1. General Introduction

This doctoral thesis is organised in four chapters with Chapter 1 being a general introduction into the topics investigated in this study. Chapter 2 presents and discusses findings on the reproductive activity and the breeding success of free-ranging female cheetahs on Namibian farmland. In Chapter 3, possible mechanisms for the discovered differences in reproductive activity between free-ranging and captive female cheetahs are investigated. In Chapter 4 results on the health status and of a serological survey on Namibian cheetahs are presented.

The cheetah in southern Africa (*Acinonyx jubatus jubatus*) belongs to the order of Carnivora, family Felidae and is as one of five subspecies recognised (Marker 1998). The distribution of cheetahs 2000 years ago was much wider than at present and covered most parts of Africa and the arid zones from India through Iran to Arabia (Joubert 1984; Caro 1994; Hunter & Hamman 2003). In Africa, the estimated cheetah number has declined from about 100.000 in 1900 (Myers 1975) to currently about 15.000 individuals in 29 African countries (Marker 1998), with Namibia containing the largest population of 3.100 - 5.800 individuals (Hanssen & Stander 2003). As a consequence of their drastic decline, the cheetah was listed in 1975 in Appendix I of the Convention on International Trade in Endangered Species of Fauna and Flora (CITES 1984), granting it the highest protection status for international trade, and the International Union for the Conservation of Nature (IUCN) classified the species in 1986 as Vulnerable (IUCN 2004).

In the 1980s, a number of studies on the genetic characteristics of cheetahs were conducted. Studies on the basis of allelic isozymes (O'Brien et al. 1983; O'Brien et al. 1985), soluble proteins (O'Brien et al. 1983), skin grafts (O'Brien et al. 1985), fluctuating scull asymmetry (Wayne et al. 1986), major histocompatibility complex (Yuhki & O'Brien 1990), mitochondrial DNA (Menotti-Raymond & O'Brien 1993; Freeman et al. 2001), hypervariable minisatellite loci (Menotti-Raymond & O'Brien 1993) and microsatellite DNA (Menotti-Raymond & O'Brien 1995) came to the result that the cheetah is genetically monomorphic. Although several authors have criticised some of the methods, analyses and interpretations of these studies (e.g. (Willig & Owen 1987; Merola 1994; Caughley 1994) it is generally accepted that the cheetah shows a low level of genetic variation. As an explanation for this lack of genetic diversity it has been suggested that at the end of the late Pleistocene about 10.000 years ago, when large numbers of mammal species became extinct, the cheetah population went through one or several population 'bottleneck(s)', reducing its number drastically (O'Brien et al. 1987; Menotti-Raymond & O'Brien 1993). The surviving individuals were assumed to have formed the founder population(s) of the present cheetahs, which are suggested to be inbred descendants of the founder animals (O'Brien et al.

1987). It was further suggested that the more recent population decline during the last one hundred years, owing to various anthropogenic activities, led to further inbreeding between related individuals in small isolated populations (O'Brien et al. 1987). Since it is currently not possible to obtain direct evidence to test the proposed 'bottleneck hypothesis', indirect evidence has to be used to test this idea. Inbred individuals are known to suffer from a variety of drawbacks owing to reduced heterozygosity. Such drawbacks generally are

1. Low reproductive performance (e.g. Wildt et al. 1993) – first part of Chapter 2 and Chapter 3
2. High juvenile mortality (e.g. Ralls et al. 1979; Hedrick 1987) – second part of Chapter 2
3. High susceptibility to infectious diseases (Evermann et al. 1988) – Chapter 4.

In the following sections these three aspects are briefly discussed and the hypotheses and predictions investigated in this thesis presented.

1.1 Low reproductive performance

Cheetahs in captivity are known for their low reproductive performance. In North American zoos regular breeding success was an exception for a long time (Marker & O'Brien 1989). A comprehensive study revealed that only a third of the females ever produced offspring and that a third of the females showed minimal or no ovarian activity (Wildt et al. 1993). This comparatively low reproductive performance for a captive felid was suggested to be the consequences of inbreeding and genetic monomorphism of cheetahs (Marker & O'Brien 1989; Wildt et al. 1993). Subsequent studies, however, suggested that the difficulties of founding a self-sustaining cheetah population in captivity are likely to be due to inappropriate husbandry and management conditions (Wielebnowski 1996).

