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## The Cranial Morphology of The Black-Footed Ferret: A Comparison of Wild and Captive Specimens

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The cranial morphology of the black-footed ferret: A comparison of wild  
and captive specimens

by

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Bachelor of Science  
University of South Carolina, 2015

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## ABSTRACT

The black-footed ferret (*Mustela nigripes*), a North American mustelid species, was once found abundantly throughout the Midwest until extreme decline in prairie dogs, the black-footed ferrets primary food source, brought the species to near-extinction. This resulted in the development of the Black-Footed Ferret Recovery Program which included bringing the entire species into captivity with the intention of breeding the species back to sufficient numbers to allow for successful reintroduction. While in captivity, many components of the ferrets' health were accounted for, but the current study aims to address the effects that captivity may have had on the cranial morphology of the black-footed ferrets, a factor that has not been widely regarded in the species. Specifically, that wild and captive ferrets are significantly different in terms of cranial morphology, and that a relatively recent change in the captive diet helped to return to alleviate the effects of captive. For this study, 23 cranial measurements were taken on 271 black-footed ferret skulls and 53 close congener skulls. Skulls were separated based on sex and captivity status and compared for all measurements as well as principal components derived from the measurements. Results found that there were significant differences between captive and wild specimens, in some ways to a greater extent than between wild specimens and the congeners, and that a diet change in the captive specimens likely helped decrease some of these differences. Based on the results, it is

determined that captivity can cause unnatural cranial development and that diet likely has a major impact on cranial morphology.

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## CHAPTER 1

### INTRODUCTION

#### *Background on the Black-footed ferret*

The black-footed ferret (*Mustela nigripes*) is a member of the Mustelidae family that is endemic to North America (Hillman and Clark, 1980). These nocturnal carnivores were originally found throughout the central portion of the United States spanning states from Montana to New Mexico and were also found in the Canadian province of Saskatchewan and in parts of Mexico (Hillman and Clark, 1980). This range was defined by the range of the five species of prairie dogs (*Cynomys gunnisoni*, *C. leucurus*, *C. ludovicianus*, *C. mexicanus*, *C. parvidens*) which black-footed ferrets have evolved to become entirely dependent upon for both food and shelter (Campbell III et al., 1987; Hillman and Clark, 1980; Jachowski et al., 2011). Observations of wild ferrets have shown that not only do prairie dogs make-up 90% of the black-footed ferret's diet, but that black-footed ferrets also rely on burrows dug by prairie dogs for shelter and denning (Campbell III et al., 1987; Hillman and Clark, 1980; Jachowski et al., 2011). It is because of this dependent relationship that black-footed ferrets nearly went extinct during the 20<sup>th</sup> century.

First described by Audubon and Bachman (1851), the black-footed ferret was commonly found throughout the grasslands of the central United States during the 19<sup>th</sup> century and the early 20<sup>th</sup> century until prairie dog populations began to rapidly decline. It is estimated, that the total population of prairie dogs numbered around 5 billion at one

point, but in the 1880s, as ranchers began to settle the land inhabited by prairie dogs, they also began eradicating the animals under the pretense that prairie dogs competed with livestock for forage (Miller et al., 1996). Government support for the wide-scale poisoning of prairie dogs began after C.H. Merriam, director of the U.S. Biological Survey, released an inaccurate report claiming that the productive capacity of land was decreased by 50-75% because of the presence of prairie dogs (Merriam, 1902; Miller et al., 1996). In 1915, the federal government began funding prairie dog eradication programs, and within the next decade prairie dogs were being exterminated at a rate of millions per year (Miller et al., 1996). By 1960, estimates approximated that the prairie dog occupied only 2% of its original range (Anderson et al., 1986; Marsh, 1984). Due to its close ecological relationship with the rapidly disappearing prairie dog, black-footed ferrets suffered an equally precarious decline in population numbers.

In the 1960s, Black-footed ferrets were only regularly sighted in South Dakota, and even these sightings were infrequent (Clark, 1978). By 1964, black-footed ferrets located in Mellette County, South Dakota were the only observed members of the species and were assumed the last remaining population of black-footed ferrets (Clark, 1994; Henderson et al., 1974; Vargas et al., 1996). Nine of these animals were captured between 1971 and 1973 and were transported to the Fish and Wildlife Service's Patuxent Wildlife Research Center in Laurel, Md. for breeding (Lockhart et al., 2006). In 1974, the last wild ferret died and the species only existed at the Patuxent Wildlife Research Center. These captive ferrets were treated with a canine distemper vaccine after scientist deemed it safe for use following successful injections in domestic ferrets. However, the black-footed ferrets proved more susceptible to the vaccine and four of the nine ferrets

died as a result (Lockhart et al., 2006). The remaining ferrets were bred and produced two litters of five kits each. However, no kits survived more than a few days and the last animal of the group died in 1979 (Lockhart et al., 2006).

The species was assumed extinct until 1981 when a rancher's dog in Wyoming brought a ferret carcass to its owner. The rancher recognized the species and reported it to the Wyoming Fish and Game Department leading to the discovery of the last wild population of black-footed ferrets near Meeteetse, Wyoming (Forrest et al., 1988; Lockhart et al., 2006) This population of over 100 ferrets was monitored in the wild until 1984 when the U.S. Fish and Wildlife Service, as part of the Black-Footed Ferret Recovery Act originally developed in 1978, began capturing individuals for another captive breeding program (Lockhart et al., 2006; Miller et al., 1996). In 1987, what is believed to be the last wild ferret was captured resulting in a final captive population of seven males and eleven females (Ballou and Oakleaf, 1989; Wisely et al., 2002b). These animals were successfully bred and the husbandry proved so successful that starting in 1991, ferrets were reintroduced into the wild in Shirley Basin, Wyoming (Marinari et al., 2006).

