-23.000 jaar geleden) populatie-expansie gepostuleerd, zoals gesuggereerd door vier verschillende statistische tests. Het gehypothetiseerde begin van de populatie-expansie viel samen met dat van de evolutionaire scheiding tussen de Kaapse buffel en de buffels uit centraal en zuidwestelijk Afrika. Reeds eerder werd in andere studies op basis van fossiele vondsten gesuggereerd dat in deze periode de Afrikaanse buffel zijn verspreidingsgebied uitbreidde van de regenwouden in centraal Afrika naar de savannes van oostelijk en zuidelijk Afrika. De genetische data kunnen derhalve beschouwd worden als een ondersteuning voor deze expansiehypothese. Verder lijken de recente voorouder-allelen van veel mtDNA-allelen uit zuidelijk Afrika te komen uit oostelijk Afrika, wat er op duidt dat tenminste een deel van de populatie-expansie in zuidwaartse richting liep. Deze zuidwaartse expansie is mogelijk gerelateerd aan vegetatieveranderingen in Afrika. Gedurende vele ijstijden hadden grote gedeeltes van Afrika een droog klimaat en hadden savannes en bosgebieden een veel noordelijker distributie dan tegenwoordig. Ten tijde van een interglaciaal breidden deze savannes en bosgebieden zich zuidwaarts uit, samen met de zoogdieren die ze herbergden.

Van twee buffelpopulaties werd de genetische kuddestructuur onderzocht. Analyse van het mitochondriaal DNA uit een van deze populaties liet hoogst significante differentiatie zien, met meer dan 10% van de genetische variatie tussen kuddes. Deze resultaten ondersteunen de ecologische waarnemingen dat vrouwelijke buffels gedurende hun hele leven in een kudde blijven. Bovendien waren er verschillende aanwijzingen dat er binnen populaties minder genetische substructuring is met autosomale microsatellieten dan met mtDNA, wat de veldwaarnemingen ondersteund van relatief hoge mate van migratie onder mannelijke buffels.

Volgens ieder van de drie typen loci vormen buffels uit centraal (Kameroen en Gabon) en zuidwestelijk Afrika (Angola en Namibië) een aparte genetische lijn. Het is opvallend dat de buffels uit zuidwestelijk Afrika niet nauwer verwant zijn aan de Kaapse buffels uit zuidelijk Afrika (Botswana, Zimbabwe en Zuid Afrika).

Morfologisch lijken ze sterk op elkaar en heden ten dagen zijn er geen sterke geografische barrières tussen deze twee gebieden, behalve de droge Kalahari woestijn en Karoo in het uiterste zuiden van Afrika. Deze gebieden kunnen echter in het verleden gescheiden zijn geweest door een droogtecorridor die tijdens ijstijden liep van Namibië in een noordoostelijke richting naar Tanzania. Op regionaal niveau hebben de buffels uit centraal en zuidwestelijk Afrika voor ieder van de drie typen loci een aanzienlijke hogere genetische diversiteit dan de Kaapse buffels. Sterker nog, de mtDNA diversiteit is hoog vergeleken met de meeste andere zoogdieren op aarde. Dit is waarschijnlijk het gevolg van langdurige populatiefragmentatie in het verleden, resulterend in de ontwikkeling van verschillende genetische lijnen, gevolgd door secundair contact tussen populaties. Dit scenario is niet onwaarschijnlijk gezien het feit dat in het evolutionaire verleden dit deel van Afrika gekarakteriseerd werd door lange perioden van droogte, wat resulteerde in natte habitat-eilanden.

Tenslotte wordt in dit proefschrift besproken hoe de nieuwe inzichten in Afrikaanse buffel die dit onderzoek heeft opgeleverd van direct belang kunnen zijn voor het beheer en de bescherming van deze diersoort. Vanwege de voortdurende vernietiging van natuurgebieden in Afrika, kan in de toekomst een meer actief beheer van natuurlijke populaties op dit continent noodzakelijk worden.

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Curriculum vitae

Wilhelmus Franciscus van Hooft werd geboren op 3 april 1969 te Hedel. In 1987 behaalde hij het diploma Gymnasium B aan het Jeroen Bosch College te 's-Hertogenbosch. Van 1987 tot en met 1991 studeerde hij Biologie aan de Universiteit Utrecht. Hierbij heeft hij afstudeervakken gevolgd bij de vakgroep Biologische Toxicologie aan de Universiteit Utrecht en de vakgroep Medische Chemie aan de Vrije Universiteit Amsterdam. Het eerste afstudeervak betrof toxicologisch onderzoek naar de fysiologische basis van de immunologische bijwerkingen van het antidepressivum zimeldine. Het tweede afstudeervak betrof moleculair biologisch onderzoek naar de vorming en identiteit van het Golgi-apparaat en zijn rol in vesiculair transport en glycosylering. Van 1991 tot en met 1993 vervolgde hij zijn universitaire opleiding met de studie Milieukunde bij de gelijknamige vakgroep aan de Universiteit Utrecht. Als afstudeervak verrichtte hij onderzoek naar de mogelijkheden om satellietbeelden te gebruiken als hulpmiddel bij vegetatiekartering van de Biebrza vallei in Polen. Van 1994 tot en met 1995 werkte hij als onderzoeksmedewerker bij de afdeling Inspectie en Milieuongevallen Dienst van het RIVM, dat onder leiding stond van F. Brinkmann en J. Kliest. Hierbij werkte hij aan de ontwikkeling van een in de praktijk hanteerbare methode waarmee de risico's voor de volksgezondheid zijn te kwantificeren van beweiding van runderen op met sporenelementen verontreinigde gronden. In juni 1996 begon hij als onderzoeker-in-opleiding bij de Wageningen Universiteit aan het promotie-onderzoek zoals beschreven staat in dit proefschrift. Het onderzoek vond plaats bij de leerstoelgroepen Natuurbeheer in de Tropen en Ecologie van Vertebraten onder leiding van Prof. dr. H.H.T. Prins en Fokkerij en Genetica onder leiding van Prof. dr. E.W. Brascamp. Sinds januari 2001 is hij werkzaam als universitair docent op het gebied van de ecologische risicoanalyse van genetisch gemodificeerde organismen bij het Centrum voor Milieukunde aan de Universiteit Leiden.