

**A REPORT FROM THE SAHELO SAHARAN INTEREST GROUP - PARC NATIONAL DE L'AHAGGAR SURVEY, ALGERIA (March 2005), Part 5: Using Molecular Genetics to study the Presence of Endangered Carnivores (Nov.2006).**



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Front cover: [Clockwise from top] Illamane peak, Ahaggar Mountains near Tamanrasset; OPNA-SSIG survey team in the field, March 2005; Captured cheetah near Tefedest, April 2004 (OPNA); Cheetah habitat; Rüppell’s fox at camera trap.

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## ABSTRACT

The status of the cheetah, *Acinonyx jubatus*, in Northern Africa is poorly known. Study of this species has concentrated on the two major populations of the Serengeti in Tanzania and in Namibia. A lack of detailed baseline data has led to an increasingly detached and unsure view of the present status of this animal in its most northern reaches of Africa. This paper represents the first steps to use multiple techniques to confirm the presence of cheetahs in Algeria and to show the power and importance of the genetics.

A joint 2005 expedition to the Ahaggar region of the Algerian Sahara collected over 40 putative carnivore scat samples for further analysis. The first major objective of this analysis was to assign species identity to the scat. This was done through genetic analyses of the samples. Among other carnivores, eight cheetahs and a leopard were found. This is the first time leopards have been recorded in this part of Algeria. Thus, this paper has an ancillary purpose in presenting a new way of using non-invasive molecular ecological techniques to compile a species list in remote areas where resources only allow for short reconnaissance studies.

Having identified the species present, the second objective of this study was to analyse the genetic structure of the cheetah samples through microsatellite studies. Cheetah from Tanzania were used as reference samples and combined in the analysis with the Algerian cheetahs, and the number of unique genotypes and possible kinship relationships were ascertained. The cheetah samples were then geo-referenced on a map containing information gathered on the 2005 expedition.

This paper, therefore, demonstrates the existence of cheetahs and leopards in Algeria and provides impetus for future work in this remote region.

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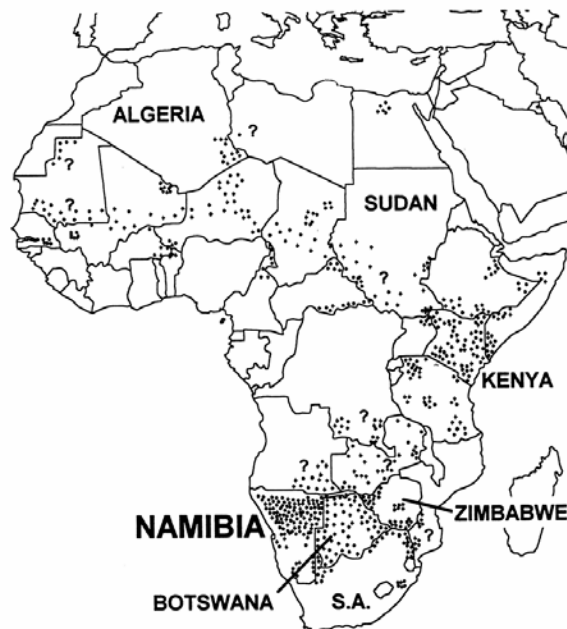
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## 1. Introduction

The cheetah's, *Acinonyx jubatus*, past distribution spanned from the tip of southern Africa, up to the north of the continent and well into Asia (Marker 1998). Apart from some cheetahs in Iran and possibly Pakistan (Nowell and Jackson 1996; Marker 1998), the species is now only found in Africa. Among the 32 African countries in which cheetahs have been reported as historically present, it is now extinct in at least four of them (Marker 1998), all in North Africa. The cheetah is listed as Vulnerable on the 2006 IUCN Red List, and Endangered (subsp. *hecki*) in North Africa and Critically Endangered (subsp. *venaticus*) in Iran<sup>1</sup> (Cat Specialist Group 1996 in IUCN 2006). Because of small populations, remote home ranges, habitat fragmentation (Marker 2000) and a lack of research, the cheetah in North Africa is barely understood.

### 1.1. North African Cheetah

The status of cheetahs in North Africa is poorly known, although they predominantly inhabit the more mountainous regions of the Sahara where water and gazelles are more easily found (Kowalski and Rzebik-Kowalska 1991). This region is intensely arid, which affects the availability of suitable prey species and therefore the numbers of cheetah that might occupy it.



**Figure 1.1. The distribution of cheetah in Africa**

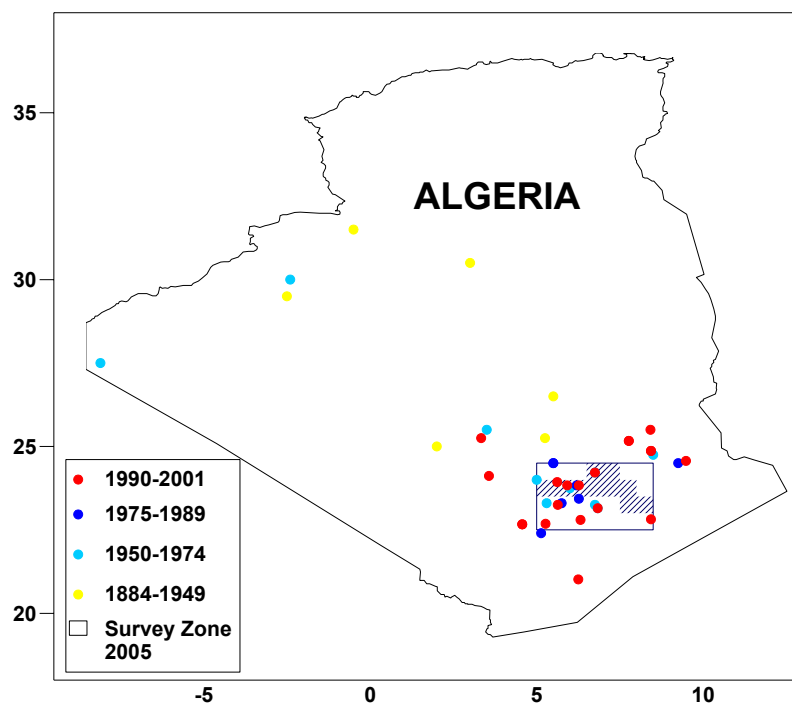
The dots represent cheetah records (From Marker 1998)

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<sup>1</sup> [www.iucnredlist.org](http://www.iucnredlist.org)

Figure 1.1. shows the distribution of cheetah in Africa (Marker 1998). As can be seen, the numbers in North Africa are uncertain and low. The evolutionary relationships of the North African population with other cheetah subspecies are unresolved (Cat Specialist Group 1996 in IUCN 2006); thus, this population also represents a subspecies of cheetah about which little is known.