During the early goings of the captive breeding program, the black-footed ferrets were fed a high calorie diet to maximize fecundity (Marinari et al., 2006; Santymire et al., 2010). This high calorie diet was a soft mixture of 60% commercial mink chow pellets and 40% skinned, eviscerated, headless ground rabbit or prairie dog (Marinari et al., 2006). A small amount of additives was also added to the diet to improve nutrition and a freshly killed hamster was provided to ferrets once a week (Marinari et al., 2006; Vargas and Anderson, 1999) In 1994, a report found that many of the nutrients present in

the “60/40 diet” provided to ferrets held at the Toronto Metro Zoo actually exceeded quantities found in the natural diet of black-footed ferrets; there was specific concern surrounding the amount of polyunsaturated fatty acids (Marinari et al., 2006). As a result of the report, the nutritional components of the diet were continuously altered during the mid- to late-1990s until the diet was entirely replaced by a new diet known as the “Toronto Zoo Diet” in 2000 (Marinari et al., 2006). This new diet (widely fed to captive carnivores throughout North America) is made of vitamin-supplemented, ground horsemeat and contains “no bones, cartilage, organs, skin or connective tissues” (De Rosa, 2014). It was preferred by ferret caretakers because of its convenience and ability to maintain acceptable reproductive health (Marinari et al., 2006). Although the “Toronto Zoo Diet” was better nutritionally for ferrets, previous research has found that it was likely the cause of increased calculus development in the oral cavities of black-footed ferrets (Antonelli et al., 2016).

In the wild, the black-footed ferret’s diet is almost entirely comprised of prairie dogs. Observations by both Campbell (1987) and Sheets (1972) of wild black-footed ferret scat found that around 90% of the diet was strictly prairie dog. Sheets (1972) also observed mice (species unidentified) in 26% of scat. In captive feeding trials, black-footed ferrets willingly ate thirteen-lined ground squirrels (*Spermophilus tridecemlineatus*), cottontail rabbits (*Sylvilagus floridanus*), deer mice (*Peromyscus spp.*), and various species of birds or insects (Hillman, 1968; Vargas and Anderson, 1996). However, feeding trials conducted by Vargas (1996) found that although black-footed ferrets did consume insects, lagomorphs, and other rodents, they prefer prairie dogs when

available suggesting that other animals are merely consumed as an alternative when prairie dogs are not available in the wild.

### *Oral Health*

The adult dental formula of the black-footed ferret is I3/3 C1/1 P3/3 M1/2 (Anderson et al., 1986). The incisors are primarily used to tear flesh, while the canines are used to hold prey and to assist in tearing flesh (Berkovitz and Poole, 1977; Miller et al., 1996). The premolars and molars are used for slicing flesh, as evidenced by both maxillary and mandibular pairs of carnassial teeth, P4 and M1 (Berkovitz and Poole, 1977). Black-footed ferret kits begin erupting deciduous teeth around 16 postnatal days. Deciduous teeth are usually fully erupted around 7 postnatal weeks and by 8 weeks, permanent teeth begin to erupt – this is approximately the time kits begin weaning. By week 12 the permanent teeth are fully erupted (Vargas and Anderson, 1996).

Periodontal disease is one of the most common health defects in captive animals (Watson, 1994). Extended presence of supragingival calculus can result in gingival recession that allows for the adhesion of subgingival calculus, calculus that develops below the free gingival margin (Niemiec, 2008a). Once below the free gingival margin, calculus can very easily disrupt the periodontium and lead to rise of periodontal disease, loosely defined as “any disorder that affects the tissues that surround or support the teeth” (Pihlstrom et al., 2005). Periodontal disease was found in 65.3% of domestic ferrets (*Mustela putorius*) in a recent report by Eroshin et al. (2010) and has been found to be a greater problem among captive black-footed ferrets than wild ones (Antonelli et al., 2016). Periodontal disease begins in the gingivitis stage, which is simply inflammation to

the gingival region. At this stage, with intervention, the effects of periodontal disease are reversible (Niemic, 2008b), but without intervention, periodontal disease progresses to the stage of periodontitis where irreversible damage to the periodontium is inflicted. Because the periodontium is responsible for the attachment of teeth, severe periodontitis may ultimately result in tooth loss (Niemic, 2008b).

### *Cranial Musculature and Morphology in the Black-Footed Ferret*

For the purpose of this current study, it is useful to describe cranial morphology in relation to the four jaw adductor muscles. These muscles - the masseter, temporalis, medial pterygoid, and lateral pterygoid – elevate the jaw and provide power for mastication along with the jaw abductors which function to open the jaw. The masseter originates both at the zygomatic arch and at the zygomatic bone, and attaches to the ramus of the mandible. Thus, increased size of any of the aforementioned regions would suggest greater area of attachment for the masseter and thus greater force generation. Interestingly, mustelids as a whole are considered to have generally weak masseter muscles, most obviously noted by a small zygomatic arch (Ewer, 1973). For this reason it has been previously hypothesized that in mustelids, the masseter may not be as important as other muscles for mastication (Lee and Mill, 2004).

