CHAPTER 5

Microsatellites reveal patterns of relatedness in a local African wild cat (*Felis silvestris*) population from the southern Kalahari, with limited evidence of hybridisation with the domestic cat (*F. s. catus*)

1. Abstract

The African wild cat (*Felis silvestris*) has a wide geographic range, stretching throughout most of the African continent, except in the tropical forests and true desert areas. Hybridisation with feral domestic cats is thought to be a threat to the genetic integrity of wild cats throughout their range. Several admixture studies on the European wild cat have been reported, but there is limited information available on the status of the African wild cat. Here we report the genetic variation and admixture analysis of 57 wild living African wild cats and 46 domestic cats using 18 microsatellite loci. Cats were morphologically identified as African wild cats (*F. s. cafra*) and two geographically separated domestic cat populations (*F. s. catus*), independent of any prior genetic information. Significant genetic differentiation between these groups confirms earlier suggestions of the distinctiveness of African wild cats and domestic cats. Bayesian cluster analysis also showed evidence of these two distinct entities and identified four cryptic hybrids among the wild cats. All hybrids were either outside or on the periphery of the Kgalagadi Transfrontier Park, suggesting that the level of introgression is low, yet still of concern for the genetic integrity of the African wild cat. The genetic diversity within our wild cat population was significantly higher than in the domestic cat populations and relatedness values were compared with results from direct observations.

Keywords: Felis silvestris, African wild cat, domestic cat, hybridisation, microsatellites, admixture, Bayesian clustering, relatedness

2. Introduction

The wild cat (*Felis silvestris*) is classified as a polytypic species with three or more distinct subspecies: African or Sardinian wild cat (*F. s. lybica*), European wild cat (*F. s. silvestris*), Asian wild cat (*F. s. ornata*) (Nowell & Jackson, 1996; Sunquist & Sunquist, 2002) and possibly the Chinese sand cat (*F. s. bieti*) (Driscoll, Menotti-Raymond, Roca, Hupe, Johnson, Geffen, Harley, Delibes, Pontier, Kitchener, Yamaguchi, O'Brien & Macdonald, 2007), as well as a domesticated form (*F. s. catus*) (Ragni & Randi, 1986; Randi & Ragni, 1991; Wozencraft, 1993; Johnson & O'Brien, 1997). Wild cats are widely distributed in Europe, Asia and Africa, they are closely related and form the so-called 'domestic lineage' in the genus *Felis* (Sunquist & Sunquist, 2002). The domestic lineage diverged around 6.2 million years ago and resulted in seven species (the black-footed cat: *F. nigripes*, the jungle cat: *F. chaus*,

the Chinese desert cat: *F. bieti*, the desert of sand cat: *F. margarita*, the African wild cat: *F. silvestris cafra, the European wild cat: F. s. silvestris* as well as the domestic cat: *F. s. catus*) (Ragni & Randi, 1986; Randi & Ragni, 1991; Masuda, Lopez, Slattery, Yuhki & O'Brien, 1996; Nowell & Jackson, 1996; Johnson, Eizirik, Pecon-Slattery, Murphy, Antunes, Teeling & O'Brien, 2006; Johnson & O'Brien, 1997; Johnson & O'Brien, 2007; Randi, Pierpaoli, Beaumont, Ragni & Sforzi, 2001). The domestication of the wild cat most likely occurred in the Near East and probably in parallel with agricultural village development in the Fertile Crescent (Driscoll *et al*., 2007; Driscoll, Macdonald & O'Brien, 2009) 8,000 to 10,000 years ago (O'Brien & Johnson, 2007). Today about 600 million domestic cats are distributed worldwide; they can interbreed with wild cats and produce fertile offspring, both in the wild and in captivity (O'Brien & Johnson, 2007; Robinson, 1977; Ragni, 1993).

The problematic description and classification of the species together with morphological similarities makes it difficult to distinguish between tabby-like domestic cats, true wild cats and, in particular, their hybrid forms. This leads to increased confusion about the subspecific status of *F. silvestris* populations (Clutton Brock, 1999; Allendorf, Leary, Spruell & Wenburg, 2001; Sunquist & Sunquist, 2002; Driscoll *et al*., 2007). Furthermore, continued co-existence of domestic- and wild cats as well as increased habitat reduction for wild cats, raised the fear that widespread interbreeding would lead to genetic extinction through hybridisation and introgression (Nowell & Jackson, 1996; Rhymer & Simberloff, 1996; Randi, 2003; 2008) of populations in Europe (Suminski, 1962), the Near East (Mendelssohn, 1999) and in South Africa (Smithers, 1983; Stuart & Stuart, 1991).

European studies revealed that wild cats and domestic cats are genetically distinct, with different rates of admixture, from recent and frequently hybridising populations in Scotland and Hungary (Beaumont, Barratt, Gottelli, Kitchener, Daniels, Pritchard & Bruford, 2001; Daniels, Beaumont, Johnson, Balharry, Macdonald & Barratt, 2001; Pierpaoli, Biró, Herrmann, Hupe, Fernandes & Ragni, 2003; Lecis, Pierpaoli, Biró, Szemethy, Ragni, Vercillo & Randi, 2006), to contrasting low genetic introgression in Italy, Germany and Portugal populations (Randi *et al*., 2001; Pierpaoli *et al*., 2003; Randi 2003; Lecis *et al*., 2006; Oliveira, Godinho, Randi & Alves, 2008*b*). In the studies where African wild cat samples were analysed (Randi *et al*., 2001; Driscoll *et al*., 2007), wild cats and domestic cats were classified as genetically distinct from each other. The African wild cat is not a protected species; however, hybridisation with domestic cats is a real concern (Smithers, 1983; Nowell & Jackson, 1996). Although genetic introgression has not been fully studied locally, a recent study by Wiseman, O'Ryan & Harley (2000) suggests that introgression appears to be lower than previously thought and occur mainly from the wild to domestic cats.

Apart from concerns regarding the genetic purity of African wild cat populations, especially in and near urbanised areas, very little is known about the biology of this widespread small predator. The southern Kalahari population was selected as the model study population, not only because the open dune habitat is ideal for radio tracking and observing individual cats, but also the remoteness of the area that has been declared a national park since 1931 made the possibility of identifying a genetically pure African wild cat population likely.

Hybrid zones are regions where two genetically differentiated taxa overlap and admixture events occur and have received substantial attention in recent years (Barton & Hewitt, 1989). Hybridisation occurs more frequently than originally believed (Mallet, 2005; Meyer, 2006) and may be due to human induced, such as between domestic and wild species, or domestic and captive species (Nijman, Otsen, Verkaar, de Ruiter, Hanekamp, Ochieng, Shamshad, Rege, Hanotte, Barwegen, Sulawati & Lenstra, 2003; Lecis *et al*. 2006), or between introduced and native species (Goodman, Barton, Swanson, Abernethy & Pemberton, 1999; Riley, Shaffer, Voss & Fitzpatrick, 2003). Natural hybridisation has been described across the zootaxa, including in insects (Beltran, Jiggins, Bull, Linares, Mallet, McMillan & Bermingham, 2002), fish (Saltzburger, Baric & Sturmbauer, 2002), amphibians (Szymura & Barton, 1991), birds (Grant, Grant, Markert, Keller & Petren, 2005) and carnivores (Lehman, Eisenhawer, Hansen, Mech, Peterson, Gogan & Wayne, 1991). In particular the question of hybridisation in domestic and wild cat populations has been extensively studied (Hubbard, McOrist, Jones, Biod, Scott & Easterbee, 1992; Daniels, Balharry, Hirst, Kitchener & Aspinall, 1998; Randi *et al*., 2001; Beaumont *et al*., 2001; Pierpaoli *et al*., 2003; Lecis *et al*., 2006; Oliveira *et al*., 2008*b*). The methods and procedures to identify cryptic population structure and admixture have advanced from mitochondrial DNA and allozyme analysis (Randi & Ragni, 1991; Hubbard *et al*., 1992) to improved accuracy through the use of microsatellites, especially when combining highly polymorphic markers with recently developed Bayesian clustering models (Lecis *et al*., 2006).

