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Patterns of population subdivision, gene flow and genetic variability in the African wild dog (*Lycaon pictus*)

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Abstract

African wild dogs are large, highly mobile carnivores that are known to disperse over considerable distances and are rare throughout much of their geographical range. Consequently, genetic variation within and differentiation between geographically separated populations is predicted to be minimal. We determined the genetic diversity of mitochondrial DNA (mtDNA) control region sequences and microsatellite loci in seven populations of African wild dogs. Analysis of mtDNA nucleotide diversity suggests that, historically, wild dog populations have been small relative to other large carnivores. However, population declines due to recent habitat loss have not caused a dramatic reduction in genetic diversity. We found one historical and eight recent mtDNA genotypes in 280 individuals that defined two highly divergent clades. In contrast to a previous, more limited, mtDNA analysis, sequences from these clades are not geographically restricted to eastern or southern African populations. Rather, we found a large admixture zone spanning populations from Botswana, Zimbabwe and south-eastern Tanzania. Mitochondrial and microsatellite differentiation between populations was significant and unique mtDNA genotypes and alleles characterized the populations. However, gene flow estimates (*Nm*) based on microsatellite data were generally greater than one migrant per generation. In contrast, gene flow estimates based on the mtDNA control region were lower than expected given differences in the mode of inheritance of mitochondrial and nuclear markers which suggests a male bias in long-distance dispersal.

Keywords: demography, microsatellites, mitochondrial DNA, nucleotide diversity, phylogeography, population structure

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Introduction

Commonly invoked models of population differentiation and speciation may not be valid for large, highly mobile carnivores that can disperse over great distances and across dramatic geographical barriers. Among large carnivores, long- and short-distance dispersal events of varying frequency may impede or complicate processes of differentiation that would otherwise allow divergence among populations of less mobile species (Lansman *et al*. 1981; Patton *et al*. 1994; Cooper *et al*. 1995). As predicted, genetic studies of highly mobile species, such as North American coyotes (*Canis latrans*) and gray wolves (*Canis lupus*), show that population differentiation is not correlated with distance where geographical ranges are relatively continuous (Lehman & Wayne 1991; Roy *et al*. 1994; but see Forbes & Boyd 1997). European gray wolf populations, in contrast, are genetically more differentiated, but this is likely due to recent habitat fragmentation (Wayne *et al*. 1992; Vilà *et al*. 1999).

In this study, we quantify variation in mitochondrial DNA (mtDNA) and nuclear dinucleotide repeat loci

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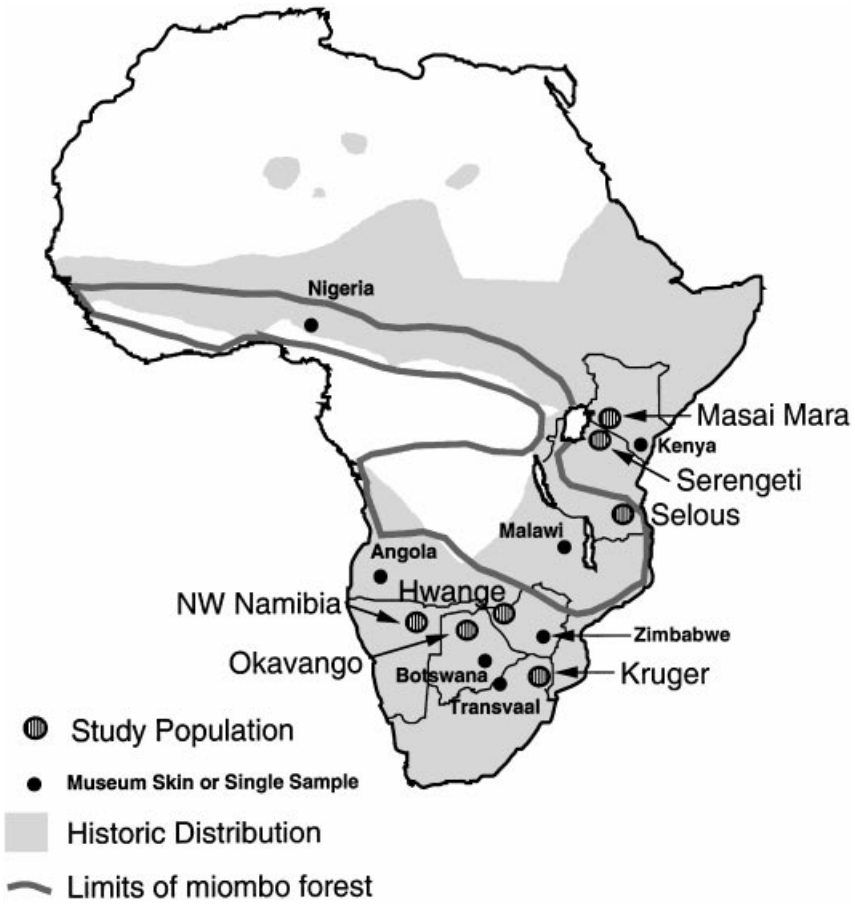

Fig. 1 Locations of study populations and museum samples. Historic distribution of wild dogs according to Creel & Creel (1998). Limits of the distribution of 'miombo' (*Brachystegia-Julbernardia*) forests modified from Matthee & Robinson (1997).

(microsatellites) to estimate genetic variation and population subdivision in the African wild dog (*Lycaon pictus*), an endangered wolf-like canid. Historically, wild dogs probably ranged over most of the savannah and acacia woodland habitats of sub-Saharan Africa (Fig. 1; Nowak 1999). During the past century, wild dog populations have declined dramatically (Ginsberg & Macdonald 1990; Woodroffe *et al*. 1997) and fragmented and declining populations of wild dogs are currently found in western, central and eastern Africa (e.g. Senegal, Chad, Kenya and Tanzania; Fanshawe *et al*. 1991, 1997). Large and relatively stable populations are limited to the region bounded by southern Tanzania, Okavango, and Kruger (Fig. 1), but even there, populations are restricted primarily to protected areas (Fanshawe *et al*. 1991). Consequently, historic levels of genetic diversity are expected to be much higher than in extant populations.

African wild dogs are known to be highly mobile and have home ranges estimated to be as large as 2000 km^2 (Frame *et al*. 1979; Fuller *et al*. 1992a). In addition, long-distance dispersal occurs in wild dogs, although the extent of female sex-bias and the frequency of dispersal is uncertain (Frame & Frame 1976; Frame *et al*. 1979; Fuller *et al*. 1992b; McNutt 1996; Girman *et al*. 1997). Previous research on the genetic structure of wild dog populations using an analysis of mtDNA restriction fragment length polymorphisms (RFLPs) and morphological measurements suggested that eastern and southern African populations are genetically and morphologically distinct (Girman *et al*. 1993). This pattern has been found in studies of other African vertebrates and is usually attributed to historical isolation of ancestral populations followed in some cases, by secondary contact (Avise *et al*. 1987, 1992; Wayne *et al*. 1990; Freitag & Robinson 1993; Arctander *et al*. 1999). For example, subspecies of the ostrich (*Struthio camelus*) with genetically divergent mtDNA lineages may have originated due to separation by the Great Rift Valley system (Freitag & Robinson 1993).

Previous allozyme studies of highly mobile African species have found only limited genetic variation even between populations having distinct mitochondrial genotypes (Brown *et al*. 1978; Wayne & O'Brien 1987; Merlander *et al*. 1989; Ashley *et al*. 1990; Georgiadis *et al*. 1994). Allozyme loci generally have low mutation rates and low levels of polymorphism (Nei 1987). In fact, an analysis of 15 allozyme loci used successfully in a study of North American gray wolf populations (Wayne *et al*. 1991) was conducted using samples from eastern and southern African wild dogs and little differentiation among populations was found (Girman, unpublished data). The use of more

variable nuclear loci, such as microsatellites, seems necessary to discern the nuclear genetic structure of African wild dog populations (Bruford & Wayne 1993; Roy *et al*. 1994).

Consequently, in this study, we assessed patterns of genetic variation and differentiation of African wild dog populations from eastern and southern Africa through the analyses of mtDNA control region sequences and 11 dinucleotide repeat loci. This analysis permits a test of the following predictions: (i) past levels of genetic diversity are much higher than the present-day reflecting recent demographic declines; (ii) eastern and southern African populations are genetically distinct (Girman *et al*. 1993); and (ii) a female sex bias exists in dispersal (Frame & Frame 1976; Frame *et al*. 1979). To test for differences in recent and historical diversity, we compared genealogical and frequency-based estimates of nucleotide diversity (e.g. Vilà *et al*. 1999). In addition, we examined control region sequences from museum skin samples to assess the effects of a recent population bottleneck and to clarify the historical connections between populations from areas that could not be sampled or where wild dogs are now extinct. Finally, we typed samples from three captive wild dog colonies to assist in the development of reintroduction strategies.

Materials and methods

Genetic samples

Blood, tissue or faecal ($n = 5$) samples were collected from 229 African wild dogs. One sample was collected from a captive individual from southern Angola, but all others were collected from free-ranging animals in seven populations (Fig. 1, Table 1): Masai Mara National Park in Kenya (Masai Mara, $n = 15$); Serengeti National Park in northern Tanzania (Serengeti, $n = 12$); Selous Game Reserve in southern Tanzania (Selous, $n = 31$); Hwange National Park in Zimbabwe (Hwange, $n = 28$); Moremi Wildlife Reserve in the Okavango Delta region of Botswana (Okavango, $n = 42$); north-west Namibia (NW Namibia, $n = 6$) and Kruger National Park in the South Africa (Kruger, $n = 94$). The Masai Mara and Serengeti populations are contiguous and straddle the Kenya–Tanzania border. In addition, mtDNA was analysed from 14 skin samples from the British Museum of Natural History, London, UK (BMNH, $n = 7$), the Transvaal Museum of Natural History, Pretoria, South Africa (TM, $n = 5$) and the Smithsonian Museum of Natural History, Washington DC, USA (SMNH,

Table 1 Mitochondrial DNA genotypes found in extant wild dog populations in eastern and southern Africa (percentage of population in parentheses), from museum skins, and from captive populations

	Haplotype									Sample size
	S1	S2	S3	Z1	Z2	E1	E2	E3	W1	
Extant populations										
Masai Mara						11 (73)		4 (27)		15
Serengeti						7 (58)		5 (42)		12
Selous			24 (77)				7 (23)			31
Okavango		6 (14)		1 (2)	3 (7)	29 (69)	3 (7)			42
Hwange		12 (43)		1 (4)	13 (46)	1 (4)	1 (4)			28
Angola							1			1
NW Namibia		6 (100)								6
Kruger	37 (39)	57 (61)								94
Subtotal	37	81	24	2	16	48	12	9	0	229
Museum skins										
Nigeria									1	1
Kenya						2				2
Malawi		1		1						2
Botswana				1						1
Zimbabwe		1								1
Transvaal	2	4		1						7
Subtotal	2	6	0	3	0	2	0	0	1	14
Captive colonies										
DeWildt (SA)		19								19
Kapama (SA)		16								16
Chipangali (Zim.)		2								2
Subtotal	0	37	0	0	0	0	0	0	0	37

SA, South Africa; Zim., Zimbabwe.