Figure 1.2. shows the collation of all reported cheetah sightings and signs in Algeria. In March 2005, a reconnaissance expedition to the Ahaggar Mountains of Algeria (boxed area in figure 1.2.) conducted by the Sahelo-Saharan Interest Group (SSIG), in collaboration with the Office du Parc National de l'Ahaggar, aimed to collect preliminary data on the desert wildlife of the area through fieldwork and sample collection (Wacher *et al.* 2005). Habitat, weather and wildlife observations (such as large mammal signs) were made and an extensive collection of unknown carnivore scat samples was assembled. Field observations made at the collection sites provided good reason to believe that at least some of the scat samples came from Algerian cheetahs.



**Figure 1.2. Positions of all cheetah records in Algeria from 1884 to present day**

The boxed area is the zone of Algeria in which the 2005 SSIG-OPNA expedition to the Ahaggar National Park has been carried out. The hashed lines show the half degree squares visited on the survey. The dots represent records collected before the expedition from the literature (Kowalski and Rzebik-Kowalska 1991; Hamdine *et al.* 2003)



## 1.2. Non-invasive genetic sampling

Genetic data can be obtained with increasing ease from animal hair and scat samples. Studies, however, tend to concentrate on samples collected from known individuals or species. With the growing database of sequence data now available online<sup>2</sup>, it should also be possible to obtain gene sequences from unknown samples, and assign specific identity to them through comparison searches and phylogenetic analyses.

Molecular ecological techniques are becoming increasingly powerful. Specifically, gene sequence data can be used to infer phylogenetic and phylogeographic relationships both within and between species. Microsatellites can also be used to assess genetic variation of different populations (e.g., Driscoll 1992; Gottelli *et al.* 2004).

Through work on cheetahs from Eastern and Southern Africa, it has been suggested that cheetahs are genetically impoverished (O'Brien *et al.* 1983; O'Brien *et al.* 1985; Wayne *et al.* 1986; O'Brien *et al.* 1987), probably due to inbreeding (but see Caughley 1994; Merola 1994). It is of vital interest to examine the genetics of all cheetah populations if necessary conservation management decisions are to be made. Therefore, given that the world population is Vulnerable, and that cheetahs are genetically depauperate, studies on the more marginal and smaller populations of cheetah are necessary. This paper provides emphasis for future genetic studies of cheetah from North Africa.

## 1.3. Aims

This project aims to investigate the faecal samples collected in Algeria in order to:

1. Identify some of the carnivore species present in the Parc National de l'Ahaggar, in Saharan Algeria, through genomic analysis.
2. Use all positive cheetah DNA samples to investigate the Ahaggar population structure.
3. Present a novel use for non-invasive sampling to type samples of an unknown origin.
4. Highlight the need for further long term work in Algeria and its importance in felid conservation.

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<sup>2</sup> [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)

## **2. Materials and Methods**

### **2.1. Samples**

The samples used in this study were collected during the 2005 SSIG-OPNA expedition to the Ahaggar Mountains of Southern Algeria (Wacher *et al.* 2005). Fifty-one faecal samples were collected from different areas of the massif, of which 42 were available for analysis. DNA was also extracted from the tissue of 7 animals from the Zoological Society of London (ZSL) tissue bank to be used as references, including a cheetah, two sand cats, a Persian leopard, a dwarf mongoose, a banded mongoose and a fennec fox. Sequences were also collected from the literature (table 2.1.) and via the internet (Benson *et al.* 2005). In order to compare between North and East African populations, 7 cheetah samples from Serengeti National Park in Tanzania were included in this study. These individuals were randomly selected from samples collected over the last 9 years by the Serengeti Cheetah Project (Gottelli *et al.* under review).

### **2.2. DNA extraction, amplification, and production**

DNA was extracted from the samples using the QIAGEN QIAmp Stool, QIAGEN Tissue extraction and the QIAGEN DNA purification kits; all according to the manufacturer's guidelines. Two genes were then amplified, 12S and cytochrome *b*, and subsequently sequenced using the ABI BigDye chemistry and 3100 Automatic Sequencer. This enabled the species of animal that produced the scat to be identified.

### **Phylogenetic analysis**

The program SEQUENCING ANALYSIS (Applied Biosystems) was used to produce sequences for the genes for each of the samples. Sequences were also compared to other sequences of the same genes from known species of animals. The 12S and cytochrome *b* sequences were analysed separately in the computer program PAUP (Swofford 1998). Sequences were also BLASTED on the NCBI website<sup>3</sup> to look for homologous sequences within the database. The identity of the sample was assigned from the combination of these two analyses.

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<sup>3</sup> <http://www.ncbi.nlm.nih.gov/blast/>

### 2.3. Microsatellites

The positively identified Algerian cheetah samples and 7 Serengeti cheetahs were screened for the presence of 9 dinucleotide microsatellite loci, following the tube approach of Taberlet *et al.* (1996). Eight loci, characterized in the domestic cat *Felis catus* (Menotti-Raymond *et al.* 1999), chosen on the basis of their high polymorphism and easy scorability, were used. Microsatellites are short sequences of DNA inherited from an animal's parents and can be used to assess relatedness. Eight of these microsatellites were used to investigate this relatedness. After production of the microsatellites, they were analysed on computers. Two programs were used to investigate this: GIMLET (Valière 2002) and Mer (Wang 2004).

### 2.4. Spatial analysis

The geographical position for each of the cheetah samples was known and plotted onto a map of the area using SURFER (Golden Software Colorado). This was combined with various other geo-referenced data collated by SSIG-OPNA during the 2005 expedition, such as cheetah signs and reports, the positions of large scats, and gazelle densities.

**Table 2.1. Numbers and origins of the reference sequences used in the phylogenetic analyses**

The sequences cited from this study all originated as samples taken from the ZSL tissue bank. Therefore none of these samples were collected from this study.

