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Paper CH2: MORPHOLOGICAL ABNORMALITIES REPORTED

IN NAMIBIAN CHEETAHS (Acinonyx jubatus)

Author : MARKER-KRAUS L\*

Institution / Address: Cheetah Conservation Fund, P.O. Box 247, Windhoek,

Namibia

Abstract: Extensive genetic studies have shown that cheetahs are genetically homozygous, a condition that makes their survival more vulnerable to environmental and ecological changes. Three distinct morphological abnormalities have been observed in the free-ranging Namibian cheetah population. Two dental anomalies, focal palatine erosion (FPE) and crowded incisors, as well as a distinct kink in cheetahs tails.

Cheetah Conservation Fund has developed an extensive data base on over 250 free-ranging Namibia cheetah. Morphological abnormalities have been included in this data-base. Opportunistically, captured cheetahs are anesthetized, during which time physical exams and biological samples are collected for over-all health, disease and genetic analysis. A high incidence of cheetahs have been recorded with deep impressions in the upper palate, possibly a predisposition to FPE, a condition where the lower molars break through the upper palate. FPE is a serious problem that can lead to fatal disease. Previously, FPE has only been reported in captive cheetah and was thought to be a result of lack of bones in captive diets. This is the first reporting of FPE in free-ranging cheetahs. Behavioural signs accompanying FPE and treatment are presented.

Another anomaly includes a high incidence of cheetahs with crowded lower incisors. The crowding varies from slight to severe where incisors are arranged in two parallel rows. This anomaly has not yet been reported in literature, which makes it difficult for those who work with these animals to recognize the defect and study it further. This defect is significant due to the challenges the species face in the wild. Incisor teeth are used by cheetah to skin their prey, so a malocclusion may theoretically make skinning more difficult thus allowing more time for other predators to steal their food. Being able to quantify abnormalities is important for the conservation of species, as defects may have long-reaching affects on the survival of endangered species. If morphological abnormalities, such as those discussed in this paper, are a localized problem and continue to be passed on, they could rapidly become widespread throughout the population.

# MORPHOLOGICAL ABNORMALITIES REPORTED IN NAMIBIAN CHEETAHS (Acinonyx jubatus)

## MARKER-KRAUS L\*

Cheetah Conservation Fund, P.O. Box 247, Windhoek, Namibia

#### INTRODUCTION

Cheetahs are highly specialized, cursorial predators. Although classified in the family Felidae, they are unique among cats in many aspects of their anatomy and natural history. Cheetahs are classified by CITES (1992) as vulnerable or endangered in the wild. Today, there are fewer than 15,000 of these endangered cats remaining in Africa and  $\pm$  200 in Asia (Kraus and Marker-Kraus, 1991). Loss of habitat, a declining prey base, competition with large predators and livestock interests are taking a heavy toll on wild cheetah populations throughout their range. The vast majority of cheetahs live in small, isolated groups outside protected game reserves where they are often in conflict with humans interests and livestock, and most populations continue to decline (Kraus and Marker-Kraus, 1991). Cheetah do not reproduce well in captivity (Marker and O'Brien, 1989; Marker-Kraus and Grisham, 1993; Marker-Kraus, 1997) and the species is recognized as having less genetic diversity than other felids (O'Brien et al., 1983; O'Brien et al., 1985; May, 1995), believed to have caused morphological asymmetry (Wayne et al. 1986), and a greater susceptibility to diseases thus making the species more vulnerable to ecological and environmental changes.

Namibia is home to the largest population of cheetahs in the world (Kraus and Marker-Kraus, 1991). However, in the last 15 years their numbers have been reduced by half to  $\pm$  2,500. From 1980 to 1996, well over 9 000 cheetahs were legally reported to have been removed from the wild Namibian population shooting (CITES, 1992; Nowell,1996; Marker-Kraus et al., 1996), mainly through indiscriminate catching in live traps and shooting by livestock and game farmers. Significant declines in the population continue as farmers capture and remove large numbers of cheetahs as "pests" and pets. The consequences of these removals may not merely be loosing individual animals from the wild population, but may possibly be encouraging/causing even greater genetic problems for the species in its struggle for survival.

In 1991, in order to work directly with the people most affecting the cheetahs chance for survival, the Cheetah Conservation Fund (CCF) established a permanent base of operations on a farm in north central Namibia, the major cheetah area of the country. CCF's conservation and educational efforts are supported by on-going field research. These field research projects are designed to learn about distribution, behaviour, biology, demographics and overall health of wild cheetah populations.