Possible evolutionary outcomes of hybridisation could include (i) that two hybrid taxa may merge, (ii) reproductive barriers may be reinforced between parental taxa, (iii) the transfer of genetic material into both parental taxa (this may facilitate adaptive evolution) (iv) a new species of hybrid origin may evolve or, (v) the hybrid zone may become established without any major impact on the parental taxa (Arnold, 1992; Seehausen, 2004). Therefore the studies of hybrids can give important insights into evolutionary processes and adaptation of species (Pastorini, Zaramody, Curtis, Nievergelt & Mundy, 2009).

Knowledge of relatedness and relationships between individuals is important to describe the behaviour and social structure of a species (Ralls, Pilgrim, White, Paxinos, Schwartz & Fleischer, 2001). Social structures are characterised by territoriality, social behaviour, tolerance, dispersal patterns, mating systems and the relatedness of the individuals (Gompper, Gittleman & Wayne, 1998). The African wild cat is described as a solitary felid (Smithers, 1983; Nowell & Jackson, 1996; Sunquist & Sunquist, 2002). Its social organisation shows large home range overlap between females but little overlap between males, although the home ranges of males typically overlap with several females in their home ranges (Chapter 4).

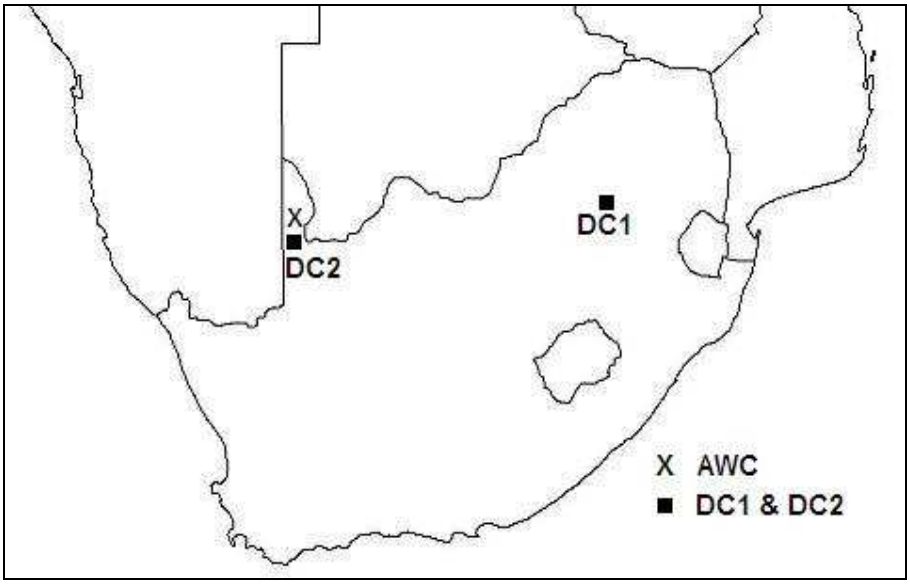
Molecular techniques have been applied widely to investigate social organisation in social carnivores, for example in African lions, *Panthera leo* (Packer, Gilbert, Pusey & O'Brien, 1991); African wild dogs, *Lycaon pictus* (Girman, Mills, Geffen & Wayne, 1997); gray wolves, *Canis lupus* (Smith, Meier, Geffen, Mech, Burch, Adams & Wayne, 1997); swift foxes, *Vulpes velox* (Kitchen, Gese, Waits, Karki & Schauster, 2005); kit foxes, *Vulpes macrotis* (Ralls *et al*., 2001) and raccoons, *Procyon lotor* (Nielsen & Nielsen, 2007) but only very recently in solitary felids e.g. bobcats, *Lynx rufus* (Janečka, Blankenship, Hirth, Tewes, Kilpatrick & Grassman, 2004) and cougars, *Puma concolor* (Biek, Akamine, Schwartz, Ruth, Murphy & Poss, 2006). In our study we used 18 microsatellite loci to analyse: (i) The extent of genetic variation among African wild cats in the southern Kalahari, (ii) the genetic purity of African wild cats, mostly sampled from the KTP, (iii) genetic structure in the wild cat population, and (iv) relatedness between African wild cat individuals of which the spatial organisation were recorded through intense behavioural observations.

3. Materials and Methods

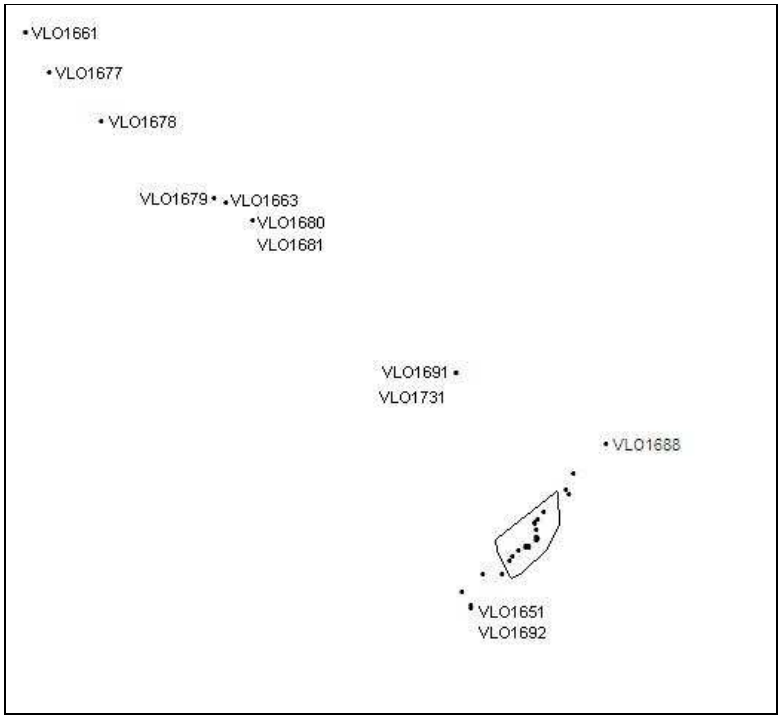
3.1 Sample collection and DNA extraction

We analysed a total of 103 tissue and hair samples, including 57 African wild cats (AWC), 25 Kalahari domestic cat (DC1) and a reference collection of 21 domestic cat (DC2; Veterinary Genetics Laboratory, University of Pretoria; C. Harper pers. comm.) samples (Figure 5.1a). Of the wild cat samples 47 were collected from April 2003 – December 2006 in the KTP (Figure 5.1b and Figure 5.1c), South Africa and Botswana and ten were collected from road kills outside the Transfrontier Park and stored in 95% ethanol. Wild cats were morphologically identified by coat-patterns, long legs and the characteristic reddish tint at the back of their ears (Smithers, 1983). Tissue samples were preserved in 95% ethanol and hair samples in plastic bags. All hair samples consisted of cat whiskers with the root visible at the tip.