The temporalis is a fan shaped muscles that originates along the temporal fossa and inserts as a tendon on the coronoid process of the mandible. As noted by Ewer (1973), in smaller mustelids the temporalis is actually enlarged in contrast to the diminished masseter. Lee and Mill (2004) hypothesized that this is likely due to the relatively large prey size of most mustelid species, and that in order to effectively deliver

a kill bite to the prey, smaller mustelids need quick and powerful contraction of the jaw. The black-footed ferret is certainly no exception to this, as it relies on this kill bite to dispatch of its prey the prairie dog, which is of comparable length and mass to the black-footed ferret. Morphologically, the larger temporalis is most evidenced in black-footed ferrets by the well-defined sagittal crest, which increases the area for the temporalis to originate (Miller et al., 1996). The skull is also broad in comparison to its length which allows for further attachment of temporalis musculature.

The medial and lateral pterygoids both attach to the mandible, but differ in many other regards. The medial pterygoid has origins on the maxilla and the sphenoid bone, whereas the lateral pterygoid originates on the greater sphenoid wing and on the sphenoid bone. The medial pterygoid runs vertically and assist with closing the jaw, while the lateral pterygoid actually runs horizontally and primarily functions to protract the mandible. The function of these muscles specifically in mustelids is not as well studied, likely because their roles in mastication are not as important as either the masseter or the temporalis. Yet, development of these muscles could affect cranial measurements particularly in regards to the mandible or to width measurements of the skull.

#### *Previous Studies of the Effects of Captivity*

Captive centers strive to provide animals with the most nutritious diets available, yet often times they overlook another key element of diet: mechanical properties.

Nutrition is valuable for health and development but improper mechanical properties can cause detrimental effects as well (Gawor et al., 2006; O'Regan and Kitchener, 2005).

Numerous reports have found that unnatural mechanical properties like the kinds found in

captive diets can lead to oral health problems (Fitch and Fagan, 1982; Haberstroh et al., 1984; King and Glover, 1945; O'Regan and Kitchener, 2005). Soft diets that lack abrasion have been found to lead to increased cases of calculus build up and periodontal disease in species-specific studies (Haberstroh et al., 1984) and in general surveys of various captive mammals (Gawor et al., 2006). Research on the subject has also extended to mustelids. King and Glover (1945) reported that altering the physical properties of a diet fed to domestic ferrets (*Mustela putorius*) changed the prevalence of calculus development. Ferrets fed soft diets supplemented with short pieces of horse rib bone experienced minimal calculus development, if any, yet all ferrets fed the same soft diet but without the additional rib bone, which allows for abrasive action with the surface of the teeth, developed calculus, in many cases in severe proportions.

Captivity has also been shown to cause abnormal effects to cranial shape and development, likely resulting from the unnatural softness of diets. Anomalies such as cranial thickening in baboons (*Papio spp.*) (Cordy, 1957), and decreased skull sizes in Indian rhinoceros (*Rhinoceros unicornis*) (Groves, 1982) have been observed in captive animals. More specifically, previous research has found a correlation between captive diets and cranial morphology (Duckler, 1997; Hartstone-Rose et al., 2014; Saragusty et al., 2014; Yamaguchi et al., 2009; Zuccarelli, 2004). The most comprehensive of these studies, conducted by Hartstone-Rose and colleagues (2014), found evidence that captivity dramatically altered the shape of the skull of lions (*Panthera leo*) and tigers (*Panthera tigris*) based on 3-dimensional analyses of forty-three landmarks. Results from that study found that captivity was actually a stronger driving factor for cranial morphology than even sex. The study also found that cranial morphological differences

in the large felids was due in most part to zygomatic arch breadth and biangular width, in relation to rostral length measurements (Hartstone-Rose et al., 2014).

### *Previous Studies of Captive Black-Footed Ferrets*

Black-footed ferrets have been the subject of numerous studies, mostly regarding their reproduction and disease prevention. One of the main goals of the Black-Footed Ferret Recovery Act was to restore the population size of the black-footed ferret. To this end, reproduction and disease prevention were rigorously tracked and studied, and rightly so. Studies on reproduction span a wide variety, covering topics from genetic diversity (Wisely et al., 2002a), courtship (Miller and Anderson, 1990), and age-dependent male fertility (Wolf et al., 2000), among other topics. Similarly, research into disease and the black-footed ferret has been thorough and groundbreaking, generally focusing on canine distemper (Carpenter et al., 1976; Williams et al., 1996; Williams et al., 1988) and sylvatic plague (Abbott et al., 2012; Antolin et al., 2002; Matchett et al., 2010; Rocke et al., 2008), as these have undoubtedly been the deadliest diseases in regards to black-footed ferrets. This research is of course extremely important to the black-footed ferret and its recovery and reintroduction, but further research into other areas of health, such as cranial morphology and oral health, especially as to how these areas are affected by captivity, will only serve to continue to improve the conservation efforts of this species.

A previous study concerning the effects of captivity on black-footed ferrets found that ferrets in captivity showed higher levels of oral health defects (Antonelli et al., 2016). This study analyzed both captive and wild ferrets for calculus build-up and periodontal disease. Results were significant in determining that both calculus and

periodontal disease appeared worse in captive animals than in wild animals. This paper also found that the specimens accessioned after the diet change in 2000 had increased calculus development compared to the specimens accessioned before 2000, and were significantly separated based on periodontal disease (Antonelli et al., 2016). This paper offered valuable insight into the effects of captivity on this species and the effectiveness of the diet change in regards to oral health.