$n = 2$). These samples included individuals from Nigeria (BMNH 13.3.8.1), Kenya ($n = 2$, BMNH 96.3.4.2, SMNH 181508), Malawi ($n = 2$, BMNH 97.10.1.70, BMNH 2.1.6.34), Zimbabwe (BMNH 61.988), Botswana (SMNH 368443) and Transvaal ($n = 7$, BMNH 6.11.7.10, BMNH 6.11.7.12, TM11898-901, TM11895a, TM11895b, TM8867 and TM3211). Finally, 37 samples from three captive colonies where analysed: DeWildt Breeding Colony, South Africa ($n = 19$); Kapama Breeding Colony, South Africa ($n = 16$); and Chipangali Wildlife Breeding Centre in Zimbabwe ($n = 2$).

DNA extraction

For blood and frozen tissue samples, DNA was extracted by proteinase K digestion followed by isolation of the DNA with phenol/chloroform/isoamyl alcohol (Sambrook *et al*. 1989). Alternatively, for museum skin samples, faecal samples and blood that did not yield sufficient DNA using the previous protocol, the sample was homogenized with sterile equipment and digested in 8 mL of TNE (10 mM Tris pH = 8.0, 2 mM EDTA, and 10 mM NaCl) with 4 mg collagenase, 4 mg proteinase K, 80 mg dithiothreitol (DTT) and 880 μL 10% SDS for 20 h at 37 °C. The DNA then was isolated using a guanidium thiocyanate/silica extraction protocol (Boom *et al*. 1990).

Control region sequencing

The polymerase chain reaction (PCR) was used to isolate and amplify a 381-bp region of the control region I of the mitochondrial genome (Saiki *et al*. 1988). Primers were based on Kocher *et al*. (1989) (ThrL 5′-CGAAGCTTGATATG-AAAAACCATC-3′) and a consensus sequence of human, mouse and cow (DLH 5′-CCTGAAGTAGGAACCAGATG-3′). Double-stranded product was obtained in a symmetric PCR amplification containing ≈ 10 ng of genomic DNA in a reaction buffer of 50 mM KCl, 2.0 mM $MgCl_2$, 10 mM Tris HCl (pH = 8.8), 1 mM dNTP mix, 2.5–12.5 units of *Taq* DNA polymerase, and 25 pmoles of each primer in a total volume of 50 μL. A Perkin-Elmer Cetus 9600 DNA thermocycler was programmed for 35–40 amplification cycles with denaturation at 94 °C for 25 s, annealing at 45–55 °C for 30 s, and extension at 72 °C for 45 s. The DNA was isolated on a 2% low melting point agarose gel, recovered using a Gene Clean Kit (Bio 101), and its sequence was determined using a variation of the dideoxynucleotide chain termination reaction with Sequenase 2.0 DNA polymerase (US Biochemical).

Single-stranded conformation polymorphisms

Two internal canid-specific primers were designed for the mtDNA control region from consensus sequences of six African wild dogs and a gray wolf: CDLH: 5′-CCCTTATTGGACTAGGTGATATGCAT-3′ and LDLL: 5′-CCCCTATGTACGTCGTGCATT-3′. Following initial sequencing of wild dog samples collected from locations throughout their range, 175 samples were screened through the analysis of single-stranded conformation polymorphisms (SSCPs) (Lessa & Applebaum 1993; Girman 1996). Primers were end-labelled with [γ^{32}P]-ATP in 25 μL polynucleotide kinase reaction. The end-labelled primers were then included in a PCR identical to that used in the sequencing reaction. The PCR products were added in a 1:5 ratio to a 98% formamide stop solution, denatured at 84 °C for 5 min and loaded onto a 6% nondenaturing polyacrylamide gel. Products were electrophoresed for 3 h at 40 W in a refrigerated room such that the gel temperature remained at ≈ 10 °C. Genotype standards representing each control region sequence found in an initial survey were included on every gel. The gels were then dried and exposed to autoradiographic film (Kodak Biomax) for 6–18 h. Two individuals from each gel were sequenced directly to confirm genotype scores. Control region sequences were aligned using the CLUSTAL V DNA sequence alignment program and confirmed by eye (Higgins & Sharp 1989).

Identification of microsatellite alleles

Wild dog samples collected from eastern and southern Africa were screened for variation in 60 $CA_{(n)}$ microsatellite loci, originally isolated from a domestic dog genomic library (Ostrander *et al*. 1993). Eleven microsatellite were identified that consistently gave PCR product, were polymorphic in wild dogs, and had low levels of polymerase stutter (see list in Appendix I). These microsatellites were scored in a subsample of 203 individuals (Table 2). Detection of microsatellite alleles from genomic DNA was achieved by end-labelling one primer by a standard [γ^{32}P]-ATP and T4 polynucleotide kinase (Amersham) reaction (Sambrook *et al*. 1989) and performing 28 cycles of PCR amplification in a 25-μL reaction volume using 50 ng of target DNA, 2 mM $MgCl_2$ and 0.8 units of *Taq* DNA polymerase (Promega). Single-stranded alleles were visualized by fractionating heat-denatured (94 °C for 5 min) PCR products onto a 6% sequencing gel containing 50% w/v urea. A M13 control sequencing reaction was run adjacent to the samples to provide an absolute size marker for the microsatellite alleles. Because absolute allele sizes were determined with reference to a size marker we could combine data for different gels.

mtDNA control region diversity

To assess differences in recent and historical diversity, two measures of nucleotide diversity were used. First, the nucleotide diversity, π (Nei & Li 1979), and its standard

	mtDNA			Microsatellites				
Population	*N*	# *Hp.*	$\hat{\theta}_T$	*N*	# *Loci*	*N/locus*	*A*	H_E (SE)
Kruger	94	2	0.0013	94	11	93.8	4.1	0.556 (0.146)
Hwange	28	5	0.0077	22	11	21.0	4.0	0.655 (0.105)
Okavango	42	5	0.0120	31	11	29.7	4.0	0.605 (0.124)
NW Namibia	6	1	0.0000	6	11	5.8	3.4	0.618 (0.177)
Selous	31	2	0.0114	22	11	17.8	4.4	0.665 (0.162)
Mara/Serengeti	27	2	0.0048	28	11	26.6	4.2	0.622 (0.106)
Captive	37	1	0.0000	29	11	28.5	3.3	0.504 (0.172)
Wild dogs	228	8	0.0145	203	11	194.7	4.0	0.643 (0.126)
Coyotes	16	14	0.0460	92	10	—	5.9	0.675 (0.067)*
Gray wolves	259	34	0.0260	103	10	—	4.8	0.649 (0.040)*

*Average across populations.

Table 2 Genetic variability in wild dog populations and in other canid species. For mitochondrial DNA (mtDNA), sample size (*N*), number of mtDNA genotypes (# *Hp.*) and nucleotide diversity ($\hat{\theta}_T$) are shown. For microsatellites: sample size (*N*), number of loci studied (# *Loci*), average sample size studied for each microsatellite locus (*N/locus*), mean number of alleles per locus (*A*), and mean expected heterozygosity (H_E; unbiased estimate, Nei 1978; standard error in parenthesis, *SE*). Data for coyote and wolf mtDNA from Vilà *et al*. (1999), and for microsatellites from Roy *et al*. (1994)

deviation were estimated using the program DNASP (Rozas & Rozas 1997). For mtDNA data, the parameter θ equals $N_f\mu$, where N_f is the female effective population size and μ is the mutation rate per site per generation. An estimate of the effective number of females can be derived from this relationship if θ is estimated and a mutation rate is assumed. Tajima (1983) showed that the $E(\theta) = \pi$, and we denote this estimate as θ_T (Vilà *et al*. 1999). However, this estimator does not use genealogical information, and therefore is not efficient (Felsenstein 1992). Consequently, a maximum likelihood estimator of θ denoted θ_F was calculated that utilizes genealogical information and allows for variable population size (Kuhner *et al*. 1995). While the population of inference for θ_T is the current population, the population of inference for θ_F is the historical population (Crandall *et al*. 1999). The comparison of both estimates has proven useful in inferring long-term population trends in wolves and coyotes (Vilà *et al*. 1999). To estimate θ_F and the growth parameter *g* the computer program FLUCTUATE 1.3 was used (Kuhner *et al*. 1998).

Population relationships based on mtDNA control region sequences

Phylogenetic relationships among control region sequences were analysed using three approaches. First, the relationships of the genotypes were determined by maximum parsimony (MP) analysis using PAUP* (Swofford 1998) with a gray wolf sequence as an outgroup. Second, genetic distances between genotypes were computed assuming a Tamura–Nei model of sequence evolution and heterogeneity in the substitution rates across nucleotide sites following a gamma distribution with a value of $\alpha = 0.5$ (Tamura & Nei 1993; Wakeley 1993). These distances were then used to construct a neighbour-joining (NJ) tree (Saitou & Nei 1987) using the same computer program. Values of the gamma distance parameter, α, from 0.2 to 0.9 were also tried and found to give NJ trees with identical topologies. One thousand bootstrap replicate trees were generated from the sequence data to evaluate the support for each node in the NJ and MP trees. Third, the pattern of sequence evolution was portrayed using a minimum spanning tree, in which sequences are the nodes of a network rather than the terminal tips of a tree. Using a program supplied by L. Excoffier (MINSPAN, Department of Anthropology, University of Geneva), all possible minimum spanning networks were determined using the pairwise number of substitutions.