(a)


X
DC2
DC1
X AWC
DC1 & DC2

(b)


VLO1661
VLO1677
VLO1678
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VLO1680
VLO1681
VLO1691
VLO1731
VLO1688
VLO1651
VLO1692

(c)

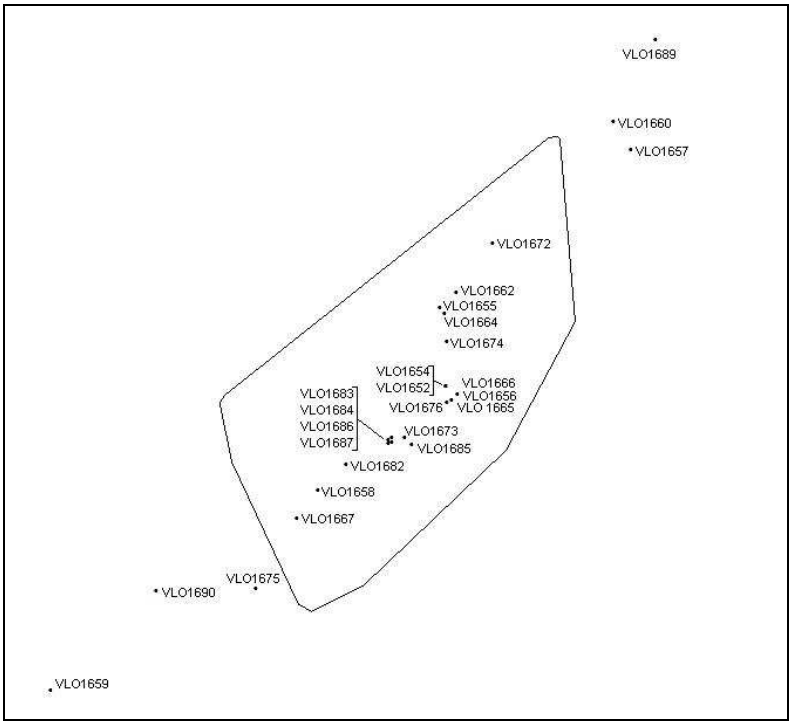

Figure 5.1 (a) Map of South Africa with locations of where all samples were collected, DC = domestic cat populations, AWC = African wild cat population (b and c) the core study site, indicating 38 African wild cats that were sampled and analysed for relatedness and population structure from March 2003 to December 2006

DNA extractions and microsatellite genotyping were conducted at the Veterinary Genetics Laboratory, Faculty of Veterinary Science, University of Pretoria (Onderstepoort). DNA tissue samples were extracted with a Cell Lysis stock solution (10mM Tris–HCl pH 8.0, 50mM NaCl, 10mM EDTA) and Phenol-Chloroform-Isoamylalcohol (Sigma kit) (C. Harper pers. comm.). DNA from hair samples were extracted with 200mM NaOH and 200mM HCl, 100mM Tris–HCl, pH 8.5 (C. Harper pers. comm.). Eighteen microsatellite markers that forms part of an international parentage panel (International Society of Animal Genetics (ISAG)) and developed by the laboratory of Leslie Lyons (University of California Davis) were used for genotyping (Lipinski, Amigues, Blasi, Broad, Cherbonnel, Cho, Corley, Daftari, Delattre, Dileanis, Flynn, Grattapaglia, Guthrie, Harper, Karttunen, Kimura, Lewis, Longeri, Meriaux, Morita, Morrin-O'Donnell, Niini, Pedersen, Perrotta, Polli, Rittler, Schubbert, Strillacci, Van Haeringen, Van Haeringen & Lyons, 2007). These loci were initially chosen based on their variability in various cat breeds, their probability of exclusion in parentage testing of closely

related individuals, their robustness in multiplex polymerase chain reaction (PCR) testing and their consistency during testing (Lipinski *et al*., 2007). Table 5.1 provides the initial test panel results from ISAG 2004 discussions (Harper pers. comm.). A comparison with Menotti-Raymond, David, Lyons, Schaffer, Tomlin, Hutton & O'Brien (1999) indicated that although some of these markers map to the same chromosome they are unlinked.

The PCR amplifications were performed in 10µl reaction volume multiplex reactions using AmpliTaq Gold DNA polymerase (Applied Biosystems). PCR conditions were: 95ºC for 5min, followed by 35 cycles of 95ºC for 1 min, 58ºC for 30 s, 72ºC for 30 s, and followed by a final 72ºC for 30min. PCR products along with LIZ 500 size standard were run on a 3130xl Genetic Analyzer (Applied Biosystems) and analysed with STRand Software (version 2.3.94, Board of Regents, University of California, Davis).

Table 5.1 Population data of genetic markers in the domestic cat parentage and identification panel (C. Harper pers. comm.). PIC = polymorphism information content, Chr. = chromosome

Marker	Number (breeds)	Number (all)	Allele range	PIC (breeds)	PIC (all cats)	H (breeds)	H (all cats)	Chr.
FCA005	239	299	130-154	0.7	0.69	0.55	0.56	E1
FCA026	332	407	128-160	0.78	0.79	0.48	0.51	D3
FCA069	307	401	96-116	0.79	0.79	0.53	0.55	B4
FCA075	482	609	104-146	0.75	0.75	0.57	0.59	E2
FCA097	272	355	136-156	0.75	0.77	0.54	0.58	B1
FCA105	362	443	173-205	0.82	0.83	0.51	0.56	A2
FCA149	-	-	-	-	-	-	-	B1
FCA201	358	456	133-161	0.78	0.79	0.58	0.61	B3
FCA220	411	513	210-224	0.43	0.45	0.25	0.26	F2
FCA224	297	382	148-180	0.66	0.63	0.4	0.41	A3
FCA229	374	482	150-176	0.69	0.69	0.51	0.54	A1
FCA240	-	-	-	-	-	-	-	X
FCA293	308	412	179-201	0.8	0.8	0.54	0.54	C1
FCA310	291	394	112-140	0.74	0.74	0.54	0.57	C2
FCA441	399	483	145-173	0.71	0.71	0.56	0.58	D3
FCA453	278	352	184-208	0.67	0.66	0.32	0.36	A1
FCA651	213	306	135-141	0.21	0.23	0.13	0.14	X
FCA678	298	392	216-236	0.7	0.7	0.43	0.45	A1

3.2 Analyses of genetic variation

Allele frequencies, observed (H_O) and expected (H_E) heterozygosity for each locus and for each population were calculated using Genepop 3.4 (Raymond & Rouset, 1995) to determine significant deviations from Hardy-Weinberg Equilibrium (HWE) for all locus-population combinations and to statistically infer Linkage Equilibrium (LE) among loci. Significance levels were adjusted using sequential Bonferroni corrections for multiple comparisons in the same data set (Rice, 1989). We estimated the genetic variation between wild and domestic populations through a hierarchical Analysis of Molecular Variance (AMOVA) with the software GenAlEx (Peakall & Smouse, 2006) using F_{ST} and R_{ST}. The significance of genetic differentiation was tested by random permutation, under the null hypothesis that all individuals belong to a single population. Wilcoxon signed rank tests were used to evaluate the differences in allelic diversity (number of alleles: Na), the allelic richness (effective number of alleles: Ne) and H_E between pairs of geographical groups (Statistica 7.0).