Since most data suggesting a low reproductive potential in cheetah females originated from captive individuals, previous studies could not differentiate whether the low fertility in females was mainly due to consequences of genetic monomorphism or to inappropriate husbandry and management conditions. Such a differentiation is possible when investigating free-ranging cheetahs in their natural habitat. A previous study on free-ranging female cheetahs in the Serengeti National Park, Tanzania, suggested that husbandry conditions may impair female reproduction, since all free-ranging adult females (N = 14) in the study reproduced (Laurenson et al. 1992).

Thus, if reproduction of female cheetahs is affected by husbandry conditions, free-ranging female cheetahs should show active reproductive organs and

high fertility. In Chapter 2 of this thesis the reproductive activity and fertility of free-ranging female cheetahs on Namibian farmland was investigated. Data on the reproductive organs were obtained from immobilised animals using the minimally invasive technique of ultrasonography (Hildebrandt & Göritz 1998). Results were compared with respective data from female cheetahs kept in captivity in enclosures in their natural habitat in Namibia. If captivity in general affects reproductive performance, then also captive cheetahs kept in enclosures in their natural habitat were expected to exhibit a lower reproductive performance than their free-ranging counterparts.

In Chapter 3 predictions derived from previously hypothesised mechanisms for reduced reproductive activity in captive cheetah females were investigated in detail. In cheetah females kept in zoos or in their natural environment, the occurrence of periods of anoestrous as well as reproductive inactivity has been previously demonstrated (Wildt et al. 1993; Brown et al. 1996; Jurke et al. 1997; Wielebnowski & Brown 1998; Wielebnowski et al. 2002; Terio et al. 2003). Such irregularities were suggested to be caused by unnatural and stressful conditions in captivity (Brown et al. 1996; Jurke et al. 1997; Wielebnowski et al. 2002) or be triggered by an endogenous circannual rhythm related to onset and intensity of the rainy season in their African regions of origin (Terio et al. 2003). Recently, a model of 'asymmetric aging' has been developed (Hermes et al. 2004) to explain reproductive failures in captive white rhinoceros (*Ceratotherium simum sp.*) (Hermes et al. 2004; Hermes et al. 2006) and African and Asian elephants (*Loxodonta africana* and *Elephas maximus*) (Hildebrandt et al. 2000). This model suggests that reproductive organs in non-breeding captive females may experience accelerated aging compared to breeding captive or breeding free-ranging females owing to continuous maturation of follicles, ovulation and luteal phases of the former. During this asymmetric aging process, the reproductive organs might develop pathologies and at the end of the process ovaries might turn into irreversible acyclicity.

To investigate whether the reproductive inactivity in captive female cheetahs in Namibia is due to stressful captive conditions the size of the adrenal glands, an indicator for a stress response, were measured in free-ranging and captive cheetah females using ultrasonography. To test the idea of a circannual rhythm underlying the reproductive irregularities in captive Namibian cheetahs, ultrasonographic results on the reproductive tracts were related to the rainy season in Namibia. To investigate whether the model of asymmetric aging is applicable for captive cheetahs, the reproductive organs of free-ranging and captive Namibia cheetah females were examined with ultrasonography to detect pathologies and liquid filled structures in close proximity to the ovaries.

1.2 High juvenile mortality

Between 1956 and 1982 cub mortality in three cheetahs breeding centres was reported to be on average 29.1% by the age of 6 months (O'Brien et al. 1985). This relatively high cub mortality compared to other exotic animal species was also suggested to be the consequence of the genetic monomorphism of the cheetah (O'Brien et al. 1985). However, the analysis of the underlying data has been questioned repeatedly and re-calculation of these data and analysis of new data revealed that cub mortality differed for inbred and non-inbred offspring, suggesting that cheetahs do have substantial variability of those loci influencing cub survival (Caughley 1994; Wielebnowski 1996). Furthermore, it was observed that breeding success varied highly among breeding facilities, indicating that husbandry conditions had an influence on cub mortality rates (Wielebnowski 1996).