Family	Scientific name	Common Name	12S N	Cytb N	GenBank accession numbers	Citation
Felidae	<i>Acinonyx jubatus</i>	Cheetah	2	2	AY463959	This study; Burger <i>et al.</i> 2004
	<i>Felis margarita</i>	Sand cat	1	-		This study
	<i>Felis catus</i>	Domestic cat	1	1	D28892	Masuda <i>et al.</i> 1994; Meece <i>et al.</i> 2005
	<i>Panthera pardus</i>	Leopard	1	1	AB211405	This study ; Sugimoto <i>et al.</i> 2005
	<i>Panthera leo</i>	Lion	1	1	S79300; AY928670	Janczewski <i>et al.</i> 1995; Koepfli <i>et al.</i> 2006
Viverridae	<i>Genetta maculata</i>	Blotched genet	-	1	AY241921	Gaubert <i>et al.</i> 2004
Hyaenidae	<i>Hyaena hyaena</i>	Striped hyaena	-	1	AY928678	Koepfli <i>et al.</i> 2006
Herpestidae	<i>Herpestes javanicus</i>	Indian mongoose	1	1	AY873843;AY928675	Penny and McLenachan 2005; Koepfli <i>et al.</i> 2006
	<i>Mungos mungo</i>	Banded mongoose	1	1		This study
	<i>Helogale parvula</i>	Dwarf mongoose	1	1		This study
Canidae	<i>Vulpes zerda</i>	Fennec fox	1	1	AF028146	This study ; Wayne <i>et al.</i> 1997
	<i>Vulpes vulpes</i>	Red fox	1	1	Y08508; AF028146	Ledje and Arnason 1996; Yoo <i>et al.</i> 2006
	<i>Canis familiaris</i>	Domestic dog	1	1	DQ780500	Bjornerfeldt <i>et al.</i> 2006
	<i>Canis latrans</i>	Coyote	1	1	DQ480511	Bjornerfeldt <i>et al.</i> 2006
	<i>Canis aureus</i>	Common jackal	1	1	DQ102371;AF028138	Das <i>et al.</i> 2005; Wayne <i>et al.</i> 1997
Mustelidae	<i>Mustela nivalis</i>	weasel	1	1	Y08515; AB026106	Ledje and Arnason 1996; Kurose <i>et al.</i> 2000

### 3. Results

#### 3.1. Assigning species identity to the samples

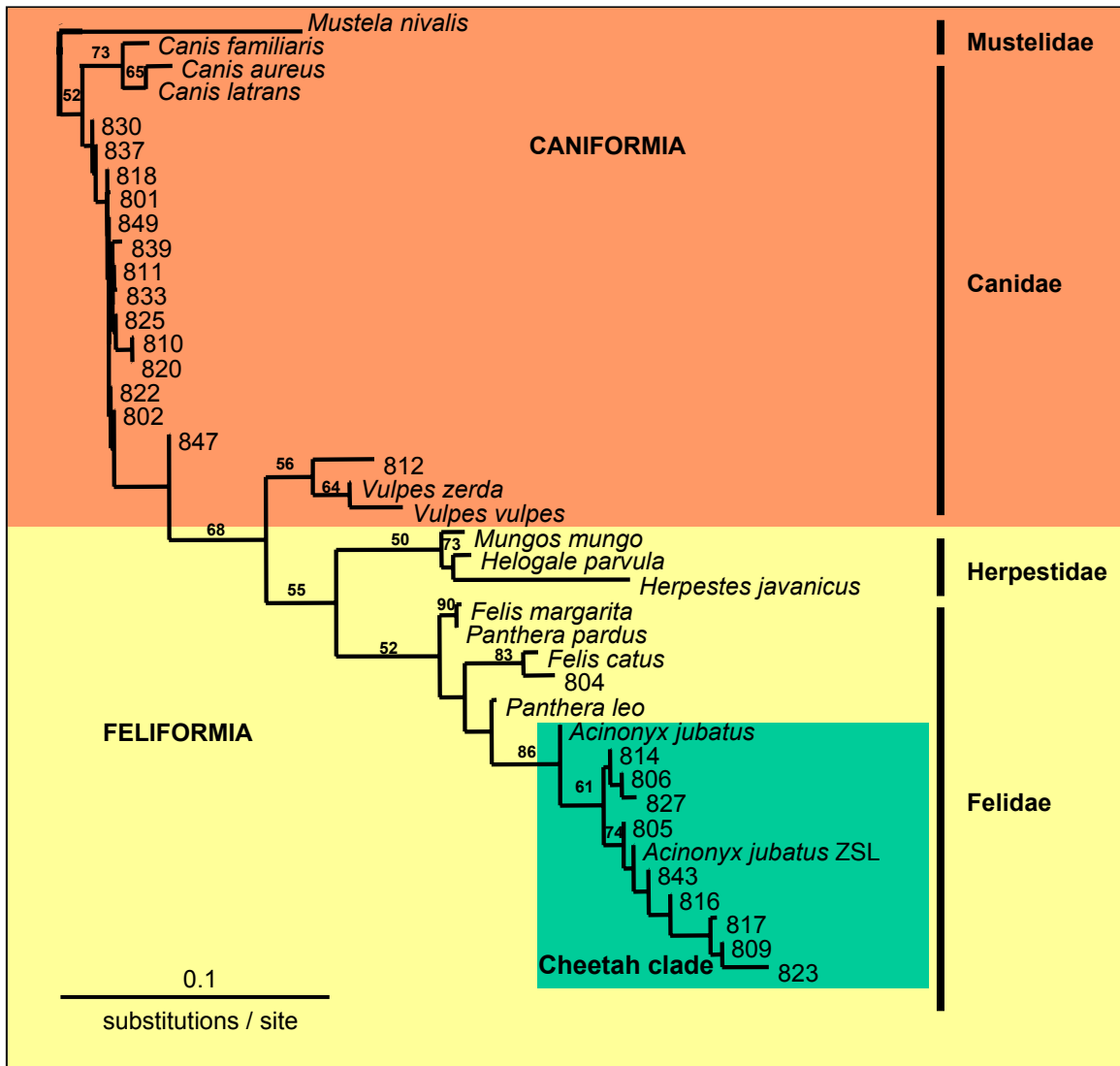
Thirty of the 42 analysed faecal samples gave good sequences for 12S and/or cytochrome *b*. The table 3.1. shows the results of the genetic analysis. The species were diagnosed through the comparison of genes with known reference samples and by grouping similar sequences together. In total, 8 cheetahs, *Acinonyx jubatus*, 1 wildcat *Felis* sp., 1 leopard, *Panthera pardus*, 5 genets, *Genetta* spp., 1 banded mongoose *Mungos mungo* and 14 dogs *Canis* spp. were identified. A computer program, PAUP, was used to group similar samples together. The results of this are shown hereafter (figures 3.1. and 3.2.). The numbers on the branches represent the confidence of that branch (100 = very confident). The trees combine the Algerian samples with reference sequences from known ZSL animals and from the internet (see table 2.1.). The two trees differ slightly (see legend accompanying figures).