3.3 Population structure and admixture analyses using Bayesian cluster analysis and Principal Component Analysis

Population structure, individual assignments and admixture proportions were estimated through a Bayesian approach implemented in Structure 2.2 (Falush, Stephans & Pritchard, 2007). The number of putative populations, *K*, was determined by comparing the log-likelihood values and *ΔK* (Evanno, Regnout & Goudet, 2005; Waples & Gaggiotti, 2006) over multiple runs (20 iterations each with a 10,000 chain burn-in and 100,000 MCMC chains) for values of *K* ranging from 1 to 8. An admixed model with correlated allele frequencies was used (other model parameters yielded the same results). For assignment of individuals to the inferred clusters, chains of 1×10^6, following a burn-in of 100,000, were run three times to ensure convergence. Following Lecis *et al*. (2006) individuals assigned with a probability of membership of $q_i \geq 0.8$ were regarded as belonging to a single cluster, while values of < 0.8 were inferred as an indication of admixture.

Allele frequencies from known or unknown source populations are modelled to assign individuals to one or more populations (Lecis *et al*., 2006), with the assumption that admixture leads to Hardy-Weinberg- and linkage disequilibrium (Pritchard, Stephens & Donnelly, 2000). The programme Structure 2.2 model correlations between loci in an admixed population, to detect more ancient admixture events and identifies population structure where populations are connected by gene flow or has diverged recently (Falush, Stephens & Pritchard, 2003).

A Principal Component Analysis (PCA) was performed using GenAlEx 6 software (Peakall & Smouse, 2006) which is a multivariate technique that plots the major patterns within a multivariate dataset and indicate the relationship between distance matrix elements based on their first two principal coordinates.

3.4 Relatedness estimates within the African wild cat population

Relatedness between individuals in the wild cat population was calculated using the programme GenAlEx (Peakall & Smouse, 2006). The software uses genetic distances from codominant data for a single population. The level of relatedness (*R*) described in Queller & Goodnight (1989) was used: for first order relatives (full sibs and parent-offspring) R-values ~0.5 are expected, second order relatives should on average show values of 0.25, while values below 0.125 indicate unrelated individuals. Individual inbreeding coefficients, kinship and relatedness coefficients were also compared in SPAGeDi version 1.2 (Hardy & Vekemans, 2002). Known relationships, obtained from behavioural observations done on the study population, were used to evaluate these results.

4. Results

4.1 Genetic diversity in wild and domestic cats

We determined individual genotypes for 57 morphologically classified African wild cats, 25 Kalahari domestic cats and the reference collection of 21 domestic cats. All microsatellite loci were polymorphic in both the 57 genotyped wild- and 46 domestic cats with six (FCA453 and FCA651) to 17 (FCA075) alleles per locus (average: 11.61 ± 3.13) (Appendix 4). The number of private alleles (alleles unique to a single population) within the wild cat population was 4.06 ± 0.59, the Kalahari domestic population 0.11 ± 0.08 and the domestic cat reference collection 0.44 ± 0.17. Twelve combinations between pairs of loci disclosed a significant deviation from linkage disequilibrium after Bonferroni correction for 18 independent replications ($P < 0.0028$). The microsatellite loci in this study map on different cat chromosomes (Menotti-Raymond *et al.*, 1999) except as shown in Table 5.2. These loci should be distant enough to allow for independent allele assortment. Pairwise allelic combinations were in linkage equilibrium at all loci over the wild cat genotypes except in one case (significance probability level $p < 0.05$ Bonferroni corrected for 14 comparisons). A significant departure from HWE (Table 5.3) was observed at two wild cat loci: FCA240 (F_{IS} = 0.69, p = 1.00) and FCA651 (F_{IS} 0.77, p = 1.00) and one domestic cat locus FCA240 (Kalahari population F_{IS} = 0.63, p = 1.00 and reference collection F_{IS} = 0.65, p = 1.00). However, both these loci are on the X-chromosome and the large number of male individuals in our study skewed the overall level of homozygosity. Subsequent analyses were conducted

with, and excluding, these two loci and the scoring of males as homozygotes at the X-linked loci did not affect the Bayesian clustering or relatedness estimation.

Genetic diversity was significantly higher in the wild cats than in the domestic cats with higher allelic diversity and heterozygosity (Table 5.3). Moreover, Wilcoxon signed rank tests confirmed these results, showing significant differences in H_E (DC1: $Z = 3.2$, $p = 0.001$ and DC2: $Z = 2.9$, $p = 0.004$), Ne (DC1: $Z = 3.3$, $p = 0.001$ and DC2: $Z = 3.1$, $p = 0.002$) and Na (DC1: $Z = 3.7$, $p = 0.0003$ and DC2: $Z = 3.6$, $p = 0.0008$) between the wild and domestic cat populations and no significant differences between the two domestic cat populations. These results encouraged the analysis of wild cats and domestic cats as two distinct genetic entities and the two geographically separated domestic cat populations as one. The $F_{ST} = 0.10$ ($p < 0.01$) over all loci (Table 5.3) and an Analysis of Molecular Variance showed significant differentiation between the wild cat and two domestic cat populations and revealed most of the variance within rather than between the two domestic cat groups (Table 5.4).

Table 5.2 Microsatellite loci that showed linkage disequilibrium and their locations on specific chromosomes

Locus	Locus	Locus	Chromosome	Menotti-Raymond *et al*., (1999)
FCA026	FCA441		D3	Not linked
FCA097	FCA149		B1	Not linked
FCA229	FCA453	FCA678	A1	Not linked
FCA651	FCA240		X	Not linked

Table 5.3 Summary of diversity indices for each locus-population combination, observed (H_O) and expected (H_E) heterozygosities, (Na) number of alleles, (Ne) effective number of alleles, the fixation index (F), the inbreeding coefficient (F_{IS}) and the coefficient of genetic differentiation (F_{ST}) between wild (AWC) and domestic populations (DC)