Three distinct morphological abnormalities have been observed in the free-ranging Namibian cheetah population. A distinct kink in cheetahs tails, as well as two dental anomalies, focal palatine erosion (FPE) and crowded lower incisors. Before investigation into the impact, prevalence, and etiology of the dental abnormalities can be undertaken, the anomaly must be properly defined, characterized, and described in literature.

#### **METHODS**

To date CCF has collected data on 250 cheetah which has been added to an expanding database. Opportunistically, captured cheetahs are anesthetized using intramuscular Telazol, during which time physical exams, a full set of measurements, blood samples and skin biopsies are collected on all animals for evaluating the over-all health of the cheetah population, monitoring genetics, virology, morphology, reproduction, and contributing to a genome resource bank. Morphological abnormalities are also included in this data-base.

An oral examination is performed on each animal. The animal's occlusion is scored, during which the depth of the upper palate is rated for Focal Palatine Erosion. The depth of the indent is rated as follows: 1= slight indent; 2= medium indent; and 3= deep indent and often showing signs of loss of pigmentation, inflammation and sparse cellulitis. Irregular wear patterns, broken teeth, missing teeth, presence or absence of upper first premolars, and other obvious signs of poor dental health are noted. Established methods in veterinary dentistry, are currently being used to define the incisor defect quantitatively and establish whether it and the molar defect are widespread or confined to a few animals. Calipers are used to measure particular teeth and inter-oral photographs are taken.

Because of the extensive data collected by CCF on the dental anomalies, a cooperative project was undertaken with Tufts University Veterinary School in the USA, to investigate and document the occurrence of these dental defects in wild cheetahs. In 1996, dental impressions were taken from several live animals. A 3M Express system (a polyvinyl material) was used to make putty impressions of the mandibular dentition. These impressions have been turned into permanent plaster molds at the Tufts Dental School. A group of skulls were also selected for analysis. Skulls have been carefully examined for evidence of Focal Palatine Erosion. The skulls are being CT-scanned at the Tufts University School of Veterinary Medicine and a three-dimensional image will be generated of the teeth to obtain measurements to determine the morphological defect causing the problem, i.e. size of lower jaw, tooth size or shape, face asymmetry.

These skulls will represent an initial survey of a wild population for the effect. Dental impressions will also be made of the skulls, and measurements taken from the resulting models will be compared to actual measurements taken from skulls. This will verify our technique and indicate how accurate the models are compared to the actual animals. Since only impressions can be taken from living animals, this is a necessary step. Morphological data will be paired with other information such as genetics, health, diet, and geographical origin. This project is still in progress.

## **Description of Morphological Abnormalities**

## 1. Kinked tails

Since 1995, 19% of the cheetahs which CCF has handled have been recorded with a distinct kink in the posterior tail vertebrae. This is the first documenting of this abnormality in the wild population. Prior to this, tail abnormalities such as a short tail and a curl in the tail were occasionally noted in the captive population.

A distinct kink in the posterior tail vertebrae has been reported in isolated populations of Florida panthers (*Felis concolor coryi*) (Belden, 1986; Roelke-Parker et al., 1993), a species of North American cougar that has less genetic variation than any other cougar subspecies or other feline species, and is nearly as low as the level of allozyme variation reported in the cheetah (Roelke-Parker et al., 1993; O'Brien et al., 1985; 1987).

This morphological characteristic has only rarely been seen, and occurs at low frequencies supporting the traits' genetic basis.

#### 2. Focal Palatine Erosion

A high incidence of captive wild-caught and free-ranging cheetahs in Namibia have been recorded by CCF with deep impressions in the upper palate, possibly a predisposition to focal palatine erosion. FPE is a condition where the lower molars break through the upper palate, medial to the upper first molars. In unaffected cheetahs, there is a slight indentation of the palatal mucosa in this general area, to accommodate the cusp of the tip of the lower first molar.

Focal Palatine Erosion was first reported by Fitch and Fagen (1982) as occurring in Namibian wild-caught animals living in captivity and captive-born animals from Namibian founders, but has not previously been shown to occur in wild cheetah (Phillips et al., 1991). When first reported, 86% of the cheetahs with FPE came from one shipment from Namibia in 1970 or their descendants (Fitch and Fagen, 1982). This oral defect was thought to be related to the feeding of soft commercial diets lacking bones (Phillips et al., 1991), as well as the possibility of specific family lines, renal disease, suppurative rhinitis, and appears to often accompany, but not always, a maloccluded dentition (Fitch and Fagen, 1982).