**Table 3.1. Species identity of the Algerian samples**

	Field ID	N	E	Grid	Sector	Tree Species	Ht / cm	Pos.	Lab ID	12S	Cytb	Species	Note
1	20050310/01	23.92	6.86	4	38.5	<i>Tamarix</i>	150	branch	814	Y	Y	<i>Mungos mungo</i>	12S = <i>Acinonyx</i>
2	20050311/02	23.93	6.77	4	39.5	<i>Tamarix</i>	-	-	801	Y	N	<i>Canis</i> sp.	
3	20050311/03	23.93	6.77	4	39.5	<i>Tamarix</i>	-	-	812	Y	Y	<i>Genetta genetta</i>	12S = <i>Vulpes</i>
4	20050313/02	23.59	7.37	5	62	<i>Acacia</i>	-	-	810	Y	N	<i>Canis</i> sp.	
5	20050313/04	23.59	7.37	5	62	<i>Acacia</i>	-	-	831	N	Y	<i>Canis</i> sp.	
6	20050315/01	23.25	7.90	6	84	-	15	hole	811	Y	N	<i>Canis</i> sp.	
7	20050315/02	23.25	7.90	6	84	-	-	-	839	Y	Y	<i>Canis</i> sp.	
8	20050315/03	23.25	7.90	6	84	-	0	ground	847	Y	Y	<i>Canis</i> sp.	
9	20050316/0?	23.32	8.19	7	99	<i>Tamarix</i>	0	ground	813	N	Y	<i>Canis</i> sp.	
10	20050316/0?	23.33	8.13	7	98	-	-	-	849	Y	N	<i>Canis</i> sp.	
11	20050316/04	23.32	8.19	7	99	<i>Tamarix</i>	0	ground	837	Y	Y	<i>Genetta</i> sp.	12S = <i>Canis</i>
12	20050316/05	23.32	8.19	7	99	<i>Tamarix</i>	0	ground	804	Y	N	<i>Felis</i> sp.	
13	20050316/06	23.42	7.92	6		Rock	0	ground	802	Y	N	<i>Canis</i> sp.	
14	20050317/02	23.60	7.83	8	114	<i>Tamarix</i>	-	Ground	830	Y	Y	<i>Genetta</i> sp.	12S = <i>Canis</i>
15	20050317/03	23.60	7.83	8	114	<i>Tamarix</i>	-	branch	816	Y	Y	<i>Acinonyx jubatus</i>	
16	20050317/04	23.60	7.83	8	114	<i>Tamarix</i>	0	ground	827	Y	N	<i>Acinonyx jubatus</i>	
17	20050317/05	23.61	7.74	8	116	<i>Acacia</i>	200	branch	817	Y	Y	<i>Acinonyx jubatus</i>	
18	20050317/08	23.65	7.72	8	117	<i>Acacia</i>	100	branch	809	Y	Y	<i>Acinonyx jubatus</i>	
19	20050318/02	23.75	7.58	8	121	-	-	Cave	833	Y	N	<i>Canis</i> sp.	
20	20050318/07	23.75	7.58	8	121	-	-	Cave	818	Y	Y	<i>Canis</i> sp.	
21	20050318/11	23.78	7.54	8	122	<i>Tamarix</i>	80	branch	820	Y	N	<i>Canis</i> sp.	
22	20050319/01	24.05	7.44	9	129	<i>Tamarix</i>	160	branch	823	Y	N	<i>Acinonyx jubatus</i>	
23	20050319/03	24.05	7.44	9	129	<i>Tamarix</i>	80	branch	843	Y	Y	<i>Acinonyx jubatus</i>	
24	20050319/04	24.07	7.39	9	130	<i>Tamarix</i>	70	branch	805	Y	Y	<i>Acinonyx jubatus</i>	
25	20050319/04	24.06	7.34	9	131	<i>Tamarix</i>	-	-	870	N	Y	<i>Panthera pardus</i>	
26	20050319/06	24.07	7.39	9	130	<i>Tamarix</i>	-	-	822	Y	N	<i>Canis</i> sp.	
27	20050319/07	24.07	7.39	9	130	<i>Tamarix</i>	80	branch	806	Y	Y	<i>Acinonyx jubatus</i>	
28	20050319/08	24.07	7.39	9	130	<i>Tamarix</i>	30	branch	825	Y	N	<i>Canis</i> sp.	
29	20050320/01	24.15	7.06	9	139	<i>Tamarix</i>	0	ground	826	N	Y	<i>Genetta</i> sp.	
30	20050322/01	23.92	6.20	3	24	<i>Acacia</i>	0	ground	824	N	Y	<i>Genetta</i> sp.	

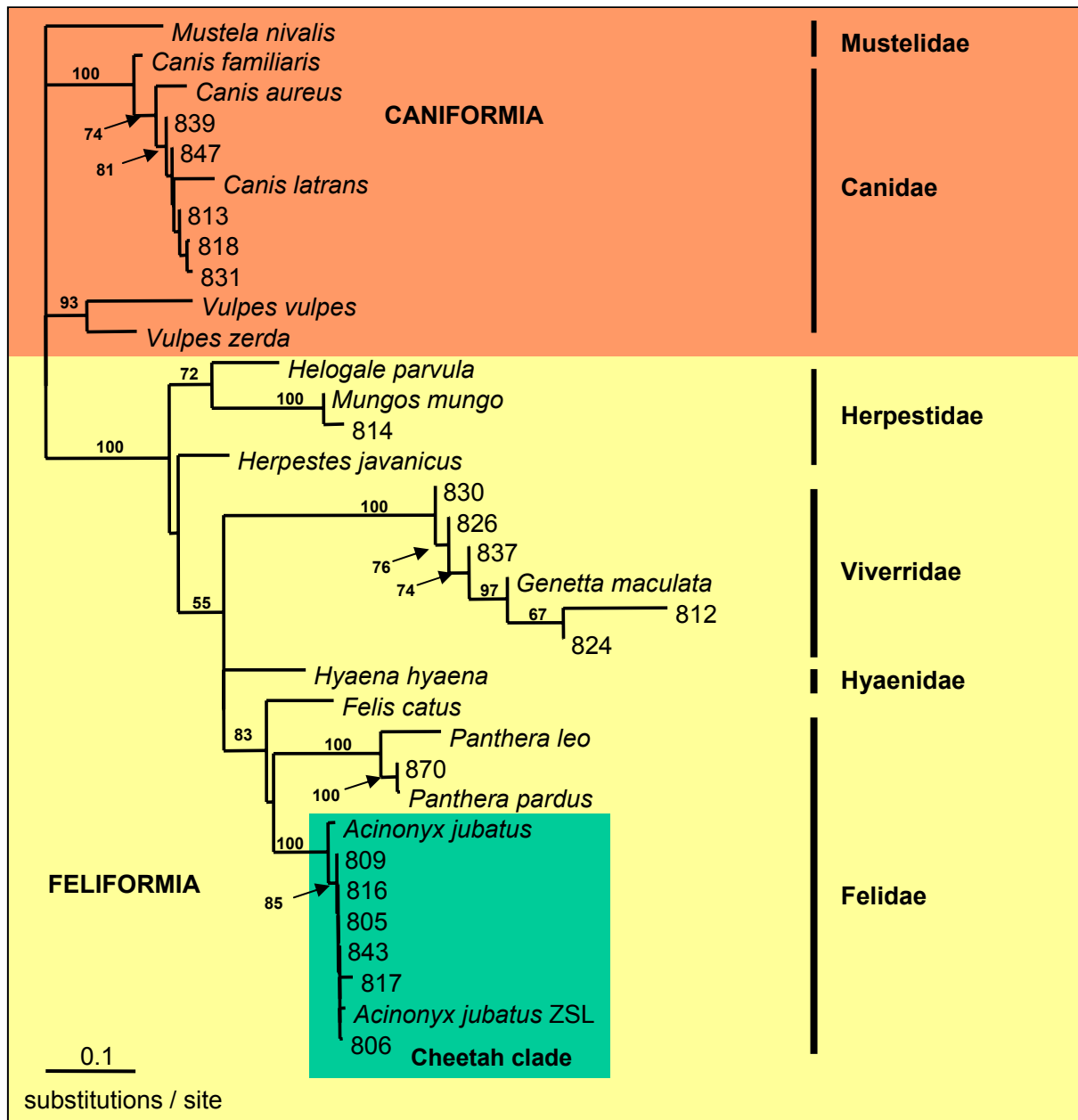
All cheetah samples are highlighted. The table also gives the information taken at the location in the Ahaggar Mountains where the sample was collected. Cheetahs are known to climb trees and often scratch the bark. Samples were collected from trees and the height on the tree and the species of tree, where known, were also noted. The lab ID refers to the sample IDs that are used in this paper. In the final notes column, the ambiguous samples are shown.