Loci	African wild cat (AWC) (n = 57)					Kalahari Domestic cats (DC1) (n = 25)					Domestic cat reference collection (DC2) (n = 21)					F_{IS}	F_{ST}
	H_O	H_E	Na	Ne	F	H_O	H_E	Na	Ne	F	H_O	H_E	Na	Ne	F		
FCA005	0.77	0.78	8	4.60	0.01	0.68	0.69	6	3.24	0.02	0.81	0.82	9	5.44	0.01	0.01	0.03
FCA026	0.86	0.88	15	8.01	0.02	0.60	0.62	7	2.60	0.02	0.67	0.79	10	4.74	0.16	0.07	0.08
FCA069	0.84	0.83	12	5.77	-0.02	0.72	0.76	7	4.11	0.05	0.62	0.64	6	2.79	0.04	0.02	0.09
FCA075	0.84	0.87	13	7.97	0.04	0.76	0.75	6	3.93	-0.02	0.71	0.78	9	4.45	0.08	0.03	0.08
FCA097	0.91	0.91	17	11.16	0.00	0.60	0.69	5	3.21	0.13	0.81	0.84	7	6.39	0.04	0.05	0.05
FCA105	0.79	0.86	12	6.90	0.08	0.86	0.84	9	6.17	-0.03	0.62	0.78	7	4.64	0.21	0.08	0.05
FCA149	0.81	0.79	8	4.76	-0.02	0.76	0.66	5	2.96	-0.15	0.76	0.79	6	4.85	0.04	-0.04	0.07
FCA201	0.82	0.87	12	7.51	0.05	0.68	0.68	5	3.15	0.00	0.86	0.82	7	5.44	-0.05	0.00	0.09
FCA220	0.89	0.86	10	6.96	-0.04	0.33	0.62	5	2.62	0.46	0.57	0.73	7	3.64	0.21	0.18	0.10
FCA224	0.93	0.86	13	7.36	-0.07	0.52	0.56	7	2.26	0.07	0.38	0.40	6	1.68	0.06	0.00	0.16
FCA229	0.79	0.81	11	5.37	0.03	0.48	0.58	6	2.39	0.18	0.62	0.60	5	2.48	-0.04	0.05	0.15
FCA240	0.25	0.79	8	4.72	0.69	0.16	0.72	7	3.53	0.78	0.29	0.77	8	4.41	0.63	0.70	0.10
FCA293	0.81	0.86	12	7.18	0.06	0.80	0.76	7	4.24	-0.05	0.71	0.77	8	4.26	0.07	0.03	0.05
FCA310	0.79	0.81	11	5.20	0.02	0.88	0.72	7	3.55	-0.22	0.67	0.76	7	4.14	0.12	-0.02	0.08
FCA441	0.72	0.73	7	3.68	0.01	0.68	0.76	5	4.10	0.10	0.62	0.76	7	4.22	0.19	0.10	0.03
FCA453	0.67	0.61	5	2.57	-0.09	0.52	0.77	6	4.34	0.32	0.43	0.73	5	3.66	0.41	0.23	0.07
FCA651	0.14	0.59	5	2.46	0.76	0.08	0.27	2	1.37	0.70	0.10	0.24	2	1.32	0.61	0.72	0.41
FCA678	0.89	0.86	12	7.20	-0.04	0.41	0.53	5	2.11	0.22	0.62	0.69	4	3.23	0.10	0.07	0.15
Average	0.75	0.81	10.61	6.08	0.08	0.58	0.66	5.94	3.33	0.14	0.60	0.71	6.67	3.99	0.16	0.13	0.10
SD	0.21	0.09	3.24	2.16	0.24	0.23	0.13	1.47	1.09	0.27	0.20	0.15	1.91	1.31	0.20		

Table 5.4 Analysis of MOlecular VAriance (AMOVA) for wild - and domestic cat groups computed using GenAlEx (d.f., degrees of freedom; SS, sum of squares; MS, mean squares; Est. Var., estimated variance)

	Source	d.f.	SS	MS	Est. Var.	%	Stat	Value	Prob
Within all populations	Among Pops	2	46399.681	23199.841	370.201	39%			
	Within Pops	203	117339.631	578.028	578.028	61%	R_{ST}	0.390	0.010
	Total	205	163739.312	23777.868	948.229				
In domestic cats	Among Pops	1	4038.260	4038.260	77.050	13%			
	Within Pops	90	46869.709	520.775	520.775	87%	R_{ST}	0.129	0.010
	Total	91	50907.968	4559.034	597.824				

4.2 Admixture analyses and identification of hybrid individuals

In the Structure simulation that considered all sampled individuals, the highest likelihood and greatest ΔK were obtained for $K = 2$ (Fig. 5.2). If the two populations (wild and domestic cats) were admixed, individual admixed samples could be identified by estimating the proportion of membership (q) of those individuals. Given two inferred clusters and with the proportion of membership $q \geq 0.8$, Cluster I grouped all the domestic cats and Cluster II all the wild cats (Fig. 5.3). Four admixed individual cats included a litter of three kittens (VL01732, VL01733 and VL01734) from a known semi tame wild cat mother on the periphery of the park and a wild cat skin sample from another region in the Northern Cape (28º14.181'S, 21º21.068'E) in South Africa (VL01742). All African wild cats collected from inside the Kgalagadi Transfrontier Park were clustered in Cluster II.

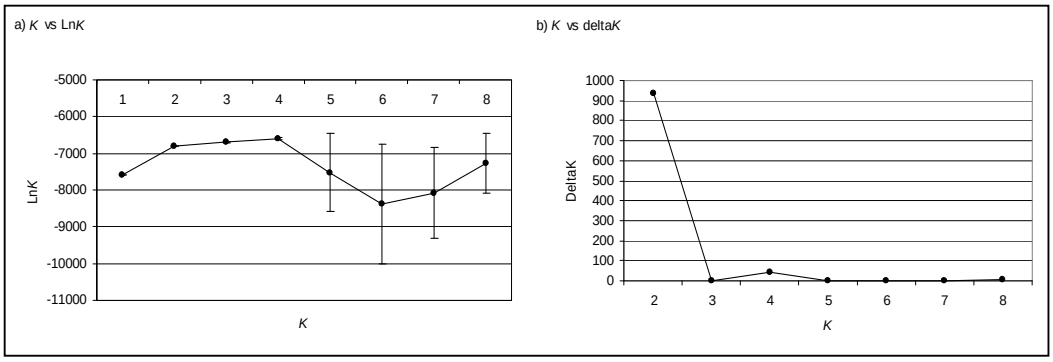

Figure 5.2 a) Probability of the data LnK and, b) ΔK against the number of K clusters in the wild and domestic cat populations

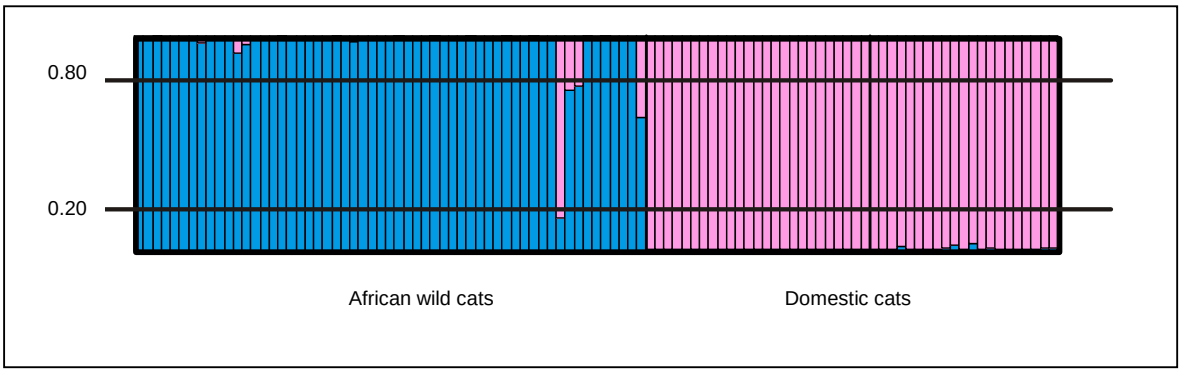

Figure 5.3 Individual assignment of domestic cats (DC1 and DC2) and wild living African wild cats (AWC) in the southern Kalahari performed using Structure 2.2 with *K* = 2. Each individual is represented as a vertical bar partitioned into K = 2 segments indicating the estimated membership to the two clusters. The horizontal black lines indicate values of individual proportion of membership *q* ≥ 0.80

The results of a Principle Component Analysis plot of all the genotypes are shown in Fig. 5.4. Individual scores were plotted onto two principle axes (PC-I and PC-II), which cumulatively explained 39% of the variance among the samples. This plot showed a clear separation into the different groups, namely wild cats (AWC) and domestic cats (DC). The two geographically separated domestic cat populations (DC1 and DC2) were almost totally overlapping. The four identified hybrids clustered intermediate between the wild and domestic cats (Fig. 5.4).