FPE is a serious problem that can lead to fatal disease. The pathogenesis of FPE occurs where the lower molar's tip makes regular contact with the palatal mucosa, the tooth eventually penetrates through the palatine bone itself, causing inflammation. The oral defects observed in affected cheetah range from sparse cellulitis, the loss of pigmentation and signs of inflammation, to large oral-nasal bony defect extending through the palatine bone, into the nasal passage. Particles of food which lodge in the focal palatine defect result in localized infection and further tissue damage. FPE has been reported in cheetahs as early as 10 months of age with a slight, localized cellulitis. In young cats it may be overlooked as a typical 'teething' disorder (Fitch and Fagen, 1982).

Behavioural signs accompanying FPE are not always evident or easily observed and in most cases only observed if an animal is immobilized. In moderate cases, a holding of the jaw unnaturally with the tongue out, excessive salivation, and bad breath accompany the disease. In extreme cases, a blood-tinged mucous discharge from the nose, snorting and sneezing in an attempt to dislodge the irritant from the nasal chamber have been reported (Fitch and Fagen, 1982).

In captive animals, FPE has been linked to renal failure and other life-threatening conditions. Radiographs have been used as a diagnostic tool to show a number of systemic diseases which have oral manifestations but are not characteristic for a particular disease (Spolnick et al., 1981). The radiographic changes associated with renal osteodystrophy include among others altered jaw density, localized destructive lesions, tooth abnormalities and extraneous calcifications (Fitch and Fagen, 1982). One of the cheetahs reported with FPE died from severe kidney failure which was associated with oral-nasal osteomyelitis from FPE. Kidney failure is one of the main causes of death for captive cheetah (Marker-Kraus, 1996; Munson, 1994). More information about the disease in wild populations is necessary in order to determine the cause and develop preventative measures.

Since FPE was first discovered in captive cheetahs, zoo veterinarians working with veterinary dental specialists have developed standard treatments and surgery to correct the problem.



Dental initial treatment should include removing the cusp tips (bilateral) of the first mandibular molar as well as the mandibular of the fourth premolar and doing a pulpotomy or pulp capping. The access opening can be sealed with amalgam or a hybrid composite (Visser, pers. comm). Following oral surgery, antibiotics are necessary to encourage soft tissue repair and regeneration. Diet recommendations include the feeding of meat on bone along with calcium and vitamin supplementation.

### 3. Crowded Lower Incisors

The third anomaly seen in Namibian cheetahs includes a high incidence of cats with crowded, crooked and maloccluded lower incisors. The crowding varies from slight to severe where incisors are arranged in two parallel rows. In domestic dogs and cats, such problems usually have a genetic basis, although nutritional status, juvenile viral infections, and metabolic disorders are possible causes (Colmery and Frost, 1986; Frost and Williams, 1986).

The frequency of the anomaly has been seen by CCF in increasing numbers of animals over the past several years in Namibia, but it has not yet been reported in literature, which makes it difficult for those who work with these animals to recognize the defect and study it further. It is possible that this is a local phenomenon due to conditions, either genetic or otherwise, specific to the Namibian cheetah population. An alternative possibility is that the defect is the result of generalized inbreeding depression and will be present in most or all cheetahs populations throughout the specie's range because of the extreme genetic homozygosity. Further research into free-ranging populations elsewhere in Africa is needed.

## Frequency and distribution of dental abnormalities in Namibian cheetahs

Overall 181 animals have been reported to have one or both of the two dental morphological abnormalities, focal palatine erosion and crowded lower incisors.

One hundred and fifty-one cheetahs have been scored for focal palatine erosion. As shown in Table 1, over-all, 45% of the cats exhibited a slight indentation, whereas 32% exhibited a medium indent and 23% a deep indent. Of these cats four had actual FPE perforations in the upper palate. Two of the cheetahs were wild-caught living in captivity, the other two were free-ranging cheetahs.

The occurrence of crowded lower incisors was recorded after CCF began to notice this abnormality in many free-ranging cheetahs examined. Lower incisor teeth of 123 animals were assessed for crowding. Sixty-four percent of these cheetahs were recorded as having crowded lower incisors (see Table 1).

Table 1 illustrates the percentage of animals per region of origin which have been scored for FPE and crowded lower incisors. From 13% to 40% of all the animals examined in the different regions exhibited a deep indentation in the upper palate and 50% to 83% of all animals exhibited crowded lower incisors. This demonstrates that the occurrence of FPE and crowded incisor is not localized but occurs at differing levels throughout the country. Differing levels of historic removals from the free-ranging population of cheetahs may contribute to the regional variation in the occurrence of FPE and the percentage of crowding throughout the population. This could possibly increase the incidence of inbreeding in some regions. Variations could also be attributed to the prevalence of specific family traits in some regions. Extensive work has already been done on cheetah genetics (O'Brien et al., 1983; 1985; 1987; Menotti-Raymond and O'Brien, 1993) and protocols exist for both DNA fingerprinting and microsatellite analysis (Gilbert et al., 1991; Menotti-Raymond and O'Brien, 1993).