### *Hypotheses*

**H1.** The main goal of this study is to determine if captivity affected the cranial morphology of black-footed ferrets. The present study aims to narrow the statement made by Wisely et al. (2002b) that “decreased [skull] sizes were likely a result of environmental conditions present in captivity”, to pinpoint diet composition as the exact reason for the morphological changes brought about in the captive specimens, and also to increase the number of cranial measurements to understand in greater depth how their skulls changed in size and shape. It is likely that physical dietary properties affect cranial morphology as Hartstone-Rose et al. (2014) found similar results in captive felids. Mechanical properties of the diet likely influenced the development of masticatory muscles, which then further resulted in the alteration of skull shape and size (Hartstone-Rose et al., 2014). Our hypothesis is that black-footed ferrets raised in captivity will have significantly different skull shapes and sizes compared to wild black-footed ferrets - especially in terms of zygomatic arch breadth, a key measurement in mastication - as a result of the unnaturally soft diets presented to captive ferrets.

**H2.** The second hypothesis of this study is that ferret specimens bred in captivity after the dietary shift in the year 2000 will have skull measurements closer to the measurements of wild specimens than captive specimens prior to the year 2000. The diet change in 2000 from the “60/40 diet” to the Toronto Zoo Diet was mainly implemented to increase the nutritional value of the diet. But the mechanical properties of the two diets have been minimally examined in regards to their effect on cranial morphology. We hypothesize that though nutrition was the focal point of the dietary change, the mechanical properties of the diet were improved as well to reflect a more natural texture.

**H3.** We also hypothesize that the extent of variation between the captive groups and the wild group will be equal to or greater than the extent of variation between the wild black-footed ferret and two of its closest congeners. *M. putorius* and *M. eversmannii* are closely related species to the black-footed ferret but observations have found cranial morphology differences between the species (Coues, 1877). We believe that captive black-footed ferrets will be less like wild black-footed ferrets by some metrics than at least one of the wild congeners.

**H4.** This study also aims to increase the understanding of the correlation between oral health and systemic health in mammals to include mustelids and to clearly define a relationship between periodontal disease and cranial morphology in black-footed ferrets. Multiple reports have found that periodontal disease can lead to complications with other health issues and can even lead to the development of pulmonary issues, cardiovascular problems and osteoporosis (Kuo et al., 2008). Unfortunately, almost all literature on the subject has focused on human patients and has not quantified cranial morphology to determine its relationship to oral health.

## CHAPTER 2

### METHODS

23 cranial measurements and oral health scores were assessed for 324 specimens, 314 from the National Museum of Natural History (specimens catalogued as USNM; Smithsonian, D.C.) and an additional 10 skulls were analyzed from the American Museum of Natural History (AMNH; NY). Of the 324 skulls, 271 are of the focal species *Mustela nigripes*, and the rest are its close wild congeners included as outgroup comparative species: 35 *M. putorius*, and 18 *M. eversmanni*. The *M. nigripes* specimens were divided into four categories based on time of death and captivity status: 1) wild specimens accessioned prior to the modern reintroduction campaign, 2) specimens kept in captivity prior to the Black-Footed Ferret Recovery Act, 3) captive bred specimens fed the 60/40 diet from 1985-2000 – “Recovery Phase I”, and 4) captive specimens fed the Toronto Zoo Diet from the breeding program from 2000 until the most recent accessions – “Recovery Phase II” (Table 2.1).

Table 2.1. Sample Specimen Distribution of the four Black-Footed Ferrets (*Mustela nigripes*) groups and the *M. putorius* and *P. eversmanii* specimens.

Group	Male	Female	Unknown*	Total
Wild	30	20	18	68
Early Captive	8	3	1	12
Recovery Phase I	53	58	46	157
Recovery Phase II	18	16	0	34
<i>M. putorius</i>	13	11	11	35
<i>M. eversmanii</i>	7	5	6	18
Total	129	113	82	324

\*Sex was determined by museum records, some of which were not recorded.

#### Oral Health Scores

Each skull was examined for the presence and severity of bone porosity around the gum line – an indication of periodontal disease. The skull was examined in six sections: maxillary right, maxillary left, maxillary anterior, mandibular right, mandibular left, and mandibular anterior. All sections were scored independently of other sections. Periodontal disease was scored in each dental section on a 0-2 scale developed from previous research (Table 2.2) (Kapoor et al., in review). The original scales, used to analyze larger (Pantherine) subjects, ranged from 0-5 but a 0-2 scale was determined to be more appropriate for the smaller *Mustela* specimens. Periodontal disease was scored based on relative observations of porosity on the skull (Figure 2.1).

Table 2.2. Periodontal Index.

PERIODONTITIS INDEX SCORE	OBSERVATIONS
0	The bone along the gum line has no visible porosity.
1	The bone along the gum line has noticeable porosity.
2	The bone along the gum line has excessive porosity OR porosity is noticeable at a substantial distance from the gum line OR tooth loss has occurred as a result of porosity.