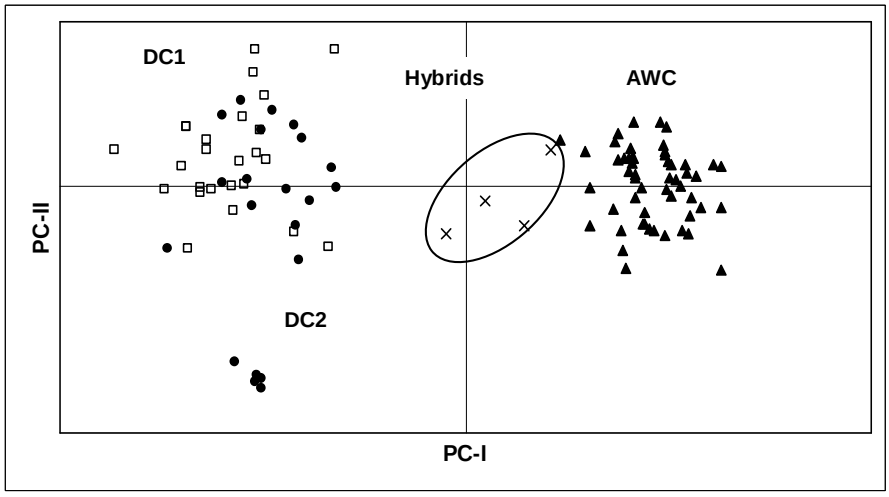

Figure 5.4 PCA of all three populations, African wild cats (AWC, solid triangle ▲), Kalahari domestic cats (DC1, open square □) and reference collection of domestic cats (DC2, solid circles ●). The four hybrids are indicated with crosses

4.3 Genetic diversity within the African wild cat population

The Principle Component Analysis of only wild cats without the hybrids shows seven individuals clustering separately. These seven individuals were shown to be all related to each other (Table 5.5). The ten geographically separated samples cluster all within the larger group of wild cat samples collected in the Kalahari (Fig. 5.5).

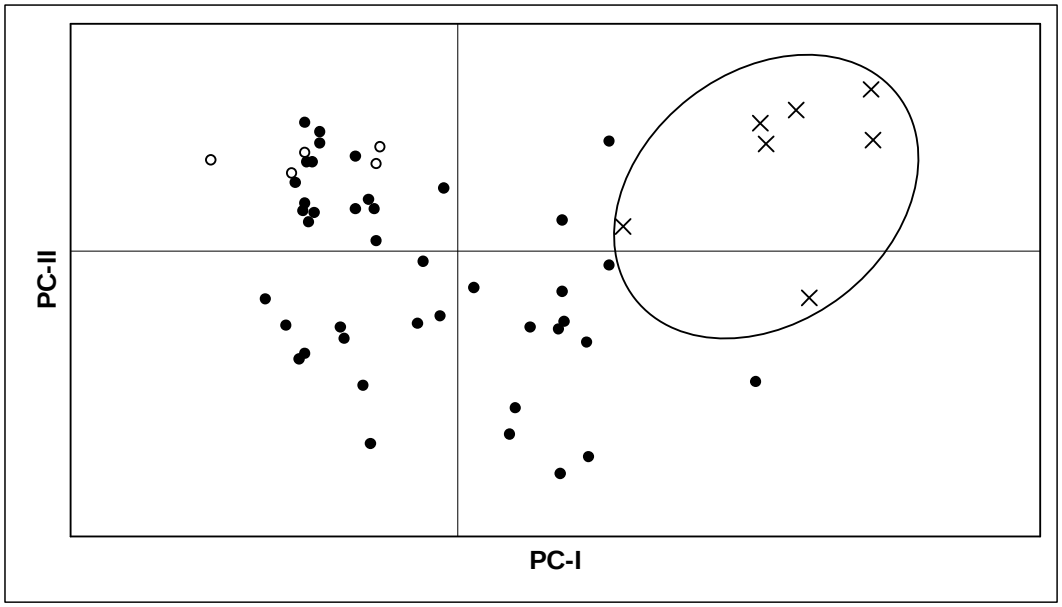

Figure 5.5 PCA of African wild cats without hybrids (solid circles ●), indicating samples collected outside the Transfrontier Park (open circle ○); related individuals from the main study site in the KTP are also indicated (crosses X)

4.4 Relatedness between Kgalagadi Transfrontier Park African wild cats

Given that only a small fraction of the KTP population was sampled, we present preliminary findings on the local population structure of wild cats. The mean relatedness values from Queller and Goodnight (1989) were used to evaluate the relationship between 38 individuals for which spatial information were available (Fig. 5.1b), including the wild cats in the core study site (Fig. 5.1c). Known relationships from behavioural observations and relatedness estimates are tabled in Table 5.5. Relatedness coefficients between adult individuals in the core study site were low (males: R = -0.02 ± 0.123, n = 8; females: R = -0.04 ± 0.113, n = 7; males versus females: R = -0.05 ± 0.138). In order to assess the accuracy of Queller-Goodnight R-values in estimating relatedness between individuals of unknown relationship,

we calculated the average *R*-values of known pairs of relationships (Fig. 5.6). The mother-offspring pairs had an average relatedness value (*R*) of 0.47 ± 0.04 and full sibling pairs had an average relatedness (*R*) of 0.42 ± 0.12.

Interestingly, many of the close relationships involved one of the males (VLO1662) that was studied over a three year period (2004-2006, Chapter 4). He is the father of at least five kittens with three different females, of which one (VLO1658) appears to be his mother or sister (*R* = 0.63). On two occasions these cats where observed mating and courting. There was also an observation where the male visited the female while she had kittens. VLO1673, a sub-adult male whom we identified through visual observations as an offspring of female VLO1658 and male VLO1662, were confirmed as such despite allelic mismatches at locus FCA005 and FCA220. This individual showed a very high inbreeding coefficient (0.412). A small kitten VLO1675 caught in the home ranges of the female VLO1654 and male VLO1662 were positively identified as an offspring of these two cats. VLO1662 also sired a litter of three kittens with female VLO1684.