Both of these techniques will be used in the near future to examine relatedness of animals with and without these morphological abnormalities.

Table 1. Percentage of FPE ratings and crowded incisors per region.

Region	Total No. FPE Scored (%)	FPE Slight (%)	FPE Medium (%)	FPE Deep (%)	Total No. Assessed for Crowding (%)	No. Crowded (%)
Gobabis	24 (16)	7 (29)	11 (46)	6 (25)	15 (12)	8 (53)
Grootfontein	15 (10)	7 (47)	6 (40)	2 (13)	15 (12)	11 (73)
Okahandja	30 (20)	18 (60)	8 (27)	4 (13)	24 (20)	20 (83)
Omaruru	20 (13)	7 (35)	5 (25)	8 (40)	12 (10)	9 (75)
Otjiwarongo	41 (27)	21 (51)	12 (29)	8 (20)	39 (32)	20 (51)
Outjo	7 (5)	1 (14)	4 (57)	2 (29)	8 (6)	6 (75)
Windhoek	. 13 (9)	7 (54)	2 (15)	4 (31)	10 (8)	5 (50)
Total	151	68	48	34	123	79 (64%)
		(45%)	(32%)	(23%)		

Focal palatine erosion has previously only been reported occurring under captive conditions, where cheetahs were fed soft captive diets. However, data collect by CCF shows that preconditions for FPE exists in the free-ranging cheetah population throughout the regions. This poses the question as to the predisposition for FPE to develop in these animals and their offspring if environmental conditions change for individuals, e.g. the animals go from a wild to captive environments and diets change.

Table 2 compares the animals less than 1 year of age to those over one year, to the length of time animals were in a captive environment, less than 30 days and greater than 30 days. Animals under one year are still developing, but permanent tooth eruption is usually completed by one year of age. Although the occurrence of a deep FPE indent in animals held in captivity more than 30 days is not higher, the length of time cheetahs are fed a non-wild diet in captivity could affect the FPE indent.

Table 2. Frequent of FPE rating to age and days in captivity

Age and Days in Captivity	Total No. animals FPE Scored	FPE Slight (%)	FPE Medium (%)	FPE Deep (%)
< 1 year, < 30 days	21	8 (38)	5 (24)	8 (38)
< 1 year, > 30 days	12	6 (50)	4 (33)	2 (17)
> 1 year, < 30 days	78	35 (45)	26 (33)	17 (22)
> 1 year, > 30 days	47	26 (55)	12 (26)	9 (19)

In order to get a better idea of the incidences of FPE and crowding of the lower incisors in the wild population, we looked at the ages of the animals. FPE often accompanies a maloccluded dentition. Crowded lower incisors and malocclusions have been recorded by CCF occurring in varying degrees in various age groups, although malocclusions may be caused during the juvenile stage by developmental or nutritional problems. We compared the animals less than one year of age to those over one year. Comparisons between the numbers of animals with FPE scoring and those cats with crowded lower incisors by age is presented in Table 3.

Table 3. Percent crowded lower incisors in FPE rating

Age	Total No. Animals	Crowded + Slight FPE (%)	Crowded + Medium FPE (%)	Crowded + Deep FPE (%)
< 1 year	33	9 (64)	7 (58)	6 (60)
> 1 year	125	45 (74)	10 (26)	7 (27)

The percentage of animals under one year of age showing lower incisor crowding within the various FPE scorings represented 67% of all animals recorded. For the animals over one year, the percentage of crowding in the FPE categories ranged from 26% to 74%. Those with the highest percentage of crowding in both age groups were scored with slight FPE palate indents. In looking at the occurrence of this in the free ranging population it becomes apparent as shown in Table 3 that crowded lower incisors occurs in both adults and juveniles age groups, with or without the occurrence of FPE. But, the high percentage of crowded lower incisors could indicate the potential for the future manifestation of FPE in these individuals as well as their progeny.

It has been reported that FPE could be a result of malocclusions or the result of missing teeth. CCF studies have shown that the occurrence of the number of first premolars is not consistent in the Namibian free-ranging cheetahs. The presence or absence of the first upper premolar was recorded in 147 cheetahs. Of those recorded, 10% of the cheetahs had no upper pre-molars, 7% had only one pre-molar and 83% had both premolars. Since it is possible that FPE could be a result of malocclusion or of teeth missing, the presence of upper premolars was correlated with the number of animals with FPE ratings. Table 4 shows the number of FPE rated animals together with those animals having their first premolars.