Studies on free-ranging cheetah cub survival are rare and the only long-term study existing so far was conducted in the Serengeti National Park, Tanzania (Laurenson 1994; Caro 1994; Kelly et al. 1998). It was shown that this population suffers from high cub mortality due to predation by lions (*Panthera leo*) and spotted hyenas (*Crocuta crocuta*). This factor accounted for 78% of cheetah cub deaths, largely contributing to the low survival of cubs (5 %) to the age of independence (Laurenson 1994). Whilst this result is very interesting, it does not allow to identify whether inbreeding / genetic monomorphism contributed to the low survival of the cubs.

On Namibian farmland, large predator species such as the African lion, spotted hyena and African wild dog (*Lycaon pictus*) were removed by people with the onset of commercial farming in the beginning of the 20th century. They were thought to be responsible for killing large numbers of livestock and were therefore eradicated (Marker-Kraus et al. 1996). As a result, the major competitors accounting for the death of a significant proportion of cheetah cubs in East Africa do not occur in the habitat of most cheetahs in Namibia. In Chapter 2, cub survival and litter sizes of free-ranging cheetahs on Namibian farmland were investigated in the absence of competitors, predicting that in Namibia on average more cubs reach independence and larger litter sizes can be observed than in Tanzania.

1.3 High susceptibility to infectious diseases

The high prevalence of diseases in captive cheetahs has been of concern in the past, because several uncommon diseases were described (Munson 1993). An outbreak of feline infectious peritonitis (FIP) in a captive cheetah population in a North American zoo was responsible for the death of 60% of the population and has repeatedly been explained by the cheetah's limited immunocompetence as a consequence of genetic impoverishment (O'Brien et

al. 1985; Evermann 1986; Evermann et al. 1988; Heeney et al. 1990; Munson 1993). This interpretation was consistent with the subsequent finding that the cheetah has a low genetic variability at class I loci of the major histocompatibility complex (MHC, Yuhki & O'Brien 1990), which encode peptides that mediate the immune response to viral infections. However, several authors have questioned the link between the FIP outbreak and cheetah genetic monomorphism, and suggested that rather the artificially increased density and stressful husbandry conditions may have led to the disease outbreak in this solitary living species (Merola 1994; Caro & Laurenson 1994).

Whether cheetahs are vulnerable to infectious diseases has not only implications for husbandry management but also for the conservation of free-ranging cheetahs. In Chapter 4 the health status and the prevalence of antibodies against viruses likely to be relevant for felids was investigated. If cheetah immunocompetence is limited as hypothesised, then free-ranging cheetahs should find it difficult to mount a competent response to the challenge of viral infections. If it is husbandry conditions that led to the observed outbreaks in captivity, then free-ranging Namibian cheetahs should be in good health and show no symptoms of acute viral infections. Due to the possible accumulation of antigens inside the enclosures of captive Namibian cheetahs and an increased exposure to domestic animals that may act as pathogen transmitters it was also expected that captive individuals show a higher prevalence of antibodies against pathogens than free-ranging ones. To investigate whether other free-ranging carnivore species might act as pathogen transmitters, the prevalence of viral antibodies in free-ranging leopards, caracals and jackals was also tested.

The health status of free-ranging Namibian cheetahs was investigated in more detail by comparing the nutritional status and body mass index (BMI) of males and females. An analysis of published data from the Serengeti National Park, Tanzania (Caro et al. 1987), revealed no difference in the body mass index of males and females. Since litter sizes in Namibia turned out to be substantially higher than those observed in Tanzania (Chapter 2), the reproductive effort by Namibian females might exceed that of Tanzanian females. It was therefore expected that Namibian cheetah females were likely to have a worse nutritional status and a lower body mass index than Namibian males.

References

Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). 1984. code federal register.part 23.

Brown,J.L., Wildt,D.E., Wielebnowski,N., Goodrowe,K.L., Graham,L.H., Wells,S. & Howard,J.G. 1996. Reproductive activity in captive female cheetahs (*Acinonyx*

jubatus) assessed by faecal steroids. Journal of Reproduction & Fertility, 106**,** 337-346.

Caro,T.M. 1994. Cheetahs of the Serengeti Plains: Group Living in an Asocial Species. The University of Chicago Press.

Caro,T.M., Holt,M.E., Fitzgibbon,C.D., Bush,M., Hawkey,C.M. & Kock,R.A. 1987. Health of adult free-living cheetahs. The Zoological Society of London, 212**,** 573-584.