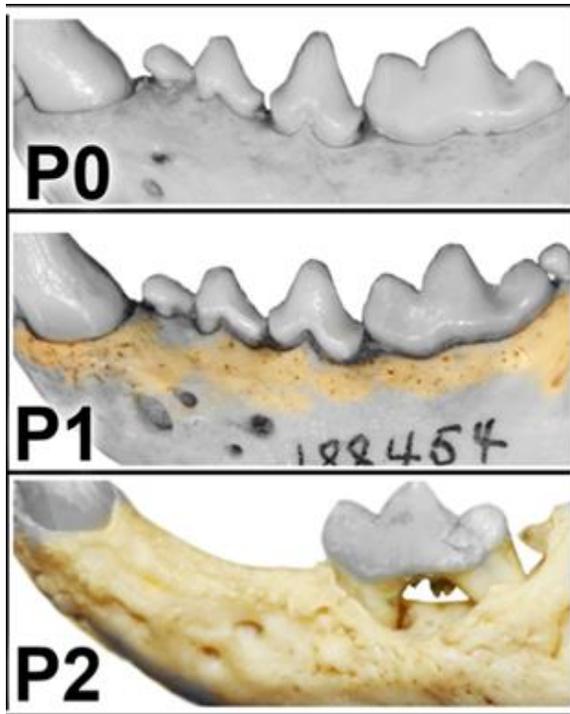


Figure 2.1. Visual Representation of our Periodontal Scales in Black-Footed Ferrets (*Mustela nigripes*). The P0 specimen is USNM A22427, P1 is USNM 188454, and P2 is USNM 592554. Taken from Antonelli et al. (2016).

### *Skull Measurements*

Skulls were measured for 23 different skull metrics, each of which is described in table 2.3. Fractured or damaged skulls were still used as long as one of the measurements could be derived without manipulation from damage. Measurements were taken using a handheld caliper and were directly loaded onto a spreadsheet in Microsoft Excel (Version 15.0, Microsoft, Corp.).

*Table 2.3. Description of Cranial Measurements*

<b>Cranial Measurement</b>	<b>Description</b>
<b>Total Length</b>	Inion to alveolare (prosthion)
<b>Basal Length</b>	Opisthion (inferior foramen magnum) to alveolare
<b>Total Height</b>	Inferior mandible to vertex
<b>Max Height</b>	Greatest dorsal-ventral height
<b>Total Width (Bizigion)</b>	Zygonion to zygonion
<b>Interorbital Constriction</b>	Supraorbitale to supraorbitale
<b>Post-orbital Constriction</b>	Cranial width at narrowest location just anterior to orbitals
<b>Bicoronion Width</b>	Coronion to Coronion
<b>Biangular Width</b>	Gonion to gonion
<b>Neurocranium Width</b>	Porion to porion
<b>Rostral Length</b>	Orbitale to alveolare
<b>Neurocranium Length</b>	Orbitale to inion
<b>Jaw Length</b>	Interdentale to condylare
<b>Coronoid Height</b>	Inferior mandible to coronion
<b>Condyle Height</b>	Inferior mandible to top of condylare
<b>Upper Canine AP</b>	Anteroposterior length of upper canine
<b>Upper Carnassial Length</b>	Anteroposterior length of upper carnassial
<b>Lower Carnassial Length</b>	Anteroposterior length of lower carnassial
<b>Palatal Length</b>	Orale to Staphylion

<b>Palatal Width</b>	Endomolare to endomolare
<b>Bicanine Width</b>	Distal surface of canine to distal surface of canine
<b>Temporal Fossa AP</b>	Anteroposterior length of the temporal fossa
<b>Temporal Fossa ML</b>	Mediolateral width of the temporal fossa

In the first analysis, only black-footed ferret specimens were compared. Specimens were divided between wild and captive specimens, which includes specimens from all three captive groups, and compared for all 23 cranial measurements and five principal components. The second analysis replicated the first, but with the captive specimens divided into the three separate captive groups: Early captive, recovery phase I and recovery phase II. The third analysis was conducted in the exact same manner of the second, but with the inclusion of the congener species, *M. putorius* and *M. eversmannii*. The final analysis compared periodontal disease scores to principal components, and only involved the recovery phase I and recovery phase II black-footed ferrets.

Because early observations found black-footed ferrets to be sexually dimorphic in terms of cranial measurements, separate analyses were conducted for male and female specimens and specimens of unknown sex were excluded for the first three analysis. Oral health scores and skull measurements were statistically analyzed using the program JMP (version 10.0.2, SAS Institute, Inc.). Variables were placed on “fit X-by-Y” graphs and analyzed using ANOVA testing when comparing two subsets. For analyses comparing more than two groups, an all pairs, Tukey-Kramer HSD analysis was used to determine variability of means. JMP was used to conduct PCA one-way analyses for all 23 measurements regarding captivity status. All results were considered statistically significant for alpha <0.05.

## CHAPTER 3

### RESULTS

For males, when comparing wild black-footed ferrets to captive black-footed ferrets for skull measurements, 12 of the 23 measurements were significantly different (Table 3.1) with basal height, coronoid height and condyle height, all being highly significant. Of the 12 differing measurements, the captive specimens had a lesser mean than the wild specimens for ten of the measurements, with the two exceptions being maximum height and biconion width.

Principal Component Analysis of the 23 measurements in males found that, as expected, PC1 was driven by overall size and accounted for a large portion of the variation in the data (50.248%). All measurements contained a positive value for PC1. PC1 was not significant (Table 3.1) in separating wild from captive specimens.

PC2 was highly significant in separating the wild ferrets from captive ferrets. PC2 was most prominently driven by condyle height (eigenvector = 0.53), post-orbital constriction (eigenvector = 0.50), and total height (eigenvector = 0.25). These measurements were in greatest contrast to biconion width (eigenvector = -0.27). PC2 contributed 9.69% of variation, which although greatly smaller than PC1, was still much larger than PC3 (5.62%) and all following principal components. No other principal components were significant in separating the wild and captive specimens.

Table 3.1. Comparative means based on ANOVA test of male captive and wild black-footed ferrets for each of the 23 cranial measurements and PC1-5.