To assess genetic differentiation between eastern and southern wild dogs populations, as well as other conceivable geographical units, an analysis of variance (ANOVA) approach modified for molecular sequence data was employed (Excoffier *et al*. 1992) that utilizes both the frequency and sequence divergence between genotypes. The divergence between genotypes was estimated, as above, assuming a Tamura–Nei model of sequence evolution and a gamma distribution of the substitution rates ($\alpha = 0.5$). This analysis of molecular variance (AMOVA) is an approach analogous to ANOVA in which the correlation among genotype distances at various hierarchical levels are used as *F*-statistic analogs, designated as ϕ statistics; ϕ_{CT} is the correlation of random genotypes within a group of populations relative to that drawn from the entire species and measures the proportion of genetic variation among groups of populations; and ϕ_{ST} is the correlation of random genotypes within a population relative to that from the whole species and is analogous to Wright's F_{ST} (Wright 1951). We used the Excoffier *et al*. (1992) method because it is not sensitive to deviations from a normal distribution of genotypes unlike other F_{ST} analogues (Takahata & Palumbi 1985; Lynch & Crease 1990; Hudson *et al*. 1992; Georgiadis *et al*. 1994) and can be used in a hierarchical framework such that the significance of population groupings can be assessed. The differentiation between populations was tested with an exact test of population differentiation (Raymond & Rousset 1995) using 10 000 Markov chain steps. These analyses were carried out using the program ARLEQUIN 1.1 (Schneider *et al*. 1997).

Gene flow based on mitochondrial control region sequences

Gene flow within and among localities was estimated as $N_f m_f$, the number of female migrants occurring between populations per generation, where N_f is the female effective population size and m_f is the female migration rate. $N_f m_f$ is estimated from the expression $F_{ST} \approx 1/(1 + 2N_f m_f)$ (Slatkin 1987, 1993; Baker *et al*. 1994). As surrogates for F_{ST} we used ϕ_{ST} among regional groupings of populations and calculated migration rates. Differentiation by distance was assessed by plotting pairwise $\log(\phi_{ST})$ values against log(geographical distance) (Slatkin 1993). The significance of the association was determined by the application of Mantel's permutation test (Mantel 1967) using 500 permutations and the software MANTEL v2.0 (Liedloff 1999).

Finally, NJ trees of populations were constructed based on the average sequence divergence between them (Nei 1987). To estimate divergence times, mean sequence divergence between populations (d_A) was corrected for polymorphisms within each population:

$$d_A = d_{xy} - (d_x + d_y)/2,$$

where d_{xy} is the mean sequence divergence between all individuals in populations x and y, and d_x and d_y are the mean sequence divergences within populations. The mean sequence divergence (d) within each population was calculated as the mean of all possible comparisons (k) of n individuals, where $k = n(n-1)/2$. In addition, 1000 data sets were generated by bootstrapping the individuals inside each population and a distance matrix was generated for each one of them with a program written by J. Stone. As a measure for the support of the population tree, the consensus of all resulting NJ trees was built using the program PHYLIP 3.572 (Felsenstein 1989).

Microsatellite diversity, population relationships and gene flow

Genetic polymorphism of each population was measured as the mean number of alleles per locus (A), the observed heterozygosity (H_O), and the heterozygosity expected from Hardy–Weinberg assumptions (H_E; Nei 1978, 1987). H_E is used because it is a less biased index of genetic variability and is highly correlated with H_O (Nei & Roychoudhary 1974). Heterozygosity statistics were calculated using the program BIOSYS (Swofford & Selander 1981). In addition, because of the presence of rare alleles, deviations from Hardy–Weinberg equilibrium (HWE) were tested by the Markov chain method implemented in GENEPOP (Guo & Thompson 1992; Rousset & Raymond 1995). Linkage disequilibrium was assessed using the program GENETIX (Belkhir *et al*. 2000) with a test based on Black & Krafsur (1985).

To assess genetic differentiation between eastern and southern wild dog populations and to identify other geographically differentiated population units, four approaches were used. First, unique alleles were identified between each pair of populations of African wild dogs (Goldstein *et al*. 1999). Rare alleles that were found in a small subset of the populations and thus supporting a shared history were also identified. Second, NJ topologies were constructed based on Nei's (1978) unbiased distance (e.g. Roy *et al*. 1994) and θ (Weir & Cockerham 1984), using the programs MICROSAT (Minch *et al*. 1995) and PAUP*. As a measure of the support for the Nei's distance tree, 1000 bootstrapped distances matrixes were generated using the program MICROSAT and a consensus tree of all resulting NJ trees was built with the program PHYLIP 3.572. These drift-based measures were used rather than distance measures assuming a stepwise mutational model such as $(\delta\mu)^2$ (Goldstein *et al*. 1995) to avoid assumptions of a specific mutational model and because only a small number of microsatellite loci was studied (Gaggiotti *et al*. 1999). Third, the differentiation between populations was tested with an exact test of population differentiation (Raymond & Rousset 1995) using 10 000 Markov chain steps (implemented in GENEPOP). Fourth, as above with the mtDNA data, an AMOVA approach was used to estimate the significance of geographical subdivisions among population groupings (Goodman 1997). Pairwise θ-values were calculated between populations and the level of significance for these values was estimated through a randomization test (as implemented by the program GENETIX). Groupings that maximized θ and were significantly different from 1000 random distributions of individuals, with group sizes held constant, were assumed to be the most probable geographical subdivisions. The number of migrants per generation Nm was estimated from θ-values by the expression $\theta = 1/(1 + 4Nm)$. Differentiation by distance was assessed by plotting these pairwise values against geographical distance and determining the significance of associations using a Mantel's permutation test (Mantel 1967). As for mtDNA, for this analysis, we used 500 permutations and the computer program MANTEL v2.0 (Liedloff 1999). The same approach was used to study the correlation between θ and ϕ_{ST}.

To study the likelihood of finding one of the observed genotypes in each of the populations an assignment test was used (Paetkau *et al*. 1995; Waser & Strobeck 1998; available at http://www.biology.ualberta.ca/jbrzusto/Doh.html). The likelihood of finding a given genotype in each population was calculated and individuals were assigned to populations for which they had the highest likelihood (e.g. Paetkau *et al*. 1998). To avoid likelihood values of zero we assigned a frequency of 0.01 to alleles missing in one population. Finally, in order to visualize the similarity between populations and their fragmentation, the distance $d'_{ij} = 1 - P_{ij}$ (where P_{ij} is the proportion of shared alleles

between individuals *i* and *j*) was used to construct a NJ tree of individuals (Goldstein *et al*. 1999). The distances between individuals were calculated using the program MICROSAT, and a NJ tree was built with these distances using the program PAUP*.

Results

mtDNA control region diversity

Seventeen variable sites defined nine control region genotypes in the 280 wild dog samples (Table 1). The nucleotide diversity θ_T and θ_F, provided similar estimates of θ for the wild dog sample of 0.0145 (± 0.0007) and 0.0102 (± 0.0006), respectively. The growth parameter estimated by the program FLUCTUATE is small and negative ($g = -1.2718$) and not significantly different from 0 (constant population; $P > 0.05$). The nucleotide diversity, θ_T, of eastern and southern genotype clades was also estimated independently considering past evidence of isolation (Girman *et al*. 1993). The nucleotide diversity for the eastern (E1–E3, $n = 69$) and southern (S1–S3, Z1, Z2, $n = 160$) genotypes was very similar having values of 0.0039 (± 0.0005) and 0.0035 (± 0.0003), respectively.

With five each, Okavango and Hwange had the largest number of mtDNA genotypes (Table 1). However, the Selous population had greater nucleotide diversity than Hwange (0.0114 and 0.0077, respectively; Table 2). This finding reflects the high frequency of genotypes from both the eastern and the southern clades in Selous (Table 1 and Fig. 2). Only a single genotype was found in the NW Namibia population of six individuals, however, sample size considerations identify it only tentatively as the least diverse population. The Kruger sample has the lowest nucleotide diversity of any well-sampled wild population, followed by the pooled sample from Masai Mara/Serengeti (see below).

Distribution and differentiation of mtDNA control region genotypes

Population specific and ubiquitous genotypes were identified in African wild dogs. Genotype W1 was found in the single museum skin from Nigeria. The S3 genotype was unique to a single sampling locality (Selous). S1 (Kruger and skins from Transvaal), Z2 (Okavango and Hwange) and E3 (Masai Mara and Serengeti), occurred only in two neighbouring populations. The remaining genotypes were found over a relatively wide area. Z1 was found in Hwange and Okavango as well as in museum skins from Malawi, Botswana and the Transvaal region of South Africa. Genotype E1 occurred in Masai Mara, Serengeti, Hwange and Okavango and was also found in historic samples from Kenya. The E2 genotype was found in samples from Selous, Okavango, Hwange and a single individual from Angola. Finally, S2 was the most widespread genotype, and was found in Kruger, NW Namibia, Hwange and Okavango and the historic samples from Malawi, Zimbabwe and the Transvaal (Table 1, Fig. 1).

North to south patterns were evident in the distribution of genotypes (Table 1, Fig. 1). Only two genotypes, E1 and E3 were found in the two northernmost populations, Masai Mara and Serengeti. Further south, in Okavango and Hwange, five genotypes (S2, Z1, Z2, E1 and E2) were observed in common. The other two southern populations, NW Namibia and Kruger, shared the most common genotype S2 with Okavango and Hwange, although in Kruger a unique genotype (S1) also was found. Finally, the most common genotype found in Selous (S3), located in east Tanzania and approximately midway between the northernmost and

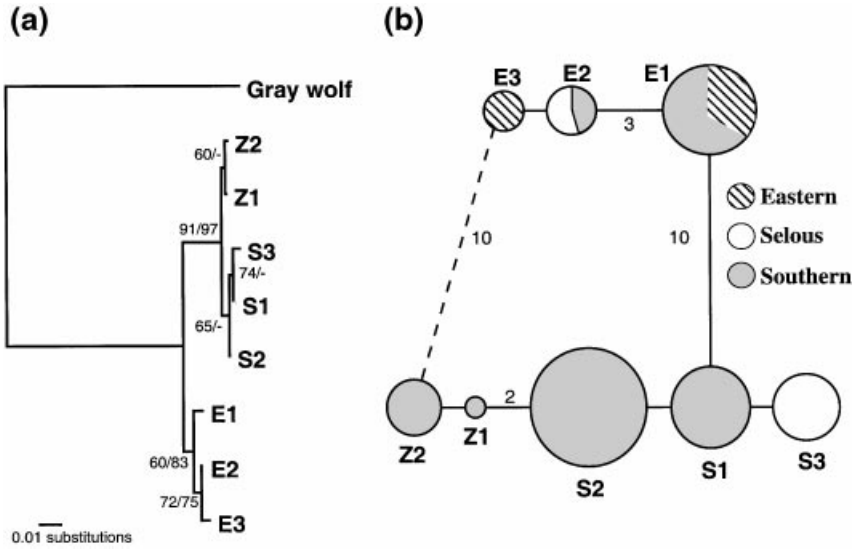

Fig. 2 Phylogenetic relationships of wild dog control region genotypes. (a) Neighbour-joining tree based on Tamura & Nei (1993) distance (gamma correction, $\alpha = 0.5$). Ratio by nodes are the bootstrap percentages of support when > 50% in 1000 neighbour-joining (numerator) and maximum parsimony (denominator) trees. (b) Minimum spanning network. The size of the nodes indicates frequency of genotype in the entire sample. The frequency of each genotype in eastern (Masai Mara and Serengeti), Selous and Southern (all others) populations is indicated by shading. The number of substitutions differentiating genotypes is shown on each branch when different from one. An alternative link between northern and southern genotypes is indicated by a dashed line.