**Figure 3.1. A phylogenetic tree made with 12S sequences from the unknown Algerian and carnivore reference samples**



The numbers on the ends of the branches refer to the identity of the samples as listed in table 3.1. The species are those listed in table 2.1. The numbers on the branches refer to the bootstrap value of that branch and include all branches supported by over 50% of the bootstrap consensus. A distinct group of cheetah samples can be seen in green in the lower right corner. The large unresolved group at the top of the tree is ambiguous. The long branch between the Mustelid outgroup and the unresolved Algerian samples suggest that they represent a more closely related Caniform taxa. However there are no representatives of the other caniforms (super-family Arctoidea) other than the Mustelidae and Lutrinae (otters) in Africa (Kingdon 1997).

Figure 3.2. A phylogenetic tree made with cytochrome *b* sequences from the unknown Algerian and carnivore reference samples



This tree uses the same terminology as figure 3.1. The numbers on the branches indicate percentage confidence values. The analysis benefited from a larger number of reference samples from more detailed lineages. Here, the cheetah clade, again in green, contains 6 of the Algerian samples, all of which were present in the gene analysis. Individual 870 is a leopard. The presence of a genet sequence has resolved some of the discrepancies in figure 3.1.

### 3.2. Cheetah microsatellite analysis

Table 3.2. shows the numbers of unique alleles for each population. The genotypes were analysed further to determine whether the Algerian samples belonged to different individuals.

**Table 3.2. Summary microsatellite data for the two cheetah populations**

Population	N° of alleles	N° of unique alleles
Tanzanian	33	17 (32%)
Algerian	36	18 (34%)
<b>TOTAL</b>	53	

Table 3.3. below shows a matrix of the number (lower diagonal) and the percentage (upper diagonal) of identical loci between each of the 8 cheetahs. The highest percentage of identical alleles loci is 67%; therefore, it can be assumed that all of the Algerian samples come from different individuals.

**Table 3.3. A pairwise matrix of Algerian cheetah genotype comparisons over the 9 microsatellite loci**

Sample	805	806	809	816	817	823	828	843
<b>805</b>	*	56	44	22	44	56	56	56
<b>806</b>	5	*	44	22	44	44	56	67
<b>809</b>	4	4	*	67	67	44	56	67
<b>816</b>	2	2	6	*	44	33	44	44
<b>817</b>	4	4	6	4	*	56	44	67
<b>823</b>	5	4	4	3	5	*	67	44
<b>827</b>	5	5	5	4	4	6	*	56
<b>843</b>	5	6	6	4	6	4	5	*

The upper diagonal is the percentage of pairwise similarity and the lower is the actual number of identical loci

### Kinship

Although in terms of number of loci amplified, the sample size was small and kinship analysis of both populations was conducted. The kinship of the Tanzanian cheetahs was known and, as such, could be used to test the reliability of any kinship inferences made about the Algerian individuals. The table 3.4. shows the combined results for two different kinship analyses. Essentially, similar results were achieved for both analyses.



**Table 3.4. Relatedness result from MER and GIMLET Programs for the Algerian cheetahs**

Individual 1	Individual 2	Possible Kinship	
		MER	GIMLET
806	843	parent-offspring	parent-offspring
809	816	full-sibs	(siblings)
809	817	parent-offspring	parent-offspring
816	817	parent-offspring	parent-offspring
817	843	half-sibs	

These results indicate the following relationship between the cheetahs found in the Ahaggar Mountains (figure 3.3.):

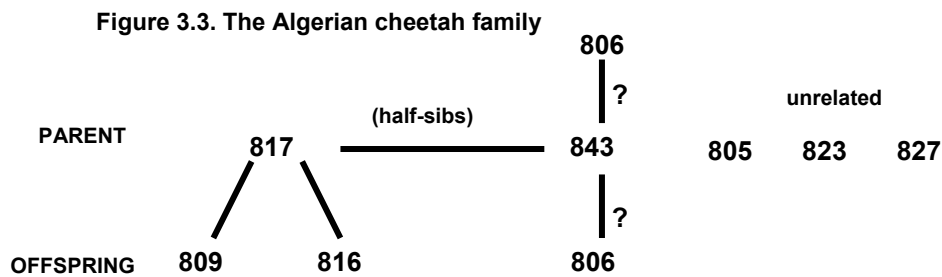


Figure 3.3. shows a related group of animals and another 3 potentially unrelated individuals within a relatively small area, suggesting a larger total population than previously identified.

### 3.3. Mapping the cheetah samples

The majority of scats were found on trees, as these locations were targeted by the expedition. Six out of the 8 cheetah samples were found on, or around, *Tamarix* trees. The figure 3.4. shows the positions of the 8 cheetah samples on a map of the study area. All cheetah samples and the leopard one were found in grids 8 and 9. The putative families of cheetah are also highlighted in different colours. The family of three (809, 817 and 816) were all found in close proximity of each other, as was the other parent-offspring pairing (806 and 843). The three remaining 'unrelated' cheetah (823, 805 and 827) were found dispersed but in the same two grids of the survey. It is worth noting that gazelle encounter rate in this region were among the highest recorded (see figure 3.4.). This area was characterised by long wadis, interspersed with *Tamarix* trees, with high mountains on either side.