Caro,T.M. & Laurenson,M.K. 1994. Ecological and Genetic Factors in Conservation: A Cautionary Tale. Science, 263**,** 485-486.

Caughley,G. 1994. Directions in conservation biology. Journal of Animal Ecology, 63**,** 215-244.

Evermann,J.F. 1986. Feline coronavirus infection of cheetahs. Feline Practice, 16**,** 21-30.

Evermann,J.F., Heeney,J.L., Roelke,M.E., McKeirnan,A.J. & O'Brien,S.J. 1988. Biological and pathological consequences of feline infectious peritonitis virus infection in the cheetah. Archives of Virology, 102**,** 155-172.

Freeman,A.R., MacHugh,D.E., McKeown,S., Walzer,C., McConnell,D.J. & Bradley,D.G. 2001. Sequence variation in the mitochondrial DNA control region of wild African cheetahs (*Acinonyx jubatus*). Heredity, 86**,** 355-362.

Hanssen,L. & Stander,P. Namibia Large Carnivore Atlas. Predator Conservation Trust. Vol.2. 2003. Windhoek.

Hedrick,P.W. 1987. Genetic bottlenecks. Science, 237**,** 963.

Heeney,J.L., Evermann,J.F., McKeirnan,A.J., Marker-Kraus,L., Roelke,M.E., Bush,M., Wildt,D.E., Meltzer,D.G. & Colly,L.P. 1990. Prevalence and Implications of Feline Coronavirus Infections of Captive and Free-Ranging Cheetahs (*Acinonyx-jubatus*). Journal of Virology, 64**,** 1964-1972.

Hermes,R., Hildebrandt,T.B. & Göritz,F. 2004. Reproductive problems directly attributable to long-term captivity-asymetric reproductive aging. Animal Reproduction Science, 82-83**,** 49-60.

Hermes,R., Hildebrandt,T.B., Walzer,C., Göritz,F., Patton,M.L., Silinski,S., Anderson,M.J., Wibbelt,G., Tomasova,K. & Schwarzenberger,F. 2006. The effect of long non-reproductive periods on the genital health in captive female white rhinoceros *(Ceratotherium simum simum, C.s. cottoni)*. Theriogenology, 65**,** 1492-1515.

Hildebrandt,T.B. & Göritz,F. 1998. Use of ultrasonography in zoo animals. (Ed. by M.E.&.R.E.Fowler), pp. 44-54. Philadelphia: WB Saunders.

Hildebrandt,T.B., Göritz,F., Pratt,N.C., Brown,J.L., Montali,R.J., Schmitt,D.L., Fritsch,G. & Hermes,R. 2000. Ultrasonography of the urogenital tract in elephants

(*Loxodonta africana* and *Elephas maximus*): an important tool for assessing female reproductive function. Zoo Biology, 19**,** 321-332.

Hunter,L. & Hamman,D. 2003. Cheetah. 1 edn. Cape Town, South Africa: Struik Publishers.

IUCN 2004. 2004 IUCN Red List of Threatened Species: http://www.iucnredlist.org

Joubert,E. 1984. The Cheetah - South West Africa's Spotted Enigma. South West Africa, 13-18.

Jurke,M.H., Czekala,N.M., Lindburg,D.G. & Millard,S.E. 1997. Fecal corticoid metabolite measurement in the cheetah (*Acinonyx jubatus*). Zoo Biology, 16**,** 133-147.

Kelly,M.J., Laurenson,M.K., Fitzgibbon,C.D., Collins,D.A., Durant,S.M., Frame,G.W., Bertram B.C.R. & Caro,T.M. 1998. Demography of the Serengeti cheetah (*Acinonyx jubatus*) population: The first 25 years. Journal of Zoology, 244**,** 473-488.

Laurenson,M.K. 1994. High juvenile mortality in cheetahs *(Acinonyx jubatus)* and its consequences for maternal care. Journal of Zoology, 234**,** 387-408.

Laurenson,M.K., Caro,T.M. & Borner,M. 1992. Female cheetah reproduction. National Geographic Research and Exploration, 8**,** 64-75.