	Kruger	Hwange	Okavango	NW Nam.	Selous	Mara/Ser.
Kruger	—	0.196	0.154	0.121	0.225	0.207
Hwange	0.0049	—	0.113	0.116	0.196	0.183
Okavango	0.0212	0.0205	—	0.065	0.296	0.251
NW Namibia	0.0007	0.0043	0.0223	—	0.222	0.304
Selous	0.0075	0.0108	0.0175	0.0090	—	0.190
Mara/Ser	0.0316	0.0298	0.0066	0.0333	0.0238	—

Table 3 Mean sequence divergence for mitochondrial DNA (mtDNA; below diagonal) and Nei's unbiased genetic distance (Nei 1978; above diagonal) for microsatellite markers for seven wild dog populations

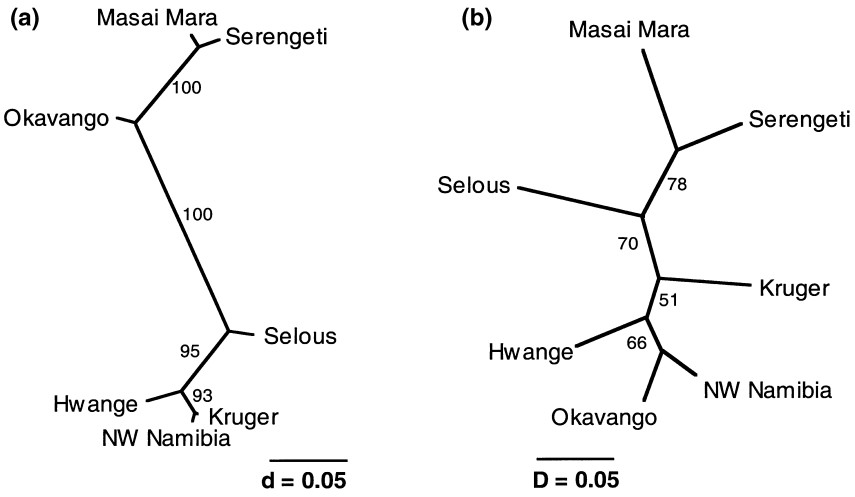

Fig. 3 Neighbour-joining trees of populations. (a) Control region topology based on the average sequence divergence between sequences in each population. (b) Microsatellite topology based on Nei's (1978) unbiased genetic distance between populations. Bootstrap support in 1000 replicates is indicated by the branches.

the southern populations, was not found anywhere else. E2, also found in Selous, is shared with the southern populations. Genotypes S1, S2, S3, Z1 and Z2 are closely related (see below) and were found only in modern and historic populations south of the Rift Valley plus Selous (Table 1). Finally, all sampled individuals from the three captive populations have genotype S2, the most common in southern wild dog populations.

Two control region sequence clades were apparent in MP and NJ trees and in a minimum spanning network (Fig. 2). One clade contained genotypes common in eastern Africa (E1, E2 and E3), and was supported, respectively, in 60 and 83% of NJ and MP bootstrap replicate trees. The second clade included sequences only observed in southern populations and Selous (S1, S2, S3, Z1 and Z2) and was supported, respectively, in 91 and 97% of NJ and MP bootstrap replicate trees. One to five sequence differences existed between any two genotypes within each major grouping and 10–13 sequence differences were found between any two genotypes from the two sequence clades. Gamma-corrected Tamura–Nei distances ranged from 0.3 to 1.4% for genotypes within each clade and from 2.9 to 3.9% between clades (mean = 3.4%, SD = 0.29).

Genotype W1 was recovered from a single museum skin sample from Nigeria (Table 1). Although only 134 bp were sequenced from this sample, this sequence has high similarity to eastern genotypes (E1, E2, E3). W1 differed from these genotypes by 3 or 4 substitutions, whereas extant 'E' genotypes differ from each other in the same fragment by 1–4 substitutions. In contrast, W1 differed from the remaining sequences by 8–10 substitutions and extant 'E' genotypes differed from them by 8–11 substitutions for the same 134 bp region.

Several population groupings are suggested by the structure of a NJ tree of African wild dog populations (Table 3; Fig. 3a). Three southern populations, Kruger, Hwange and NW Namibia, appear tightly grouped, and the Selous population is located close to them. The northernmost populations, Masai Mara and Serengeti, also are closely grouped with each other and neighbour to the southern Okavango population. Although the mtDNA genotypes found in Okavango wild dogs are the same as those found in Hwange (Table 1), the most common genotype in both Okavango and Masai Mara/Serengeti is E1 (Table 1).

In pairwise comparisons of ϕ_{ST}, only the association of the Masai Mara and Serengeti populations was not significantly different from 1000 random permutations of individuals ($P = 0.422$). Therefore, these two contiguous populations were pooled for further analyses (Table 4). We calculated ϕ_{CT} for population groupings suggested by clustering analysis (Fig. 3a) and by the geographical distribution and relatedness of populations (Fig. 1; Tables 3 and

Table 4 Differentiation between populations. (a) Pairwise ϕ_{ST} values for mitochondrial DNA (mtDNA) data below diagonal and pairwise θ (Weir & Cockerham 1984) for microsatellite data above diagonal. (b) Pairwise number of migrants per generation between populations for mtDNA data ($N_f m_f$) below diagonal and for microsatellite data (Nm) above diagonal

	Kruger	Hwange	Okavango	NW Nam.	Selous	Mara/Ser.
(a)						
Kruger	—	0.114**	0.097**	0.085**	0.127**	0.122**
Hwange	0.471**	—	0.061**	0.060*	0.084**	0.088**
Okavango	0.777**	0.567**	—	0.041*	0.131**	0.123**
NW Namibia	0.200 (ns)	0.191*	0.598**	—	0.095**	0.140**
Selous	0.505**	0.344**	0.452**	0.313*	—	0.089**
Mara/Serengeti	0.935**	0.799**	0.158*	0.883**	0.679**	—
(b)						
Kruger	—	1.94	2.33	2.70	1.71	1.81
Hwange	0.56	—	3.82	3.93	2.72	2.58
Okavango	0.14	0.38	—	5.88	1.67	1.78
NW Namibia	2.00	2.12	0.34	—	2.38	1.53
Selous	0.49	0.95	0.61	1.10	—	2.57
Mara/Serengeti	0.04	0.13	2.67	0.07	0.24	—

**$P < 0.01$; *$P < 0.05$; ns, not significant.

Table 5 Support for groupings of populations estimated using ϕ_{CT} and θ-values based on mitochondrial DNA (mtDNA) haplotype distances and microsatellite allele frequencies, respectively

	mtDNA		Microsatellites		
Group	ϕ_{CT}	P	θ	P	Groupings
Grp 1	−0.097	0.492	0.083	< 0.001	[Mara/Serengeti, Selous] [Okavango, Hwange] [NW Namibia, Kruger]
Grp 2	0.212	0.300	0.079	< 0.001	[Mara/Serengeti] [Selous, Okavango, Hwange] [NW Namibia, Kruger]
Grp 3	0.319	0.204	0.078	< 0.001	[Mara/Serengeti] [Okavango, Hwange] [Selous, NW Namibia, Kruger]
Grp 4	0.448	0.176	0.081	< 0.001	[Mara/Serengeti] [Selous, Okavango, Hwange, NW Namibia, Kruger]
Grp 5	0.132	0.331	0.064	< 0.001	[Mara/Serengeti, Selous, Okavango, Hwange] [NW Namibia, Kruger]
Grp 6	0.652	0.059	0.040	< 0.001	[Mara/Serengeti, Okavango] [Selous, Hwange, NW Namibia, Kruger]
Grp 7	0.615	0.034	0.081	< 0.001	[Mara/Serengeti, Okavango] [Selous] [Hwange] [Kruger, NW Namibia]
Grp 8	0.629	0.022	0.062	< 0.001	[Mara/Serengeti, Okavango] [Selous] [Hwange, Kruger, NW Namibia]
Grp 9	0.612	0.070	0.090	< 0.001	[Mara/Serengeti] [Okavango] [Selous] [Hwange, NW Namibia, Kruger]
Grp 10	0.684	0.060	0.103	< 0.001	[Mara/Serengeti] [Okavango] [Selous] [Hwange] [NW Namibia, Kruger]
Grp 11	0.215	0.208	0.054	< 0.001	[Selous, Kruger] [Mara/Serengeti, Hwange, Okavango, NW Namibia]
Grp 12	−0.284	0.805	0.059	< 0.001	[Mara/Serengeti, Selous, Kruger] [Okavango, Hwange, NW Namibia]
Grp 13	0.253	0.279	0.079	< 0.001	[Mara/Serengeti] [Selous, Kruger] [Okavango, Hwange, NW Namibia]
Grp 14	−0.017	0.434	0.070	< 0.001	[Mara/Serengeti, Selous] [Okavango, Hwange, NW Namibia, Kruger]
Grp 15	0.016	0.512	0.067	< 0.001	[Mara/Serengeti, Selous, Okavango, Hwange, NW Namibia] [Kruger]
Grp 16	−0.504	1.000	0.081	< 0.001	[Selous] [Kruger, Mara/Serengeti, Hwange, Okavango, NW Namibia]
Grp 17	−0.153	0.586	0.055	< 0.001	[Mara/Serengeti, Hwange] [Selous, Okavango, NW Namibia, Kruger]
Grp 18	0.432	0.105	0.043	< 0.001	[Mara/Serengeti, Selous, Okavango] [Hwange, NW Namibia, Kruger]
Grp 19	0.144	0.240	0.091	< 0.001	[Mara/Serengeti] [Selous] [Okavango, Hwange, NW Namibia, Kruger]
Grp 20	0.681	0.098	0.106	< 0.001	[Mara + Serengeti] [Selous] [Okavango] [Hwange] [NW Namibia] [Kruger]