Cheetah reports and signs were found over a greater range than the genetic data alone would suggest. One of these, in grid 3, was a camel kill with definite cheetah tracks suggesting that cheetah roam at least that far west. Cheetahs are also known to occur in the Tassili N'Ajjer Mountains to the north-east, and also in the Tefedest area, north of the

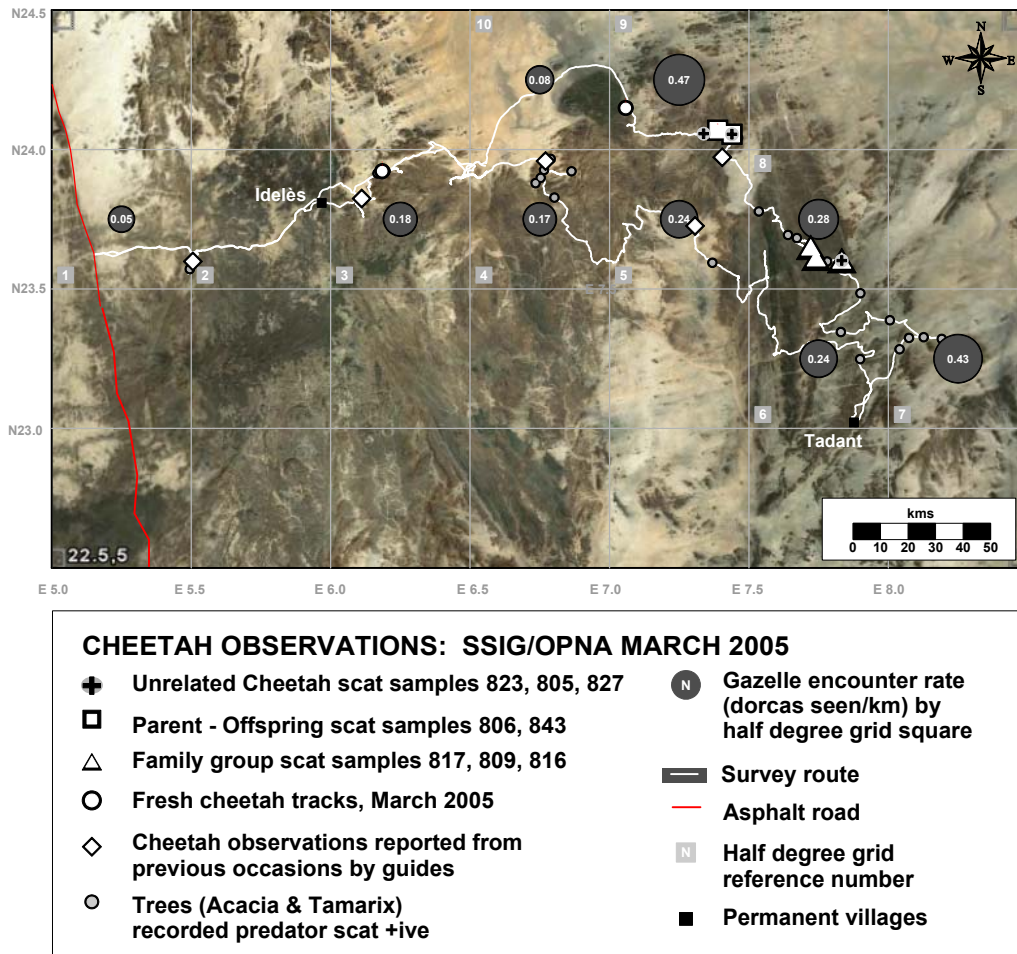


Figure 3.4. Satellite image of central Ahaggar Mountains, showing route and location of all cheetah related samples and observations. SSIG/OPNA survey, Southern Algeria, March 2005.

study site (Seddiki 1990; and see Wachter *et al.* 2005 for information on a sub-adult captured by local people in Oued Ahtes, northeast Tefedest, April 2004, documented by OPNA staff).

## 4. Discussion

### 4.1. A carnivore species list for the Ahaggar Mountains (Southern Algeria)

This report demonstrates the presence of six carnivore species in the Ahaggar Mountains (Central Sahara of Algeria).

#### 4.1.1. Non-felid species

Genets are carnivores of the Viverridae family. According to Kingdon (1997) only one species is present in the Northern reaches of Africa, and it is probable that the species found here is *Genetta genetta*, the Common genet. Indeed, in Algeria, genets have been reported surviving on small rodents and, occasionally, reptiles and insects (Larivière and Calzade 2001). Kowalski and Rzebik-Kowalska (1991) report genet in Algeria, but only in the more coastal region and the Tell Atlas. Given their relative abundance in this study (1/6 of all samples), their presence in the Ahaggar is novel. Although never documented, they might also present an opportunistic prey item for cheetah.

Individual 814 is a mongoose (Herpestidae). Banded mongoose, *Mungos mungo*, is not reported north of the Sahara and this species may probably be the Egyptian mongoose, *Herpestes ichneumon* (Kingdon 1997). Kowalski and Rzebik-Kowalska (1991) report the Egyptian mongoose as the only herpestid in Algeria. However the possible occurrence of the Endangered Slender mongoose, *Galerella sanguinea*, in Ahaggar should be considered, as this species is known from the Aïr, in Northern Niger (John Newby *pers. comm.*).

Fourteen canid specimens were found by the genetic analysis reported in this paper. Golden jackals, *Canis aureus*, are found in the Ahaggar (Kowalski and Rzebik-Kowalska 1991; Wacher *et al.* 2005), but the inclusion of a reference sample from this species shows that the canids found were not jackals. Two other canids, Fennec fox, *Vulpes zerda*, and Rüppell's fox, *Vulpes rueppellii*, are also both found in the Ahaggar (Kowalski and Rzebik-Kowalska 1991; Wacher *et al.* 2005), and Rüppell's foxes were caught in camera traps during the 2005 SSIG expedition (Wacher *et al.* 2005). When BLASTED, most samples appeared to be most similar to non-African Canids in both cytochrome *b* and 12S sequences. Therefore, although evidences of Rüppell's foxes and common jackals were found during the 2005 SSIG-OPNA expedition (Wacher *et al.* 2005), none of the samples in the present study could be unambiguously assigned to these species.