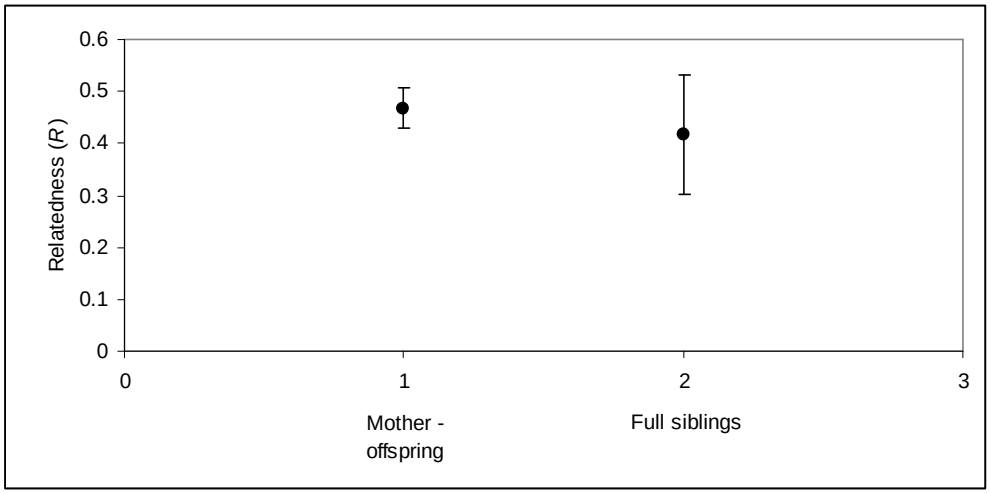

Figure 5.6 Relatedness values for known relationships among African wild cats in the Kalahari study site with the standard deviation included

Table 5.5 Relatedness values (*R*) and the expected relationships according to Queller and Goodnight (1989)

ID	Sex	ID	Sex	*R*	Relationship
VL01662	♂	VL01658	♀	0.63	Full siblings*
VL01662	♂	VL01673	♂	0.58	Parent - Offspring
VL01658	♀	VL01673	♂	0.56	Parent - Offspring
VLO1662	♂	VLO1654	♀	-0.03	Unrelated breeders
VL01662	♂	VL01675	♂	0.38	Parent - Offspring
#VL01654	♀	VL01675	♂	0.52	Parent - Offspring*
VLO1662	♂	VLO1684	♀	-0.01	Unrelated breeders
VL01662	♂	VL01683	♂	0.54	Parent - Offspring
VL01662	♂	VL01686	♂	0.53	Parent - Offspring
VL01662	♂	VL01687	♂	0.41	Parent - Offspring
#VL01684	♀	VL01683	♂	0.47	Parent - Offspring*
#VL01684	♀	VL01687	♂	0.45	Parent - Offspring*
#VL01684	♀	VL01686	♂	0.43	Parent - Offspring*
VL01683	♂	VL01686	♂	0.35	Full siblings*
VL01683	♂	VL01687	♂	0.31	Full siblings*
VL01686	♂	VL01687	♂	0.44	Full siblings*
VLO1673	♂	VLO1675	♂	0.07	Half siblings
VL01673	♂	VL01683	♂	0.36	Half siblings*
VL01673	♂	VL01686	♂	0.42	Half siblings*
VL01673	♂	VL01687	♂	0.43	Half siblings*
VL01675	♂	VL01683	♂	0.33	Half siblings
VLO1675	♂	VLO1686	♂	0.10	Half siblings
VLO1675	♂	VLO1687	♂	0.22	Half siblings
VL01658	♀	VLO1675	♂	0.19	Half sibs (aunt)*
VL01658	♀	VL01683	♂	0.38	Half sibs (aunt)*
VL01658	♀	VL01686	♂	0.42	Half sibs (aunt)*
VLO1658	♀	VLO1687	♂	0.09	Half sibs (aunt)*
VL01691	♀	VL01731	♀	0.57	Full siblings*

Known mothers

* Known relationships

5. Discussion

There is considerable controversy over what constitutes a wild cat and whether wild cats can be defined purely by morphological criteria (Daniels *et al*., 1998; Kitchener, 1998). Extensive molecular studies on the European wild cat have been published (Beaumont *et al*., 2001; Randi *et al*., 2001; Lecis *et al*., 2006; Oliveira *et al*., 2008*b*) and the phenomenon where an introduced population hybridise with a native population is not uncommon (Rhymer & Simberloff, 1996). Especially in wild cats it is difficult to estimate degrees of admixture when the gene frequencies in the native population prior to admixture are unknown (Beaumont *et al*., 2001). If an *a priori* known "pure" wild cat population do not exist there will be no reference wild cat population to be used for estimating the rate of crossbreeding between wild and domestic cats (Daniels *et al*., 1998). Domestication produced obvious changes in the domestic cat of which coat coloration is probably the most noticeable one. Coat colouration is controlled by a few genes and wild cats that are homogenous for domestic colour patterns could be classified as domestic on morphological identification alone. Alternatively natural selection against coat colour phenotypes in domestic cats may lead to a selection of wild tabby markings in feral domestic cats (Randi *et al*., 2001). Therefore it is difficult to classify cats purely on a morphological basis as wild and domestic cats (Balharry & Daniels, 1998; Daniels *et al*., 1998). We identified wild cats morphologically by their tabby markings, longer legs and reddish colour behind the ears. We studied their ecology by radio telemetry; however, we compliment our behavioural observations with molecular data from microsatellite analyses.

The genetic variability of African wild cats in the southern Kalahari was examined (H_E = 0.81) and although different loci were used the results were comparable to that found in other wild felid studies e.g. cougar, *Puma concolor* H_E = 0.66 (Sinclair, Swenson, Wolfe, Choate, Gates & Cranall, 2001); bobcat, *Lynx rufus* H_E = 0.77 (Janečka *et al*., 2004); African wild cat, *F. s. lybica* H_E = 0.80 (Wiseman *et al*., 2000) and European wild cat, *F. s. silvestris*: Portugal H_E = 0.76 (Oliveira *et al*., 2008*b*); Italy H_E = 0.72 and Hungary H_E = 0.81 (Lecis *et al*., 2006).

Our results confirm that wild and domestic cats are genetically distinct (F_{ST} = 0.14, R_{ST} = 0.39) and Structure analysis clearly group our wild cat samples separately from the two domestic cat populations, with a clear indication of admixed individuals. Despite the widespread occurrence of domestic cats on the periphery of the KTP, the genetic distinction between wild and domestic cats was high and the existence of private alleles clearly suggest that gene flow between these populations is low and that hybridisation between Kalahari wild cats and domestic cats is limited. The hybrid individuals were offspring from a semi tame wild cat mother, nonetheless, this emphasises that admixture events on the border of the KTP

could have serious implications for conservation efforts to protect the African wild cat. Hybridisation in a species can be widespread although it might be locally rare (Oliveira, Godinho, Randi, Ferrand & Alves, 2008*a*). Reports in southern Africa predict that hybridisation is widespread (Smithers, 1983), although at low levels (Wiseman *et al*., 2000). Our data highlights that the general mapping of levels of introgression are important to identify areas, such as the southern Kalahari, as focal areas for efficient conservation management strategies. In future studies, the KTP wild cats can be used as an a priori pure population, but it will be important also to assess natural levels of variation and gene flow among wild cats across their distribution range.

In general adult wild cat ranging patterns showed slight male-male overlap but extensive female-female overlap, although female core areas tend to be exclusive. The home ranges of male wild cats typically overlap with several females (Chapter 4). The grouping of closely related females has been described in many carnivores (Smith, McDougal & Sunquist, 1987; Logan & Sweanor, 2001; Janečka *et al*., 2004; Kitchen *et al*., 2005). However the lack of relatedness among our core study site females might be explained by: (i) a regular local turnover of maternal lineages that would tend to disrupt local clusters of related individuals (Biek *et al*., 2006), (ii) the frequent introductions of new alleles by immigrating males (Goudet, Perrin & Wasser, 2002), or that (iii) female dispersal might be distant enough to prevent spatial clustering of individuals (Biek *et al*., 2006).