4). As suggested by the cluster analysis, the highest value of ϕ_{CT} (0.684) grouped Kruger and NW Namibia in a single population (Table 5, Grp 10). However, similar values were obtained by grouping Hwange with these southern populations (ϕ_{CT} = 0.612, Grp 9) or by grouping Okavango with the northern populations Masai Mara/Serengeti (Grp 7 and Grp 8, Table 5). In fact, a very high ϕ_{CT} value (0.681) is obtained when all populations are considered independently (Grp 20). Finally, an exact test of population differentiation shows that all populations are significantly differentiated from each other (after pooling Masai Mara and Serengeti) in pairwise comparisons ($P < 0.001$) except for NW Namibia vs. Hwange ($P = 0.141$) and Kruger ($P = 0.076$). However, this is probably a result of the small sample size for NW Namibia ($n = 6$, Table 1).

Gene flow based on mitochondrial control region sequences

We used pairwise ϕ_{ST} values to estimate the number of females that migrate between populations per generation

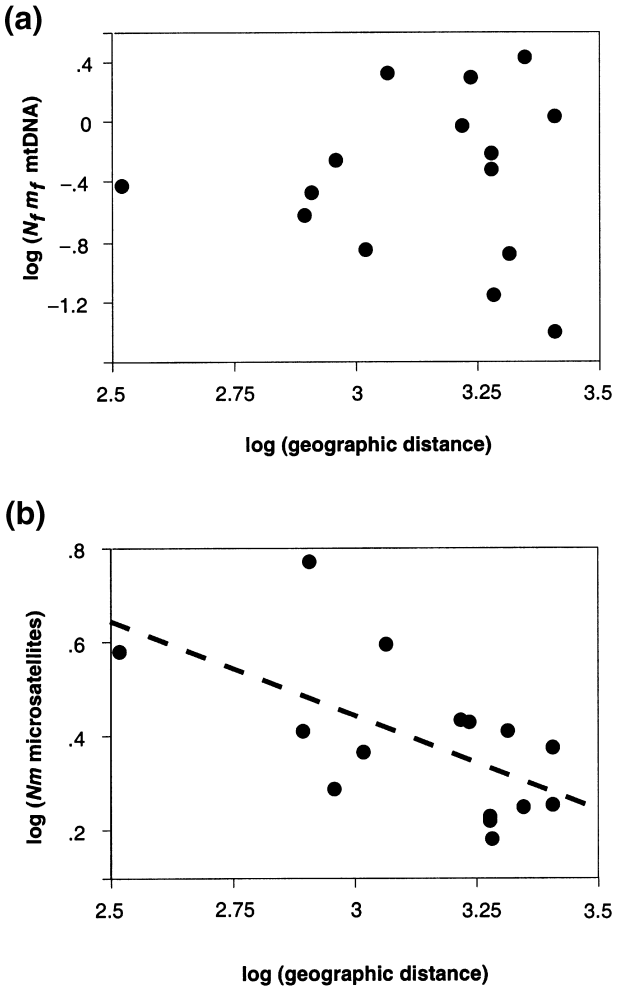

Fig. 4 Plots of *Nm* vs. the natural log of the geographical distance between wild dog populations for mitochondrial DNA (a) and microsatellite (b) data. The latter relationship is significant (Mantel's test $r = -0.579$, $P = 0.032$). The dotted line indicates the least squares regression estimate.

($N_f m_f$; Table 4b). The largest value of $N_f m_f$ occurred between the Okavango population and the Masai Mara/Serengeti population ($N_f m_f = 2.67$; Table 4b). In contrast, the lowest $N_f m_f$ value (0.04) occurred between the Masai Mara/Serengeti population and Kruger. The relationship of gene flow and geographical distance was not significant (Fig. 4a; Mantel's test $Z = 40164.36$, $r = 0.189$, $P = 0.216$).

Microsatellite diversity

With only one exception (locus *L250* in Kruger, $P = 0.015$), no significant deviation from HWE ($P > 0.05$) was found for any microsatellite locus in any population. The levels of genetic polymorphism estimated from the microsatellite data were relatively similar for all seven populations (Table 2). Although the average sample size for each population varied greatly (5.8 in the NW Namibia population to 93.8 in the Kruger population), the mean number of alleles per locus ranged between only 3.4 and 4.4. Heterozygosity values (H_E) were also similar, ranging from 0.56 for the Kruger population to 0.67 for the Selous population. The heterozygosity of the captive population was lower (0.50) and the mean number of alleles per locus was only 3.3 (similar to NW Namibia although the sample size was much larger).

Tests for linkage disequilibrium between alleles at different loci found that between 60% (for Kruger) and 15% (for Okavango) of the pairwise comparisons of loci showed significant ($P > 0.05$) linkage. No loci were consistently in linkage disequilibrium across populations. Wild dogs live in large packs that are often founded by two groups of siblings (Girman *et al*. 1997) or close relatives (Creel & Creel, in press) of the same sex. Thereafter, generally only a single pair reproduces (Girman *et al*. 1997). Consequently, packs are composed of close relatives and oversampling them could lead to high levels of apparent linkage disequilibrium (Hedrick 2000). To test this hypothesis, the alpha male and female, if known (Girman *et al*. 1997), or one adult male and one female from each pack in the Kruger population were selected. Given this sampling regime, 16% instead of 60% of the 55 possible between loci comparisons were significant ($n = 18$). The tree of individuals in Fig. 5 shows that the samples from Kruger and Masai Mara/Serengeti cluster into pack groupings. Consequently, oversampling of relatives caused by pack structure may be the cause of high levels of linkage disequilibrium (e.g. Martínez *et al*. 2000). However, further sampling is needed to determine if the absence of significance is due to smaller sample size.

Distribution and differentiation of microsatellite allele frequencies

All microsatellite loci show significant frequency differences across populations (exact test of population differentiation, $P < 0.001$). In addition, all populations had at least one unique allele as well as one allele absent that was observed in all other populations (Appendix I). The Selous population had the largest number of unique alleles (locus *L155* alleles *147*, *149* and *153*; Fig. 6a) and was missing three alleles found in all other populations (locus *L155* allele *141*, locus *L250* allele *93*, locus *L671* allele *148*). The Kruger population had three unique alleles and was missing one observed in all other populations. The Masai Mara/Serengeti population had two unique alleles and was missing two alleles that were common in other populations. One allele was unique and four common alleles were missing from the NW Namibia population, however, only six individuals had been sampled from this population.

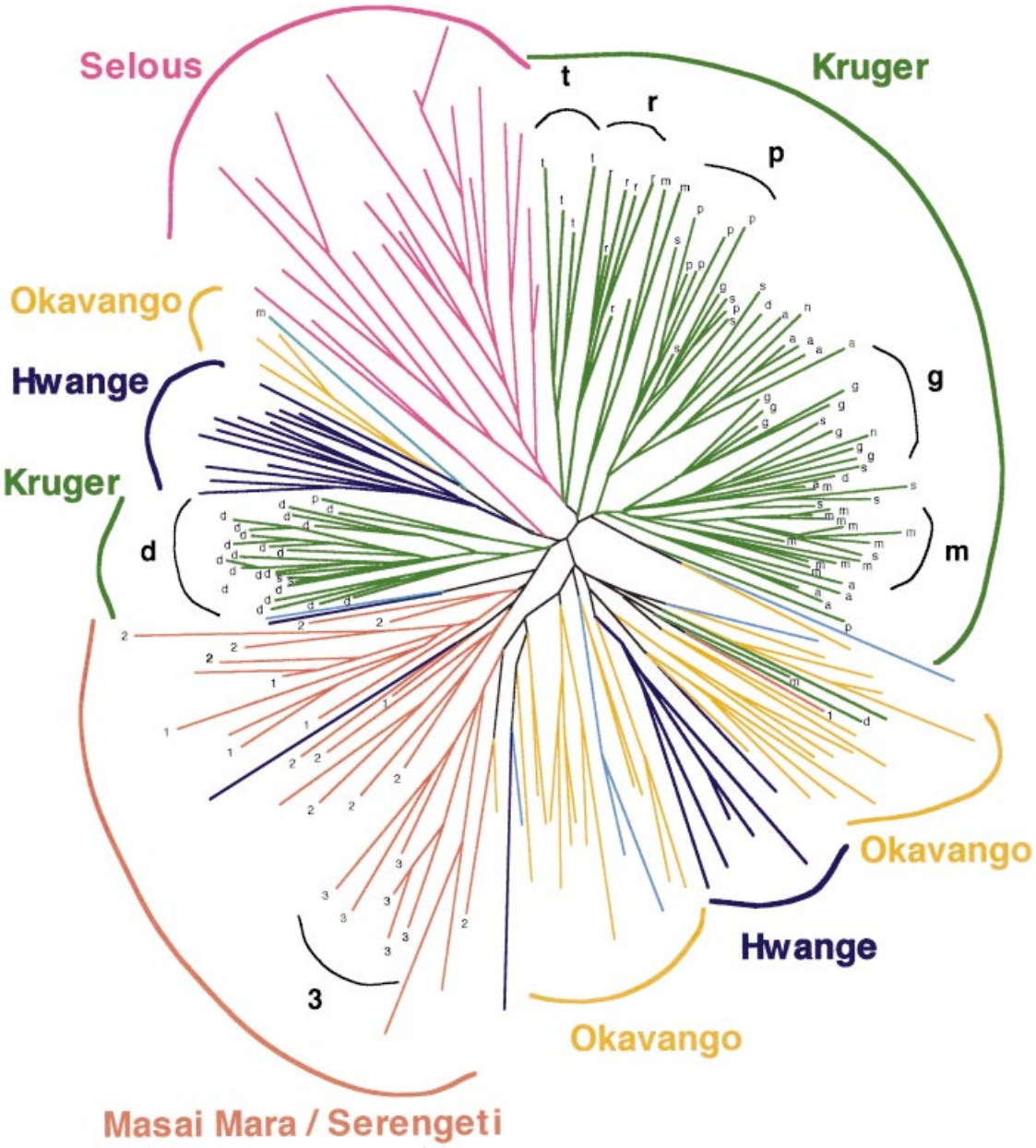

Fig. 5 Unrooted neighbour-joining tree of wild dogs from six populations based on the proportion of shared alleles between individuals. Groups of individuals from the same populations (same colour) are indicated. For Kruger and Masai Mara/Serengeti pack affiliation is indicated by a small number and letter, respectively, and some clusters of individuals with a large proportion of individuals from the same pack are also indicated.