#### 4.1.2. Felid species

Perhaps the most intriguing result is the occurrence of a leopard sample, *Panthera pardus*. Kowalski and Rzebik-Kowalska (1991) report the leopard to be extinct in Algeria. They cite that during the 19th century, the leopard was common in Northern Algeria but, other than possible migrants to Western Algeria from the Saharan Atlas in Morocco, the leopard is extinct in the country. Aside from a leopard skin seen in a private house in El-Kala in 1981 (and said to have been killed in the area by an earlier family member between 1960-1962) (De Smet 1982 *in* Kowalski & Rzebik-Kowalska 1991; De Smet *pers. comm.*), this study

reports the first direct physical evidence of wild leopards in Algeria for almost 50 years. It also provides a note of its occurrence in an area of the country where it has never been seen before: the south-eastern Ahaggar massif. It is unlikely that leopards in this region are the result of possible migrants from the Moroccan Saharan Atlas as there is no obvious connected habitat between the two areas. The most plausible explanation is that leopard may have always been present in the region, but, until now, been overlooked because of the remoteness of the region, the notorious elusiveness of the species, or due to confusion with cheetah.

A *Felis* sample was also uncovered in the present study. Unfortunately, sequencing for cytochrome *b* did not work for this sample, but the 12S analysis suggests that the cat found here was not the Sand-cat, *Felis margarita*, which is likely to be widespread in the sandy area of the Algerian Sahara (Kowalski and Rzebik-Kowalska 1991). Wild cat tracks were found during the expedition (Wacher *et al.* 2005). Records show that the African Wildcat, *Felis sylvestris lybica*, is present in much of the mountainous regions of Algeria (Kowalski and Rzebik-Kowalska 1991) and given that this species is often considered to be the ancestor of today's domestic cat (Alderton 1998), it is not surprising that it occurs in a clade with *F. catus* (figure 3.1.).

Eight different individual cheetah samples were found in the present study. Observations made during the 2005 expedition and reports collected by the team suggested that there were indeed cheetahs in this area of Algeria.

#### **4.2. The present situation of cheetah in the Ahaggar Mountains**

The figure 3.4. shows the cheetah samples plotted on a map of the region. In this study, all cheetah samples were found within two grids: 8 and 9. Interestingly, the cheetah of the putative family groups map into similar positions. Grid 9, where half of the cheetah samples, and the leopard sample, were found, is also the grid with the highest encounter rate for dorcas gazelles. This area was characterised by long and deep wadis, with patchy *Tamarix* bush. It was also one of the more remote areas of the survey. Together with the known records of cheetahs in the Tassili N'Ajjer and north of the Ahaggar (Tefedest area; Seddiki 1990; Wacher *et al.* 2005), these data suggest that a population of cheetah is distributed over a wide area in Southern Algeria.

The microsatellite analysis of the cheetah samples suggest that at least two family groups were present, which might possibly be part of one larger family. The number of unique alleles in both populations was roughly one third. Although this is based on a sample size

too small to be tested statistically, it is potentially a very important result as it implies that the two populations are genetically dissimilar.

#### **4.3. Future North African Cheetah studies**

There were no 'recaptures' during this study: all of the cheetah individuals were unique. However, more extensive collection of scat in a systematic survey, with definite 'recaptures', will potentially lead to estimates of population size and range of individual cheetahs and the population as a whole. Geographic information of the sort collected by the 2005 SSIG expedition also helps to build a better picture of the lifestyle and habit of these Saharan cheetahs.

Cheetahs are infamously genetically similar (O'Brien *et al.* 1983; O'Brien *et al.* 1985; Wayne *et al.* 1986; O'Brien *et al.* 1987). Given that around one third of the alleles over the two populations are unique, this study highlights the need for further genetic work on a larger sample size in order to corroborate this result. It is also essential for the future management of this endangered species that these results be used to increase the conservation effort in this area of Africa.

#### **4.4. Conclusions**

This paper demonstrates that genetic analysis of scat samples offers a very efficient method to detect and study rare and highly elusive species with minimum intrusion. Combined with observational evidence collected in the field, this paper shows that a population of cheetah is surviving in the Ahaggar Mountains, southern Algeria. It has also proven the existence of leopards in a previously unreported area of Africa. Therefore, this report provides impetus for further longer term and more intensive study into the felid species of this region of Africa. A combination of molecular genetic and field observation techniques is recommended as the most efficient way to improve knowledge about range, population numbers and adapted behaviours of these animals. It is in the interest of all conservationists that this lack of data be addressed now in the planning and proposing of continued desert carnivores research in Algeria.

## 5. References

- Benson, D.A., I. Karsch-Mizrachi, D.J. Lipman, J. Ostell & D.L. Wheeler. 2005.** GenBank. *Nucleic Acids Research* **33** – online issue [www.ncbi.nlm.nih.gov/Genbank/index.html](http://www.ncbi.nlm.nih.gov/Genbank/index.html)
- Bjornerfeldt, S., M.T. Webster & C. Vila. 2006.** Relaxation of selective constraint on dog mitochondrial DNA following domestication. *Genome Research* **16** (8): 990-994.
- Burger, P.A., R. Steinborn, C. Walzer, T. Petit, M. Mueller & F. Schwarzenberger. 2004.** Analysis of the mitochondrial genome of cheetahs (*Acinonyx jubatus*) with neurodegenerative disease. *Gene* **338** (1): 111-119.
- Caughley, G. 1994.** Directions in Conservation Biology. *Journal of Animal Ecology*. **63** (2): 215-244.
- Das, D.K., M. Saini, A.R. Gupta, D. Swarup, M.P. Yadav & P.K. Gupta. 2005.** *PCR amplification and sequencing of mitochondrial 12S rRNA gene fragment from Indian jackal Canis aureus* – unpublished GenBank sequence - DQ102371.
- Driscoll, C.A. 1992.** *A Characterization of Microsatellite Loci Variation in Panthera leo, Acinonyx jubatus and Felis concolor* Unpublished MSc Thesis. University of Maryland, Baltimore.
- Gaubert, P., C.A. Fernandes, M.W. Bruford & G. Veron. 2004.** Genets (Carnivora, Viverridae) in Africa: an evolutionary synthesis based on cytochrome b sequences and morphological characters. *Biological Journal of the Linnaeus Society of London* **81** (4): 589-610.
- Gottelli, D., J. Marino, C. Sillero-Zubiri & S.M. Funk. 2004.** The effect of the last glacial age on speciation and population genetic structure of the endangered Ethiopian wolf (*Canis simensis*). *Molecular Ecology* **13**: 2275-2286.
- Gottelli, D., J. Wang, S. Bashir & S.M. Durant. Under review.** Genetic benefits of promiscuity among Serengeti female cheetah. *Proceedings of the Royal Society*.