<b>Measurement</b>	<b>Captive Mean</b>	<b>Captive Std.</b>	<b>Wild Mean</b>	<b>Wild Std.</b>	<b>P value</b>
<b>Total Length</b>	64.58	0.2884	64.9	0.4284	0.5452
<b>Basal Length</b>	62.32	0.2548	64.26	0.4058	<0.001***
<b>Total Height</b>	27.69	0.1413	28.35	0.2302	0.0157*
<b>Max Height</b>	31.67	0.1411	30.97	0.2299	0.0112*
<b>Total Width</b>	40.69	0.2277	41.55	0.3919	0.061
<b>Interorbital Constriction</b>	17.19	0.1074	17.47	0.1751	0.1756
<b>Post-orbital Constriction</b>	11.81	0.1293	12.6	0.2106	0.002**
<b>Bicoronion Width</b>	30.76	0.1718	29.97	0.2864	0.0195*
<b>Biangular Width</b>	30.93	0.1852	31.25	0.3032	0.365
<b>Neurocranium Width</b>	28.68	0.1677	28.83	0.2745	0.646
<b>Rostral Length</b>	17.85	0.0991	18.28	0.1604	0.0252*
<b>Neurocranium Length</b>	51.43	0.2702	51.07	0.4014	0.4498
<b>Jaw Length</b>	42.38	0.2332	42.98	0.3816	0.1807
<b>Coronoid Height</b>	19.72	0.1147	20.69	0.1902	<0.001***
<b>Condyle Height</b>	6.17	0.0724	7.37	0.1179	<0.001***
<b>Upper Canine Anteroposterior</b>	4.26	0.0301	4.25	0.0485	0.8747
<b>Upper Carnassial Length</b>	7.41	0.0374	7.44	0.0601	0.6421
<b>Lower Carnassial Length</b>	8.15	0.0483	8.35	0.0787	0.0373*
<b>Palatal Length</b>	31.9	0.1382	32.74	0.2238	0.0019**
<b>Palatal Width</b>	24.06	0.2106	24.99	0.3431	0.0231*
<b>Bicanine Width</b>	16.28	0.1106	16.74	0.1767	0.0311*
<b>Temporal Fossa Anteroposterior</b>	17.93	0.1294	17.68	0.2146	0.3269
<b>Temporal Fossa Mediolateral</b>	16.8	0.1256	17.12	0.2121	0.1944

<b>Principle Component 1</b>	-0.09	0.4385	0.85	0.6622	0.2404
<b>Principle Component 2</b>	-0.5	0.1531	1.38	0.2312	<0.001***
<b>Principle Component 3</b>	0.15	0.1529	-0.13	0.2308	0.3101
<b>Principle Component 4</b>	0.11	0.1348	-0.104	0.2035	0.3851
<b>Principle Component 5</b>	0	0.1314	-0.25	0.1984	0.2918

Females demonstrated greater division between the captive and wild specimens, with 15 of the 23 measurements showing significant difference (Table 3.2). In all 15 instances, the mean for the wild ferrets was greater than the mean for the captive ferrets. As with the males, basal length, coronoid height, and condyle height were all highly significant, but in the female sample, total width, rostral length, palatal length, and palatal width were also highly significant.

Again, PC1 was driven by overall size, with all eigenvectors returning positive values. Unlike males though, females differed highly based on PC1 which accounted for 52.43% of the variation within the sample. In addition to PC1, significance was also found for PC2 and PC3. Similar to males, PC2, which accounted for 7.76% of variation, was mainly driven by post-orbital constriction (eigenvector = 0.52), and condyle height (eigenvector = 0.47). PC2 was most inversely driven by upper canine AP (eigenvector = -0.27). The prominent measures for PC3 were upper canine AP (eigenvector = 0.46), lower carnassial length (eigenvector = 0.25), post-orbital constriction (eigenvector = -0.34) and bicoronion width (eigenvector = -0.45).

Table 3.2. Comparative means based on ANOVA test of female captive and wild black-footed ferrets for each of the 23 cranial measurements and PC1-5.

<b>Measurement</b>	<b>Captive Mean</b>	<b>Captive Std.</b>	<b>Wild Mean</b>	<b>Wild Std.</b>	<b>P value</b>
<b>Total Length</b>	59.89	0.267	60.85	0.425	0.0607
<b>Basal Length</b>	58.3	0.221	60.59	0.421	<0.001***
<b>Total Height</b>	25.59	0.14	26.34	0.272	0.0161*
<b>Max Height</b>	29	0.144	29.11	0.28	0.7277
<b>Total Width</b>	37.42	0.178	39.4	0.347	<0.001***
<b>Interorbital Constriction</b>	15.82	0.096	16.32	0.187	0.0199*
<b>Post-orbital Constriction</b>	11.33	0.121	12.1	0.234	0.0045**
<b>Bicoronion Width</b>	28.61	0.141	28.7	0.272	0.7601
<b>Biangular Width</b>	29.05	0.179	29.71	0.345	0.0922
<b>Neurocranium Width</b>	26.96	0.15	27.11	0.286	0.6574
<b>Rostral Length</b>	16.63	0.089	17.38	0.173	<0.001***
<b>Neurocranium Length</b>	48.07	0.274	48.53	0.436	0.3728
<b>Jaw Length</b>	39.1	0.164	40.04	0.32	0.0103*
<b>Coronoid Height</b>	17.89	0.098	19.44	0.191	<0.001***
<b>Condyle Height</b>	5.54	0.051	6.91	0.099	<0.001***
<b>Upper Canine Anteroposterior</b>	3.75	0.032	3.86	0.063	0.1282
<b>Upper Carnassial Length</b>	7.03	0.034	7.189	0.064	0.0352*
<b>Lower Carnassial Length</b>	7.53	0.042	7.78	0.08	0.0069**
<b>Palatal Length</b>	29.61	0.131	30.63	0.255	<0.001***
<b>Palatal Width</b>	22.61	0.209	24.48	0.407	<0.001***
<b>Bicanine Width</b>	14.78	0.08	15.24	0.154	0.009**
<b>Temporal Fossa Anteroposterior</b>	16.32	0.109	16.34	0.212	0.9075
<b>Temporal Fossa Mediolateral</b>	15.4	0.104	16.12	0.202	0.0019*