Northern and southern populations differ in microsatellite allele frequencies but within each grouping, alleles are shared among populations. The Masai Mara/Serengeti population shared with the Selous population allele *145* at locus *L155*, allele *87* at *L250*, allele *124* at *L263*, allele *76* at *L423* and allele *150* at locus *L671* (Fig. 6a, Appendix I). In addition, these two populations shared allele frequency similarities (e.g. locus *L677*; Fig. 6b). The three well-sampled southern populations, Kruger, Hwange and Okavango, shared alleles not observed in other populations such as allele *137* at locus *L155* and allele *77* at locus *L173* (Fig. 6a; Appendix I). To examine overall patterns of population differentiation a NJ tree based on Nei's unbiased genetic distance was constructed (Nei 1978; Table 3, Fig. 3b). The branch length and topology of the resulting tree differed from that obtained using mtDNA sequences (Fig. 3a). The tree had longer terminal branches and all populations were well differentiated. In addition, population groupings corresponded more closely with geographical proximity. Masai Mara/Serengeti populations were closest to

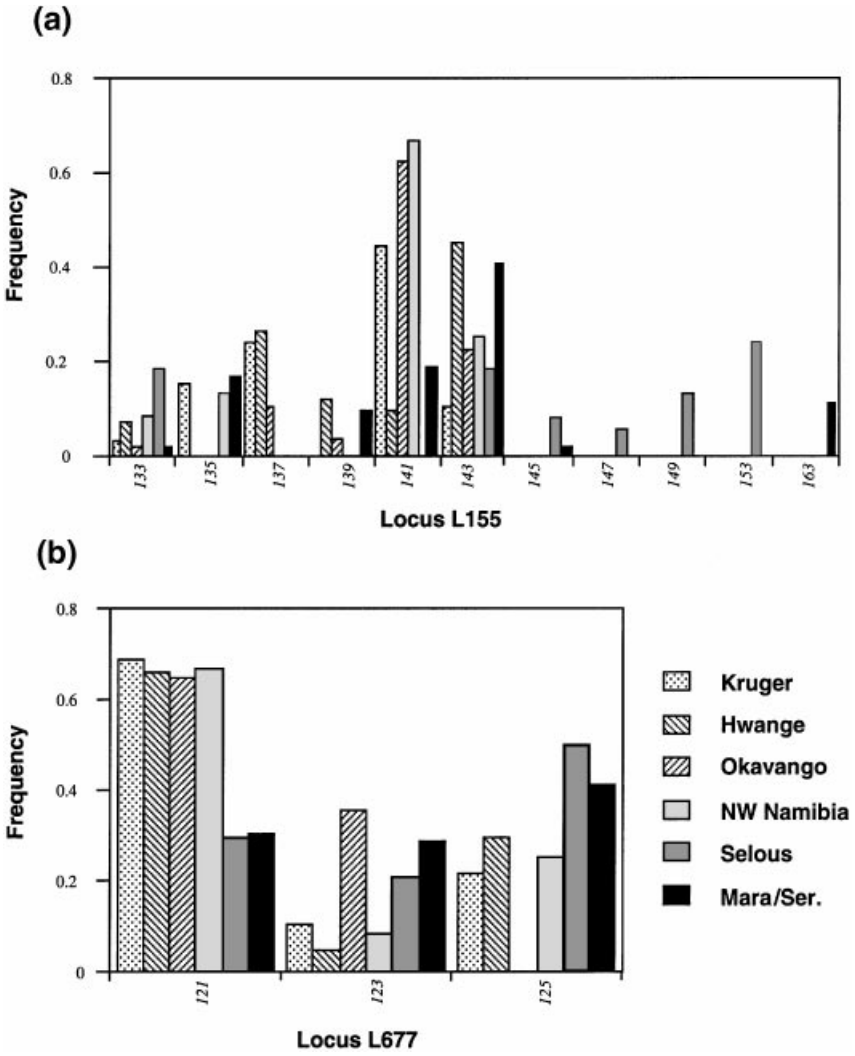

Fig. 6 Allele distribution at microsatellite locus *L155* (a) and locus *L677* (b).

Table 6 Assignment of individuals to populations and percentage of correct classifications based on microsatellite data

	Assigned to population:						
Nominal pop.	Mara/Ser.	Selous	NW Nam.	Okavan	Hwange	Kruger	Correct (%)
Masai/Ser.	27	1	0	0	0	0	96
Selous	2	19	0	0	1	0	86
NW Namibia	0	0	3	1	2	0	50
Okavango	1	0	1	29	0	0	94
Hwange	0	0	1	2	19	0	86
Kruger	0	0	0	0	0	94	100

neighbouring Selous. Similarly, NW Namibia and Okavango were geographically and genetically proximate. Hwange and Kruger populations are grouped with these two southern populations.

In a NJ tree based on allele sharing distance between individuals, wild dogs generally clustered according to population, which suggests genetic subdivision (Fig. 5). Ninety-four per cent of wild dogs were correctly classified to population of origin using an assignment test (Table 6). Nine of 12 cross-assignments (75%) involved assignments to neighbouring populations and may indicate the presence of recent migrants (Paetkau *et al*. 1998). Seven of the

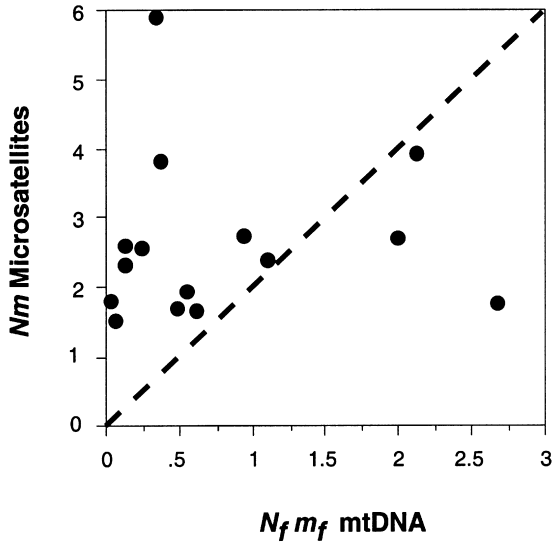

Fig. 7 Plot of the number of migrants per generation (*Nm*) estimated using mitochondrial DNA data or microsatellites for pairwise comparisons between six African wild dog populations. The dashed line indicates the expected relationship assuming equal sex ratio and equal contribution to reproduction of both sexes.

cross-assignments involved dogs from NW Namibia, Okavango and Hwange, three populations that are included in a large continuous assemblage encompassing part of Zambia, Zimbabwe, Botswana and Namibia (Fanshawe *et al*. 1997). None of the Kruger samples were misassigned although this is the largest population sample (93 individuals). Significant differentiation between populations and population groupings was supported by AMOVA (Table 5). The highest θ-values were generally obtained by treating all populations as separate except Mara/Serengeti (e.g. Grp 20, θ = 0.106) or by grouping two or more southern populations (e.g. Grp 19, θ = 0.091; Grp 10, θ = 0.103). Although both ϕ_{ST} and θ suggest that all populations were differentiated, the two indices were not correlated in pairwise comparisons (Table 4a; Mantel's test $r = 0.193$, $P = 0.244$).

Gene flow based on microsatellite loci

We used pairwise θ-values to estimate the number of individuals that migrate between each pair of sampling localities per generation (*Nm*; Table 4b). Okavango and NW Namibia populations had the highest values of *Nm* (5.88), whereas NW Namibia and Serengeti/Masai Mara populations had the lowest (1.53). A significant negative correlation was found between pairwise *Nm* values and geographical distance between localities (Mantel's test $Z = 110531.54$, $r = -0.579$, $P = 0.032$; Fig. 4b). Microsatellite and mitochondrial DNA estimates of *Nm* values were not significantly related (Mantel's test $Z = 63.08$, $r = 0.036$, $P = 0.368$) and in only one comparison was *Nm* based on the mtDNA sequence larger than the corresponding value based on microsatellites (Okavango and Masai Mara/Serengeti, Table 4b). *Nm* estimates based on microsatellite data on average were 10.7 times greater than that based on control region sequences (range: 0.67–45). Assuming equal sex ratio and equal contribution to reproduction by both sexes, the estimated number of migrants calculated from microsatellite data is expected to be twice the number of female migrants estimated from mtDNA data. However, the migration rate estimated with microsatellites is significantly more than twice the estimated number of effective female migrants between populations (Wilcoxon signed-rank test, $Z = -2.10$, $P = 0.036$; Fig. 7). Consequently, interpopulation migration might be more frequent in male than female African wild dogs.

Discussion

Genetic diversity and demographic history

The index of nucleotide diversity, π or θ_T, reflects recent, rather than historical, population size because genealogical information is not used in the calculation of the index (Crandall *et al*. 1999). Assuming a mutation rate of $\approx 5 \times 10^{-8}$ per nucleotide sites per year (Vilà *et al*. 1999) for control region I and a generation time of 3 or 5.35 years (Vilà *et al*. 1999; Creel & Creel, in press), we estimate an effective population size of 54 200–97 000 females. However, assuming that half the animals in the remaining populations of wild dogs are females, only 1500–2500 female wild dogs are thought to exist in Africa today (Fanshawe *et al*. 1997). Moreover, the female effective population size is much less than this value because wild dogs live in packs of 5–20 adults (mean pack size in Selous is 8.3 individuals, Creel *et al*. 1997; see also Creel 1998) in which only a single female generally breeds. Consequently, our results suggest a recent 50–100-fold decrease in female effective population size. However, previous genetic and morphologic results suggest that the eastern and southern African populations may be genetically independent (Girman *et al*. 1993). An estimate of the female effective population size for the eastern and southern populations based on θ_T gives values of 14 600–26 000 ($\theta_T = 0.0039$) and 13 000–23 000 ($\theta_T = 0.0035$). These more conservative second estimates still suggest that at least a 20-fold decrease in female effective population size has occurred.