- Hamdine, W., T. Meftah & A. Sekhi. 2003.** Répartition et statut du guépard (*Acinonyx jubatus* Schreber, 1776) dans le Sahara central Algérien (Ahaggar et Tassili). *Mammalia* **69** (3): 403-408.
- IUCN 2006.** 2006 IUCN Red List of Threatened Species. <<http://www.iucnredlist.org/>>. Downloaded on 26 October 2006.
- Janczewski, D., W. Modi, J. Stephens & S. O'Brien. 1995.** Molecular Evolution of Mitochondrial 12S RNA and Cytochrome B sequences in the Pantherine Lineage of Felidae. *Molecular Biology and Evolution* **12** (4): 690-707.
- Kingdon, J. 1997.** *The Kingdon Field Guide to African Mammals*. London: Academic Press, A&C Publishers.
- Koepfli, K.-P., S.M. Jenks, E. Eizirik, T. Zahirpour, B. Van Valkenburgh & R.K. Wayne. 2006.** Molecular systematics of the Hyaenidae: Relationships of a relictual lineage resolved by a molecular supermatrix. *Molecular Phylogenetic Evolution* **38** (3): 603-620.
- Kowalski, K. & B. Rzebik-Kowalska. 1991.** *Mammals of Algeria*. Wroclaw-Warszawa – Kraków. Zakład Narodowy Imienia Ossolińskich Wydawnictwo Polskiej Akademii Nauk.
- Kurose, N., A.V. Abramov & R. Masuda. 2000.** Intrageneric diversity of the cytochrome *b* gene and phylogeny of Eurasian species of the genus *Mustela* (Mustelidae, Carnivora). *Zoological Science* **17**: 673-679.
- Ledje, C. & U. Arnason. 1996.** Phylogenetic relationships within caniform carnivores based on analyses of the mitochondrial 12S rRNA gene. *Journal of Molecular Evolution* **43** (6): 641-649.
- Larivière, S. & J. Calzade. 2001** *Genetta genetta* *Mammalian Species* **680**: 1-6.
- Marker, L. 1998.** Current status of the cheetah (*Acinonyx jubatus*). In: *Proceedings of the Symposium on Cheetahs as Game Ranch Animals*, Penzhorn (ed.): 1-17. Onderstepoort, 23-24 October 1998, South-Africa.

- Marker, L. 2000.** Aspects of the Ecology of the Cheetah. *Journal of the Namibia Scientific Society* **48**: 41-48.
- Masuda, R., M.C. Yoshida, F. Shinyashiki, & G. Bando. 1994.** Molecular phylogenetic status of the iriomote cat, *Felis iriomotensis*, inferred from mitochondrial DNA sequence analysis. *Zoological Science* **11** (4): 597-604.
- Meece, J.K., C.E. Reynolds, P.J. Stockwell, J.E. Christensen & K.D. Reed. 2005.** Identification of mosquito blood meal source by terminal restriction fragment length polymorphism (T-RFLP) profile analysis of the cytochrome *b* gene. *Journal of Medical Entomology* **42** (4): 657-667.
- Menotti-Raymond, M., V.A. David, L.A. Lyons, A.A. Schäffer, J.F. Tomlin, M.K. Hutton & S.J. O'Brien. 1999.** A Genetic Linkage Map of Microsatellites in the Domestic Cat (*Felis catus*). *Genomics* **57** (1): 9-23.
- Merola, M. 1994.** A Reassessment of Homozygosity and the Case of Inbreeding Depression in the Cheetah, *Acinonyx jubatus*: Implications for Conservation. *Conservation Biology* **8** (4): 961-971.
- Nowell, K. & P. Jackson. (ed.) 1996.** *Wild Cats – Status Survey and Conservation Action Plan*. IUCN/SCC Cat Specialist Group.
- O'Brien, S. J., D.E. Wildt, D. Goldman, C. Merrill & M. Bush. 1983.** The cheetah is depauperate in genetic variation. *Science* **221**: 459-462.
- O'Brien, S. J., M.E. Roelke, L. Marker, A. Newman, C.A. Winkler, D. Meltzer, L. Colly, J.F. Evermann, M. Bush & D.E. Wildt. 1985.** Genetic basis for species vulnerability in the cheetah. *Science* **227**: 1428-1434.
- O'Brien, S. J., D.E. Wildt, M. Bush, T.M. Caro, C. FitzGibbon, I. Aggundey & R.E. Leakey. 1987.** East African cheetahs: evidence for two population bottlenecks? In: *Proceedings of the National Academy of Science of the USA* **84**: 508-511.
- Penny, D. & P. McLenachan. 2005.** Unpublished Genbank sequence - AY873843.



- Seddiki, D. 1990.** *Contribution à l'étude des mammifères et des oiseaux du massif de la Tefedest (Ahaggar)*. Unpublished Agricultural Engineer Thesis. Institut National Agronomique, Algiers.
- Sugimoto, T., J. Nagata, V.V. Aramilev, A. Belozor, S. Higashi & D.R. McCullough. 2005.** *Species and sex identification from faecal samples of sympatric carnivores, Amur Leopard and Siberian Tiger, in the Russian Far East* – unpublished GenBank sample – AB211405.
- Swofford, D.L. 1998.** *PAUP Phylogenetic analysis using parsimony and other methods*. Sunderland MA: Sinauer Associates.
- Taberlet, P., S. Griffin, B. Gossens, S. Questiau, V. Manceau, N. Escaravage, L.P. Waits & J. Bouvet. 1996.** Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acid Research* **24** (16): 3189-3194.
- Valière, N. 2002.** GIMLET: a computer program for analyzing genetic individual identification data. *Molecular Ecology Notes* **2**: 377-379.
- Wacher, T., K. De Smet, F. Belbachir, A. Belbachir-Bazi, A. Fellous, M. Belghoul & L. Marker. 2005.** *Sahelo-Saharan Interest Group Wildlife Surveys, Central Ahaggar Mountains (March 2005)*. Sahelo Saharan Interest Group.
- Wang, J. 2004.** Estimating pairwise relatedness from dominant genetic markers. *Molecular Ecology* **13**: 3169-3178.
- Wayne, R. K., W.S. Modi & S.J. O'Brien. 1986.** Morphological variability and asymmetry in the cheetah (*Acinonyx jubatus*): a genetically uniform species. *Evolution* **40**: 78-85.
- Wayne, R.K., E. Geffen, D.J. Girman, K.P. Koepfli, L.M. Lau & C.R. Marshall. 1997.** Molecular systematics of the Canidae. *Systematic Biology* **46** (4): 622-653.
- Yoo, M.H., H.S. Jeon, B.S. Kim & J.R. Rho. 2006.** *Genetic diversity of Vulpes vulpes*. unpublished GenBank sequence - DQ498127.