<b>Principle Component 1</b>	-0.52	0.465	2.51	0.7	<0.001***
<b>Principle Component 2</b>	-0.15	0.162	1.44	0.244	<0.001***
<b>Principle Component 3</b>	-0.17	0.172	0.88	0.259	0.0012**
<b>Principle Component 4</b>	-0.3	0.153	-0.07	0.23	0.4129
<b>Principle Component 5</b>	0.05	0.147	-0.02	0.221	0.782

Numerous differences arose between the four groups when comparing their mean scores for the cranial measurements and for the principal component analyses. Of the 23 measurements, significant differences were found between at least two groups in 19 of the measurements; the only measurements producing no distinction between groups were max height, neurocranium length, upper canine anteroposterior, and upper carnassial length. For the measurements that did find discrepancies between groups, recovery phase I and phase II differed on 16 measurements, recovery phase I and the wild group differed on 14 measurements, and recovery phase II and wild differed on 4 measurements (post-orbital constriction, bicoronion width, condyle height, and temporal fossa anteroposterior). The only measurement in which all three groups were significantly separated from each other was post-orbital constriction. The early captive group had no significant difference from the wild group in any of the 23 measurements but was different from the recovery phase I group in 12 measurements and differed from the recovery phase II group in 5 measurements.

Only principal components 1 and 2 found any distinction between the four groups. For principal component 1, both the wild group and recovery phase II group separated from the recovery phase I group while not separating from each other, but no other significant differences resulted for principal component 1 which, as previously

stated, accounts for skull size (Table 3.3). Principal component 2 could not distinguish the two recovery groups from each other but did distinguish both those groups from the early captive group and the wild group.

*Table 3.3. Connecting letters report for an All-pairs, Tukey comparative means test for males in the four black-footed ferret groups for all 23 measurements as well as the first five principal components.*

<b>Measurement</b>	<b>Early Captive</b>	<b>Recovery Phase I</b>	<b>Recovery Phase II</b>	<b>Wild</b>
<b>Total Length</b>	A B	B	A	A B
<b>Basal Length</b>	A	B	A	A
<b>Total Height</b>	A	C	B C	A B
<b>Max Height</b>	A	A	A	A
<b>Total Width</b>	A	B	A	A
<b>Interorbital Constriction</b>	A	B	A	A
<b>Post-orbital Constriction</b>	A B	B	C	A
<b>Bicoronion Width</b>	A B	A B	A	B
<b>Biangular Width</b>	A	B	A	A
<b>Neurocranium Width</b>	A	B	A	A B
<b>Rostral Length</b>	A	B	A	A
<b>Neurocranium Length</b>	A	A	A	A
<b>Jaw Length</b>	A B	B	A	A B
<b>Coronoid Height</b>	A	C	B	A B
<b>Condyle Height</b>	A	B	B	A
<b>Upper Canine Anteroposterior</b>	A	A	A	A
<b>Upper Carnassial Length</b>	A	A	A	A
<b>Lower Carnassial Length</b>	A B	B	A	A
<b>Palatal Length</b>	A	B	A	A
<b>Palatal Width</b>	A	B	A	A

<b>Bicanine Width</b>	A	B	A	A
<b>Temporal Fossa Anteroposterior</b>	B	B	A	B
<b>Temporal Fossa Mediolateral</b>	A B	B	A	A
<b>Principle Component 1</b>	A B	B	A	A
<b>Principle Component 2</b>	A	B	B	A
<b>Principle Component 3</b>	A	A	A	A
<b>Principle Component 4</b>	A	A	A	A
<b>Principle Component 5</b>	A	A	A	A

With females, results were not as diversified as with males, as only 14 measurements contained significant variations between means of any two groups (Table 3.4). The two recovery phase groups separated in 9 measurements, recovery phase I significantly separated from the wild group in 11 measurements, and recovery phase II separated from the wild group only 3 times. Coronoid height and condyle height were the only measurements that saw significant differences between all three groups, with recovery phase I having the lowest mean of the three and the wild group having the highest mean.

Discrepancies arose in each of the first four principal components. For PC1, recovery phase I distinguished from recovery phase II and the wild group. For both PC2 and PC3 the wild group was statistically different from both recovery groups which were indistinguishable from one another. Finally, in PC4, the recovery phase II group separated from the other two groups.

Table 3.4. Connecting letters report of an All-pairs, Tukey comparative means test for females in three\* of the four black-footed ferret groups for all 23 measurements as well as the first five principal components. \*The early captive group was excluded in this analysis due to small sample size (n=2).