Although wild dogs were once widely distributed across all sub-Saharan African ecosystems, except tropical forests (see Fig. 1), their populations have declined dramatically since European colonization and the subsequent growth of indigenous human populations (Ginsberg & Woodroffe 1997; Woodroffe & Ginsberg 1997; Creel & Creel 1998). The current distribution of African wild dogs is extremely

patchy and now measures < 1/20 of the original range, and in many of these areas wild dogs are considered vagrant or uncommon (Fanshawe *et al*. 1997). Our results indicate that the historical decline in wild dog populations has not yet resulted in a parallel loss of mtDNA diversity predicted by current census population sizes.

The value of the index of nucleotide diversity that utilizes genealogical information, θ_F, was 0.0102 and slightly lower than the value for θ_T (0.0145). Comparison of the two indices suggests that the recent population size was greater than the historical population size in African wild dogs (see above). In contrast, for other wolf-like canids, values of θ_T were significantly lower than θ_F, suggesting a 10-fold decrease in population size during the late Pleistocene (Vilà *et al*. 1999). The estimated historic female effective population of coyotes and gray wolves based on θ_F was 3.7 and 5 million, respectively. A much larger historic population size was estimated for coyotes than African wild dogs despite coyotes having a smaller historic range. In gray wolves and coyotes, values of nucleotide diversity, θ_T, also were much higher than in wild dogs and suggest an effective population size of 1 153 000 and 2 000 000, respectively. In wolves, current census population size is ≈ 300 000 individuals, which reflects hunting and habitat loss during the last century from a prehistoric size of ≈ 2 million individuals (Seton 1925; Carbyn 1987; Vilà *et al*. 1999). In contrast, coyotes have a current population size that has recently increased to ≈ 7 million individuals (Lehman & Wayne 1991; Voigt & Berg 1987; Vilà *et al*. 1999).

One possible explanation for the lower nucleotide diversity of wild dogs is that they have been consistently more limited by ecological and physiological factors than other large carnivores. Wild dogs are poor competitors, and often lose kills to hyenas and, especially, lions and may be rare or absent in areas where lions and hyenas are common (Creel & Creel 1998; Gorman *et al*. 1998). However, one of the largest wild dog populations occurs in the Okavango region, where both lions (> 1600) and hyenas (> 3000) are common (Kat, unpublished data). In contrast, gray wolves are the top carnivore over much of their geographical range and likely do not suffer interference competition to the same degree (Mech 1970). Coyotes are smaller than wild dogs and are poor competitors but currently live in habitats where other large predators are comparatively rare (Gier 1975; Macdonald 1984). In addition, wild dog habitat has periodically contracted and fragmented during Pleistocene climate changes (Hamilton 1976). Historical fluctuations in population size cause the harmonic mean of the effective population size to be much smaller than the average census population size and result in a more recent coalescence than predicted from census population size alone (Avise *et al*. 1984; Avise 1994). Consequently, current ecological and demographic data, in combination with our results, suggest that wild dogs have been historically less abundant than other large canids but have not experienced the same magnitude of historical population decline.

The recent decline in wild dog numbers has not yet caused a substantial reduction in levels of mitochondrial diversity. Similarly, the level of genetic diversity at microsatellite loci was not significantly reduced relative to other wolf-like canids. The mean number of alleles per locus and heterozygosity per population was only slightly lower than the values observed for wolves and coyotes (Roy *et al*. 1994; Forbes & Boyd 1997; Table 2). However, limited evidence for a recent loss of genetic diversity is found in historical samples from the Transvaal, which had the Z1 genotype. This sample, collected in the 1930s, presumably represented the same population as found in Kruger National Park today. Given the large sample size from Kruger ($n = 94$), the binomial probability of missing this genotype was < 1 in 10 000 if it had a frequency of 10% or more. Consequently, the Kruger populations may have lost genotype diversity in the 50 years since that sample was obtained. In fact, wild dogs were treated as vermin in parts of southern Africa, and through habitat loss and extermination programmes have disappeared from large areas. The currently large population found in the Kruger National Park is thus likely to have originated from a smaller founder population with reduced genetic diversity.

Differentiation between eastern and southern African populations

All wild dog populations are well differentiated with regard to the frequency of mitochondrial control region sequences and microsatellite alleles (Tables 4a and 6). In addition, we found two distinct mtDNA genotype clades in wild dogs (Fig. 2). One clade included genotypes S1, S2, S3, Z1 and Z2 that were not found in eastern Africa (Masai Mara-Serengeti), rather genotypes E1 and E3 were common there (Table 1). Genotype E2 also was clustered in the eastern African clade and was common in Selous, the locality closest to Masai Mara/Serengeti. However, this genotype was found in three southern African populations as well (Table 1). A museum-based Nigerian sequence had affinities to the eastern African clade suggesting that the eastern clade spanned sub-Saharan Africa north of the rainforest belt (Fig. 1). In a previous study, analysis of mtDNA restriction sites also revealed two genotype clades with similar east/south divisions (Girman *et al*. 1993). However, no intervening populations were sampled, leading to the conclusion that eastern and southern populations were isolated genetic units. Consequently, two subspecies were recognized. Our current, more extensive analysis, affirms the earlier results but defines an extensive admixture zone for genotypes that spans much of the current geographical range of the African wild dog.

The mean sequence divergence between both clades is 3.4%, and assuming a divergence rate of 10% per million years for the canid control region I (Vilà *et al*. 1999), the two clades diverged ≈ 340 000 years ago. This divergence time is similar to that of 400 000 years estimated for the two wild dog clades in the previous mtDNA RFLP study (Girman *et al*. 1993). Within each clade, mean sequence divergence values imply coalescence ≈ 70 000 years ago. However, considerable caution should be used in the literal interpretation of past population sizes above and divergence times given that mutation rates are estimated from a single gene and problems of inaccuracy in the molecular clock (Lynch & Jarrell 1993; Gibbons 1998).

We suggest three possible means by which these divergent clades could have arisen. First, the distribution of 'miombo' (*Brachystegia–Julbernardia*) forest could have influenced wild dog movements (Fig. 1). The miombo forest exhibited cycles of expansion and contraction throughout the Pleistocene (Hamilton 1976, 1982). Several species of birds and mammals have distributions interrupted by the miombo forest, including other canids, such as black-backed jackals (*Canis mesomelas*) and bat-eared foxes (*Otocyon megalotis*; Kingdon 1997). However, wild dogs can survive in forested habitats and thus the expansion of miombo woodlands alone was probably not enough to act as an effective barrier to gene flow (Verdcourt 1969; Hamilton 1976, 1982; Fanshawe *et al*. 1991). The highest densities of wild dogs are now found in miombo woodlands in the northern Selous, and these habitats also harbour the highest density populations of wild dogs in West Africa, Niokolo-Koba (S. Creel, personal observation).

Second, the Rift Valley has acted as a barrier to gene flow for bird species (Benson *et al*. 1962; Freitag & Robinson 1993), mammals (Kingdon 1997; Matthee & Robinson 1997; Arctander *et al*. 1999) and insects (Lehmann *et al*. 2000). The tropical rainforest in Africa is distributed around the Gulf of Guinea, extending to the western edge of the rift lakes. Primary rainforest is avoided by wild dogs, which are mainly restricted to savannah and acacia woodlands around the tropical rainforest belt (Fanshawe *et al*. 1997). Genotypes Z and S are found in current and historical samples south of the Rift Valley and in the Selous population, whereas in eastern Africa, genotypes E1, E2 and E3 are common (including two historic samples from Kenya, with genotype E1). Consequently, the Rift Valley could be proposed as a possible barrier to gene flow to explain the divergence between wild dog sequence clades. However, this barrier must have become effective for wild dogs subsequent to the formation of rift lakes Tanganyika and Malawi ≈ 1.5–2 Ma (Cromie 1982; Greenwood 1984) because the control region sequence data suggest that eastern and southern clades diverged 340 000 years ago. Conceivably, periods of rainforest expansion due to climate change, in combination with the development of the rift, may have been enough to decrease gene flow to a level that allowed for isolation and divergence of eastern and southern clades.

Third, the distinctiveness of eastern African clades might not necessarily mean that eastern African populations diverged *in situ*. A detailed study of three bovid species (hartebeest, *Alcelaphus buselaphus*; topi, *Damaliscus lunatus*; and wildebeest, *Connochaetes taurinus*) recently conducted by Arctander *et al*. (1999) may have relevance to the pattern of genetic divergence noted among wild dog populations. Although the oldest wildebeest fossils to date have been found in Tanzania (1.5 Ma; Vrba 1995), current populations of the species in eastern Africa represent a genetically depauperate subset of southern African wildebeest. Development of grasslands in eastern Africa was relatively recent (0.6 Ma), although a period of grassland predominance was also detected between 1.7 and 1.2 Ma (Cerling 1992). Arctander *et al*. (1999) therefore proposed that current wildebeest populations in eastern Africa were derived secondarily by migration from southern African refuges after local extinctions in eastern Africa, possibly related to loss of habitat due to Pleistocene climatic changes. Patterns of genetic differentiation among the other bovid species studied were different, but also indicated a trend of colonization of current ranges from refugia in western, central/eastern and southern Africa. Current populations of wild dogs in eastern Africa might similarly have been derived from western or central African refugia. Secondary migrations may then have occurred southwards from eastern Africa and northwards from southern Africa. This would explain the similarity among eastern African genotypes and the Nigerian genotype, as well as the presence of southern and eastern African genotypes in the Selous in Tanzania. This scenario would assume that there are no significant geographical barriers to dispersal between southern and eastern Africa, and between western and eastern Africa, which is consistent with the dispersal capabilities of highly mobile species such as wild dogs. Such apparent patterns of genetic subdivision have been shown to have a dispersal origin in North American brown bears (Leonard *et al*. 2000). However, further analyses of wild dog populations from central and western Africa are needed to more comprehensively evaluate this hypothesis.