Marker,L. Current status of the cheetah(*Acinony jubatus)*. [Proceedings of a Symposium on cheetahs as Game Ranch Animals], 1-17. 1998. Onderstepoort, South Africa. Penzhorn, B. L.

Marker,L. & O'Brien,S.J. 1989. Captive breeding of the cheetahs (*Acinonyx jubatus*) in North American Zoos (1871-1986). Zoo Biology, 8**,** 3-16.

Marker-Kraus,L., Kraus,D., Barnett,D. & Hurlbut,S. Cheetah Survival on Namibian farmlands. Cheetah Conservation Fund. 1996. Windhoek.

Menotti-Raymond,M. & O'Brien,S.J. 1993. Dating the genetic bottleneck of the African cheetah. Proceedings of the National Academy of Sciences of the United States of America, 90**,** 3172-3176.

Menotti-Raymond,M. & O'Brien,S.J. 1995. Hypervariable genomic variation to reconstruct the natural history of populations: Lessons from the big cats. Electrophoresis, 16**,** 1771-1774.

Merola,M. 1994. A reassessment of homozygosity and the case for inbreeding depression in the cheetah, Acinonyx jubatus: Implications for conservation. Conservation Biology, 8**,** 961-971.

Munson,L. 1993. Diseases of captive cheetahs (*Acinonyx jubatus*): Results of the Cheetah Research Council Pathology Survey, 1989-1992. Zoo Biology, 12**,** 105-124.

Myers,N. The Cheetah *Acinonyx jubatus* in Africa. IUCN Monograph. 1975. Switzerland, Morges.

O'Brien,S.J., Roelke,M.E., Marker,L., Newman,A., Winkler,C.A., Meltzer,D., Colly,L., Evermann,J.F., Bush,M. & Wildt,D.E. 1985. Genetic basis for species vulnerability in the cheetah. Science, 227**,** 1428-1434.

O'Brien,S.J., Wildt,D.E., Bush,M., Caro,T.M., FitzGibbon,C., Aggundey,I. & Leakey,R.E. 1987. East African cheetahs: evidence for two population bottlenecks? Proceedings of the National Academy of Sciences of the United States of America, 84**,** 508-511.

O'Brien,S.J., Wildt,D.E., Gildman,D., Merril,C.R. & Bush,M. 1983. The cheetah is depauperate in genetic variation. Science, 221**,** 459-462.

Ralls,K., Brugger,K. & Ballou,J. 1979. Inbreeding and juvenile mortality in small populations of ungulates. Science, 206**,** 1101-1103.

Terio,K.A., Marker,L., Overstrom,E.W. & Brown,J.L. 2003. Analysis of ovarian and adrenal activity in Namibian cheetahs. South African Journal of Wildlife Research, 33**,** 71-78.

Wayne,R.K., Modi,W.S. & O'Brien,S.J. 1986. Morphological variability and asymmetry in the cheetah (*Acinonyx jubatus*). Evolution, 40**,** 78-85.

Wielebnowski,N. 1996. Reassessing the relationship between juvenile mortality and genetic monomorphism in captive cheetahs. Zoo Biology, 15**,** 369.

Wielebnowski,N. & Brown,J.L. 1998. Behavioral correlates of physiological estrus in cheetahs. Zoo Biology, 17**,** -209.

Wielebnowski,N.C., Ziegler,K., Wildt,D.E., Lukas,J. & Brown,J.L. 2002. Impact of social management on reproductive, adrenal and behavioural activity in the cheetah (*Acinonyx jubatus*). Animal Conservation, 5**,** 291-301.

Wildt,D.E., Brown,J.L., Bush,M., Barone,M.A., Cooper,K.A., Grisham,J. & Howard,J.G. 1993. Reproductive status of cheetahs (*Acinonyx jubatus*) in North American zoos: The benefits of physiological surveys for strategic planning. Zoo Biology, 12**,** 45-80.

Willig,M.R. & Owen,R.D. 1987. Fluctuating asymmetry in the cheetah:methodological and interpretive concerns. Evolution, 41**,** 225-227.

Yuhki,N. & O'Brien,S.J. 1990. DNA variation of the mammalian major histocompatibility complex reflects genomic diversity and population history. Proceedings of the National Academy of Sciences of the United States of America, 87**,** 836-840.