Table 4. Frequency of FPE ratings with number of upper first premolars

No. Upper 1st Premolar	FPE Slight (%)	FPE Medium (%)	FPE Deep (%)	Total No. Animals
None	8 (53)	3 (20)	4 (27)	15 (10.2) 10 (6.8)
One	3 (30)	3 (30)	4 (40)	10 (6.8)
Two	53 (43)	41 (34)	23 (23)	122 (83)
Total	Ì		, ,	147

Of the animals that had no premolars, 27% of the animals had a FPE scoring of 3; of those with only one premolar, 40% had a FPE scoring of 3; and those with both premolars had a FPE scoring of 23%, indicating that the number of premolars did not necessarily correlate with the FPE scoring, but the occurrence of FPE is considered relatively high in all three groups.

Of the 147 animals that were recorded having none, one or both upper first premolar, 48% were also recorded with having crowded incisors. Table 5 presents the frequency of animals with crowded incisors to the number of upper first premolars. The number of premolars does not appear to correlate with the occurrence of crowded lower incisors.

Table 5. Frequency of upper 1st pre-molars to crowded incisors

No. Upper 1st Premolars	No. Animals	No. Crowded Incisors (%)
None	20	11(55)
Ône	10	2 (20)
Two	117	58 (50)

#### **DISCUSSION**

Dental abnormalities may be significant due to the challenges cheetahs face in the wild. Severe problems have already been attributed to FPE in captive cheetah collections therefore it is important to establish whether these defects have a negative impact on their ecology in the wild. Since incisor teeth are used by cheetah to skin their prey, a malocclusion may theoretically make skinning more difficult thus allowing more time for other predators to steal their food.

The history of drastic reduction of this cheetah population over a relatively short period of time, along with the morphological features are consistent with the occurrence during the cheetah's recent history of one or more population bottlenecks, followed by period of inbreeding. These preliminary observations may reveal a reduction in genetic diversity correlated with skeletal and congenital abnormalities that severely threaten the survival of the cheetah. A high degree of genetic homozygosity is thought to have many effects on the phenotype of a given animal. This high level of homozygosity carries with it many consequences.

Developmental and morphological defects have been reported in highly inbred animals. Dental anomalies which we have seen in wild cheetahs have been observed in the highly inbred populations of captive white tigers (Emily, P., pers. comm.). The combined allozyme study on Namibian cheetahs and the morphological data suggests a genetic explanation (O'Brien et al., 1983; Wayne, et al., 1986). The dental abnormalities as described in this paper may prove to be a genetic condition that predisposes an individual to developing advanced focal palatine erosion. The predisposition to developing this disorder may make individuals more vulnerable to changing environmental conditions which could precipitate dental disease.

The genetic assumption that irregularity in size, shape, or absence of teeth can be inherited is based on fact (Tiecke et al., 1959]. All animals that were previously reported by Fitch and Fagen (1982) with FPE showed some degree of malocclusion, or the absence of one or both premolars. Our data illustrates a similar trend, as 55% of all cheetahs examined exhibited a FPE scoring of 2 or 3, together with 64% crowded incisors and 17% with one or both premolar missing.

Depredation by humans for nearly two decades has reduced the cheetahs numbers and the ecological assessments of the population reveal a collection of interacting factors that threaten the survival of this species. It is difficult to quantify the relative contributions of the differing factors influencing the decline of the cheetah. However, the complex problems facing the Namibian cheetah population as a result of human interference provides an example of the process of human-caused population declines. It is not yet known in what way the dental abnormalities described will impact on the cheetah's ecology, nor what the consequences will be in the long-term. For example, if cheetahs with dental abnormalities are slower in processing their kills, they may be at a distinct disadvantage in the wild; or what the implications of the physical defects, such as perforated palates as seen in extreme cases of FPE, are for over-all health of individuals.

Being able to quantify abnormalities is important for the conservation of species as defects may have long-reaching affects on the survival of endangered species. Indicators of inbreeding, or genetic homozygosity, in particular populations of cheetahs would be extremely useful for biologists and veterinarians developing both captive breeding programs and conservation strategies for free-ranging populations. If morphological abnormalities, such as those discussed in this paper, are a localized problem, this could rapidly become widespread if the trait continues to be passed on. The high occurrence of FPE in free-ranging Namibian cheetahs was first reported in captive cheetahs and contines to be prevalent in the captive population, 90% of which are descends from Namibian founders. This reiterates the need to quantify these abnormalities in populations. The management of endangered species necessitates a close link between in situ and ex situ conservation activities.

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