Male and female migration

The number of migrants per generation between populations (Table 4b) varied between 1.53 and 5.88 and 0.04 and 2.67 for nuclear and mitochondrial loci, respectively. The differences in *Nm* estimates from mitochondrial and nuclear microsatellite loci are greater than predicted by the difference in inheritance of the two markers (Fig. 7). This discrepancy can be explained if males disperse more often and/or farther than females. Studies of eastern African wild dogs suggest that although females disperse more

frequently than males, males often disperse over longer distances (Frame & Frame 1976; Frame *et al*. 1979; McNutt 1996). In addition, research in Kruger National Park indicated that male wild dogs immigrated into the populations more often than females who generally dispersed to territories near close relatives (Girman *et al*. 1997). Consequently, the results of this study are consistent with long-distance dispersal by males as the principle cause of genetic exchange between populations. However, infrequent long-distance dispersal by females may explain the large-scale pattern of admixture of control region sequences.

Implications for conservation

Our previous results (Girman *et al*. 1993) suggested that eastern and southern African wild dog populations were morphologically and genetically differentiated and formed reciprocally monophyletic units. This result supported their classification as evolutionarily significant units warranting separate conservation (Moritz 1994). However, our current more extensive survey finds that these two genotype clades co-occur over much of the current geographical range, which likely reflects natural mixing of previously isolated populations. Consequently, genetic management should aim at mimicking observed levels of gene flow between contiguous populations within this admixture zone (Crandall *et al*. 2000; Wayne & Brown, in press). Individual-based models of wild dog population dynamics also suggest that even low rates of migration between populations can demographically stabilize populations otherwise at risk of extinction (Vucetich & Creel 1999). However, in wild dogs, genetic differentiation of microsatellite loci increases with distance (see above) and eastern and southern African populations may be morphologically distinct. Consequently, translocations between distant southern and eastern populations are not advised because there may be potential fitness differences between them (see discussion in Crandall *et al*. 2000). For the Masai Mara and Serengeti, where wild dogs are endangered (Fanshawe *et al*. 1997), the Selous region would be an appropriate source of individuals for reintroduction at the level of a few migrants per generation (Table 4b). In addition, because our results suggest more frequent dispersal and/or longer dispersal distances in males than in females, the population management strategy should focus on the long-distance translocation of males to replicate natural processes (e.g. male Selous wild dogs to Masai Mara/Serengeti). All captive wild dog populations that we examined are fixed for the same control region genotype S2, the most common in southern populations (except Okavango; Table 1). Thus the captive population probably derives from southern African wild dog populations. These individuals might be suitable for reintroduction throughout southern Africa given the similarity of wild dog populations and high levels of gene flow (Table 4b). However, microsatellite variation is reduced in captive wild dogs (Table 2) suggesting outbred wild caught individuals would provide a better source for reintroduction. In addition, captive individuals are often poorly acclimated to natural environments (Carlstead 1996) and may be vectors of nonendemic diseases (Snyder *et al*. 1996). Reintroductions of captive raised wild dogs have generally failed due to their poor hunting ability and poor avoidance of lion predation (Scheepers & Venzke 1995). However, simulating pack formation by introducing captive animals of one sex to wild caught ones of the other has proved a successful way to reintroduce wild dogs (Mills, personal observation).

Finally, our genetic analyses have shown that, despite recent population declines, the genetic diversity of many populations is still high. However, the populations we studied are among the largest in Africa (Fanshawe *et al*. 1997) and thus may not be indicative of genetic diversity in smaller, more isolated populations. Given increasing habitat loss and fragmentation, a future decline in genetic variation is likely (Fanshawe *et al*. 1997; Ginsberg & Woodroffe 1997). To ameliorate this decline, population sizes should be kept as large as possible given remaining habitats. In addition, gene flow should be maintained between populations. Gene flow should be facilitated by maintaining corridors that link populations, and when this is not possible, through translocation at historic levels (Table 4b). The maintenance of genetic variation, particularly the component that influences fitness, is critical to population persistence and the future evolutionary response of wild dogs to changing environmental conditions (Crandall *et al*. 2000).

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Appendix I

Allelic frequencies for each locus and population. Sample size is given in parenthesis. Alleles, in italics, are designated by their size. Alleles that are absent from just one population are underlined. Private alleles (only in one population) are double underlined

	Kruger	**Hwange**	**Okavango**	**NW Nam.**	**Selous**	**Mar /Ser.**
L155	(93)	(21)	(29)	(6)	(19)	(27)
133	0.0323	0.0714	0.0172	0.0833	0.1842	0.0185
135	0.1505	0.0000	0.0000	0.0000	0.1316	0.1667
137	0.2742	0.2619	0.1034	0.0000	0.0000	0.0000
139	0.0000	0.1190	0.0345	0.0000	0.0000	0.0926
141	0.4409	0.0952	0.6207	0.6667	0.0000	0.1852
143	0.1022	0.4524	0.2241	0.2500	0.1842	0.4074
145	0.0000	0.0000	0.0000	0.0000	0.0789	0.0185
147	0.0000	0.0000	0.0000	0.0000	0.0526	0.0000
149	0.0000	0.0000	0.0000	0.0000	0.1316	0.0000
153	0.0000	0.0000	0.0000	0.0000	0.2368	0.0000
163	0.0000	0.0000	0.0000	0.0000	0.0000	0.1111
L173	(93)	(22)	(30)	(6)	(8)	(28)
65	0.0000	0.0000	0.0000	0.0000	0.0000	0.0893
67	0.0108	0.0000	0.0167	0.1667	0.0000	0.0357
69	0.7097	0.5682	0.3333	0.4167	0.5000	0.4821
71	0.1290	0.2273	0.3000	0.4167	0.4375	0.1964
73	0.0645	0.0682	0.2000	0.0000	0.0625	0.1964
77	0.0860	0.1364	0.1500	0.0000	0.0000	0.0000
L250	(94)	(22)	(31)	(6)	(22)	(28)
87	0.0000	0.0000	0.0000	0.0000	0.2045	0.0179
89	0.0000	0.1591	0.1774	0.0000	0.0227	0.0893
91	0.5213	0.2500	0.4194	0.3333	0.2045	0.1786
93	0.0053	0.2045	0.1774	0.0833	0.0000	0.1429
95	0.1755	0.2727	0.1935	0.5833	0.3409	0.0000
97	0.2979	0.1136	0.0323	0.0000	0.2273	0.5714
L263	(94)	(21)	(25)	(6)	(13)	(28)
124	0.0000	0.0000	0.0000	0.0000	0.0769	0.1786
132	0.0851	0.0952	0.1800	0.1667	0.2308	0.3036
134	0.4840	0.0476	0.1400	0.0000	0.3077	0.2500
136	0.1277	0.3571	0.4600	0.5000	0.0385	0.0179
138	0.2819	0.4524	0.1800	0.2500	0.1154	0.2500
140	0.0213	0.0000	0.0000	0.0000	0.1923	0.0000
142	0.0000	0.0476	0.0400	0.0833	0.0385	0.0000
L366	(94)	(22)	(29)	(5)	(13)	(18)
128	0.2606	0.2727	0.3793	0.2000	0.2692	0.2222
130	0.0160	0.2955	0.0690	0.0000	0.1538	0.5833
132	0.7234	0.3864	0.5000	0.5000	0.4231	0.1944
134	0.0000	0.0000	0.0345	0.0000	0.1538	0.0000
136	0.0000	0.0455	0.0172	0.3000	0.0000	0.0000
L423	(94)	(15)	(28)	(6)	(14)	(26)
70	0.0000	0.0000	0.1071	0.0000	0.0000	0.0000
74	0.2926	0.2000	0.3036	0.2500	0.1071	0.0577
76	0.0000	0.0000	0.0000	0.0000	0.3214	0.1346
78	0.2926	0.2667	0.0179	0.5000	0.1786	0.3077
80	0.3138	0.4000	0.5714	0.1667	0.1429	0.4808
82	0.0532	0.0667	0.0000	0.0833	0.2500	0.0192
84	0.0000	0.0667	0.0000	0.0000	0.0000	0.0000
86	0.0479	0.0000	0.0000	0.0000	0.0000	0.0000

Appendix I *Continued*

	Kruger	**Hwange**	**Okavango**	**NW Nam.**	**Selous**	**Mar /Ser.**
L442	(94)	(22)	(31)	(6)	(22)	(28)
116	0.1489	0.5682	0.3065	0.0833	0.3182	0.3214
118	0.8511	0.4318	0.6935	0.9167	0.6818	0.6786
L453	(94)	(22)	(31)	(6)	(22)	(27)
79	0.4947	0.5455	0.6129	0.4167	0.6136	0.6481
85	0.3777	0.1364	0.0645	0.0833	0.1818	0.1667
89	0.1117	0.0000	0.0484	0.0833	0.0682	0.0556
105	0.0160	0.0000	0.0000	0.0000	0.0909	0.1296
107	0.0000	0.3182	0.2742	0.4167	0.0455	0.0000
L606	(94)	(22)	(31)	(6)	(21)	(28)
119	0.0053	0.0909	0.0000	0.0000	0.0000	0.0000
121	0.4415	0.4318	0.3710	0.4167	0.7857	0.3571
123	0.5532	0.4773	0.6290	0.5000	0.2143	0.6429
125	0.0000	0.0000	0.0000	0.0833	0.0000	0.0000
L671	(93)	(20)	(31)	(5)	(20)	(27)
146	0.0851	0.0000	0.0000	0.0000	0.0000	0.0000
148	0.1170	0.1250	0.1935	0.2000	0.0000	0.0556
150	0.0000	0.0000	0.0000	0.0000	0.1000	0.0370
152	0.5053	0.1500	0.1935	0.3000	0.2000	0.5556
154	0.1543	0.3500	0.3387	0.2000	0.4000	0.2778
156	0.0000	0.2750	0.1774	0.2000	0.2750	0.0741
158	0.0160	0.1000	0.0968	0.1000	0.0250	0.0000
160	0.1223	0.0000	0.0000	0.0000	0.0000	0.0000
L677	(93)	(22)	(31)	(6)	(22)	(28)
121	0.6862	0.6591	0.6452	0.6667	0.2955	0.3036
123	0.1011	0.0455	0.3548	0.0833	0.2045	0.2857
125	0.2128	0.2955	0.0000	0.2500	0.5000	0.4107