Future more intensive sampling will be required to fully characterize local population structure and patterns of relatedness in wild cats. However, observations from our core study site suggest that a dominant male may monopolize paternity.

To conclude, admixture analyses indicate that hybridisation is not frequent in the southern Kalahari. The main threats such as persecution, accidental road killings, habitat loss and fragmentation still persists for the African wild cat in southern Africa. Habitat modification and animal translocation will increase the rate of hybridisation and introgression. The fact that evidence of admixed individuals is already present raises the conservation concerns for the protection of wild cats in southern Africa.

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CHAPTER 6
Synthesis

The African wild cat, *Felis silvestris cafra*, is one of the most widespread small predators on the African continent (Nowell & Jackson, 1996). However there is a paucity of information on virtually all aspects of its behavioural ecology. Since wild cats are the ancestor of the domestic cat, *Felis s. catus*, and the two species can freely interbreed, one of the biggest threats to wild cats over the globe is hybridisation with the domestic cat. The objective of this study was therefore to describe the feeding ecology, the spatial organisation and the population genetics of African wild cats in the southern Kalahari. This was achieved through radio telemetry and direct observations of habituated individuals that were closely followed and monitored over a period of 46 months. Throughout the study period an assertive effort was made to collect genetic material from wild cats to address the question of hybridisation as well as to supplement our understanding of population structure with molecular techniques. This chapter summarises the key aspects of every chapter and provides an overview of the behavioural ecology and population genetics of the African wild cat in the southern Kalahari.

6.1 What are the feeding habits of the African wild cat and are there sexual and seasonal differences in the diet and foraging behaviour?

African wild cats consume a large spectrum of food and prey resources depending on prey abundance and availability. This study showed that murids formed the bulk of the biomass in the diet, followed by birds and large mammals (> 500 g). Although reptiles and invertebrates were frequently caught they contributed less to the overall biomass of the diet. Fluctuations in prey abundances could be the result of seasonal rainfall and temperature fluctuations, or long term variability in rainfall resulting in wet and dry cycles. The lean season (hot-dry) was characterised by a high food-niche breadth and high species richness. Despite sexual dimorphism in size in the African wild cat, both sexes predominantly fed on smaller rodents, although there were differences in diet composition with males taking more large mammals and females favouring birds and reptiles. In support of the optimal foraging theory our results indicated that African wild cats are adaptable predators that preferred to hunt small rodents, but can change their diet according to seasonal and longer term prey abundances and availability.

6.2 What is the foraging behaviour of the Arican wild cat and does it shows sexual and seasonal differences?

The African wild cat is a successful predator with a hunting style typical of a solitary felid. Three distinct hunting behaviours were identified: (i) a slow winding walk while inspecting holes and scent trails, (ii) sitting and looking around for prey, or (iii) fast walking while spray marking with opportunistic killing of prey, typical of male cats. Both sexes show two daily peaks of activity: in the early morning and the evenings. The timing of the two active periods showed strong seasonal shifts from predominantly nocturnal during the hotter seasons to more diurnal during the colder seasons. A longer period of activity during the day was observed during the cold-dry season with corresponding low food availability, apparently a behavioural response to low prey abundances. In this wilderness area male and female African wild cats differed very little in their activity budgets, with hunting taking up most of their time. African wild cats are solitary and socialising between individuals is minimal. Cats showed gender-specific preferences for specific habitat types, with the number of prey captured corresponding closely to the time spent in each habitat. The major factors influencing the activity patterns and habitat use of the African wild cat are prey abundance and temperature extremes.

6.3 What are the spacial organisation and movement patterns of the African wild cat?

It is generally believed in carnivores that female space use is limited by resource distribution and abundance, whereas males should be strongly influenced by female spatial dyamics. Our results revealed that prey abundance plays an important role in social and spatial organisation of the African wild cat in the southern Kalahari. This also explained the lack of variability in seasonal home range sizes of both male and female cats. Minimum convex polygon (95% MCP) estimates showed male cats had larger annual home ranges (7.7 ± 3.5 km^2) than female cats (3.5 ± 1.0 km^2). Food resources in the semi desert area vary in time and space, thus females exhibited a large overlap in their home ranges, although core areas were exclusive. It seems that female cats avoid each other temporally and spacially, although only one observation of aggressive behaviour were observed it may be through scent marking and therefore female spacing pattern resembles a form of intrasexual territoriality, although ranges are not actively defended.

Since receptive females seemed to be the limiting resource for male cats, overlap between male home ranges was restricted to small areas. Male home ranges are larger than predicted from body size and metabolic considerations alone and adult males appear to be limited by receptive females as has been found in most carnivores.

6.4 What is the scent marking behaviour in African wild cats?

As predicted for a solitary carnivore, in the African wild cat scent marking is an important form of communication. For the majority of the time communication between cats occurred via a range of scent marking behaviours that increased in females to advertise their reproductive status. Males scent marked continuously during the study period to mark their home range extent to neighbouring and roaming male cats, whereas female spray marking appeared to be related to their reproductive status.

6.5 What is the reproductive bahviour of the Afican wild cat?

The African wild cat shows a polygenous mating system as suggested by the spacing patterns, sexual dimorphism and lack of parental care. In contrast to feral domestic cats that shows cooperative care of young in colonies of rich resources, this was not the case in our study, although older siblings did visit dens with smaller kittens. Food availability influenced the reproductive activity of female cats, and during a lean period no kittens were observed or reported in the Kalahari. However, as food abundances increased there was a drastic increase in kittens and two females produced up to four litters in a twelve month period. Therefore no clear breeding season was evident.

6.6 Was the African wild cat genetically distinct from the domestic cat and what were the levels of introgression in the southern Kalahari?

Molecular analyses indicate that African wild cats and domestic cats were genetically distinct. Four cryptic hybrids were identified among the wild cat samples. These hybrids were either outside or on the periphery of the park, indicating that the level of introgression was low, yet still of concern to the genetic integrity of the African wild cat. Preliminary findings on the genetic structure of our wild cat population indicated that related individuals did not cluster together. A more intense sampling of wild cats in a small area over a longer time period will be valuable to address questions of relationships between individuals and reproductive strategies in African wild cats.

6.7 What is the way forward in African wild cat conservation?

Although African wild cats are widely distributed and not protected over most of their range, little information has been available until now about their behaviour in the wild. This study provide detailed observations on feeding habits, foraging behaviour, spatial organisation, reproduction and the genetic status of the African wild cat in the southern Kalahari. These results can, in the absence of other studies, assist in understanding wild cat behaviour across distributional ranges.

Future studies shoud focus on the genetic status of African wild cats in other regions so that more genetically pure populations can be identified and the needed conservation actions implemented. Regions with a high probability of hybridisation should be identified and tested. Hybridisation is a natural process that may be very difficult to prevent, however education and public information on the role of small mesocarnivores and the threat of feral domestic cats to wild cats is important to increase awareness. Therefore reduce the risk of hybridisation events. Monitoring and research, a deeper knowledge of wild cat behaviour, abundance, population dynamics and other aspects of their ecology in other areas is essential.

It is hoped that this study will provide a basis for comparison for future studies on the African wild cat in other habitats and that it provides baseline data that can be used in comparison to other felids. Natural history knowledge of a species behaviour is the key to successful conservation efforts while ignorance of behaviour can lead to conservation failures.

References

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