<b>Measurement</b>	<b>Recovery Phase I</b>	<b>Recovery Phase II</b>	<b>Wild</b>
<b>Total Length</b>	A	A	A
<b>Basal Length</b>	B	B	A
<b>Total Height</b>	A	A	A
<b>Max Height</b>	A	A	A
<b>Total Width</b>	B	A	A
<b>Interorbital Constriction</b>	A	A	A
<b>Post-orbital Constriction</b>	A	A	A
<b>Bicoronion Width</b>	A	A	A
<b>Biangular Width</b>	B	A	A B
<b>Neurocranium Width</b>	B	A	A B
<b>Rostral Length</b>	B	A B	A
<b>Neurocranium Length</b>	A	A	A
<b>Jaw Length</b>	B	A B	A
<b>Coronoid Height</b>	C	B	A
<b>Condyle Height</b>	C	B	A
<b>Upper Canine Anteroposterior</b>	A	A	A
<b>Upper Carnassial Length</b>	A	A	A
<b>Lower Carnassial Length</b>	B	A B	A
<b>Palatal Length</b>	B	A B	A
<b>Palatal Width</b>	B	A	A
<b>Bicanine Width</b>	B	A	A
<b>Temporal Fossa Anteroposterior</b>	B	A	A B
<b>Temporal Fossa Mediolateral</b>	B	A	A

<b>Principle Component 1</b>	B	A	A
<b>Principle Component 2</b>	B	B	A
<b>Principle Component 3</b>	B	B	A
<b>Principle Component 4</b>	A	B	A
<b>Principle Component 5</b>	A	A	A

Results from the multivariate discriminate analysis indicate that the two recent captivity groups are more distinct from the wild group than one of the outgroup comparison species are to the wild group on a number of measurements. Based on figure 3.1, there is overlap between the *M. eversmanii* group and both the wild and early captive group. Based strictly off the canonical 1 axis, which is mostly driven by rostral length, condyle height, max height, and bicoronion width, *M. eversmanii* and the early captive groups are nearly identical with the wild group being less than one unit away. The *M. putorius* is easily the most distinct group sharing no overlap with any other groups and being the only group entirely scored below 0 for canonical 1. Based on the canonical 2 axis, which is driven extremely powerfully by basal length and inversely by neurocranium length, the *M. eversmanii* group actually scored between the two modern captive groups with the wild group scoring nearly two units higher than the *M. eversmanii* group. Both axes considered, the modern captive groups are clearly more separated from the wild group than the *M. eversmanii* group with the recovery phase I group being slightly more different from the wilds than the recovery phase II group.

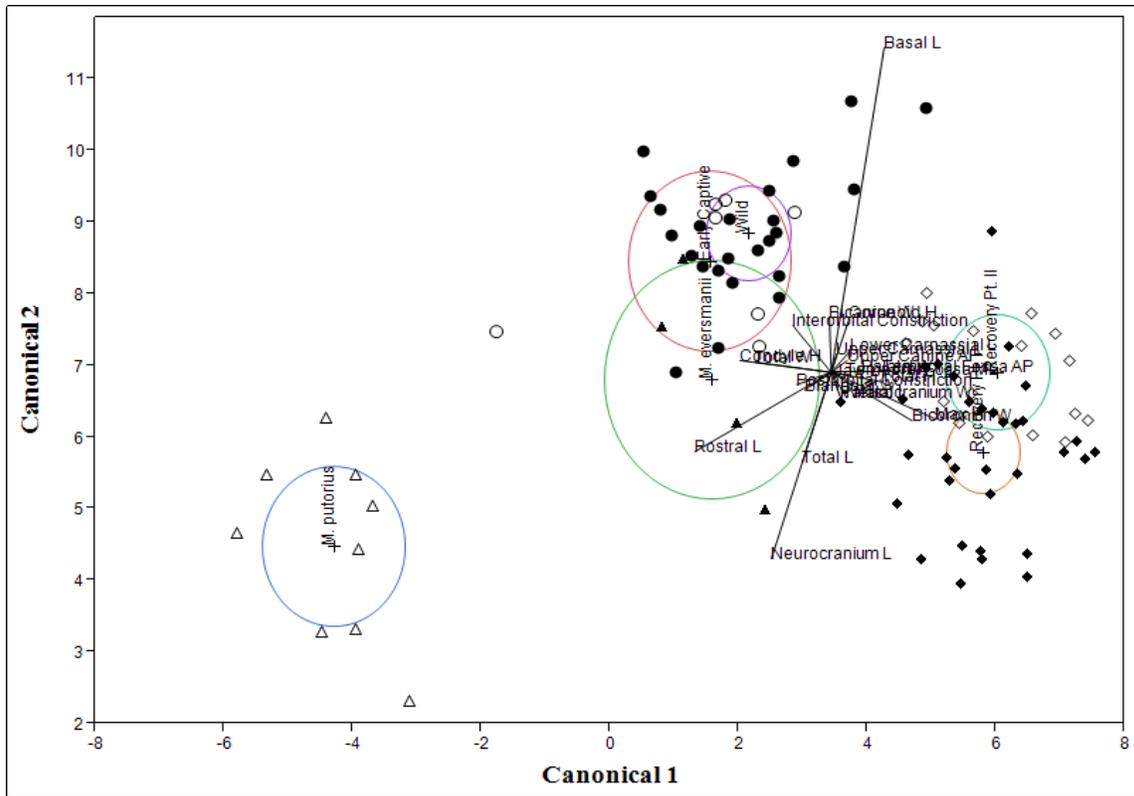


Figure 3.1. Canonical plot of discriminant analysis for males for all four black-footed ferret groups and the two comparative outgroup species *M. eversmanii* and *M. putorius*. Markers for the groups are as such, wild group - filled circle, early captive – empty circle, recovery phase I – filled diamond, recovery phase II – empty diamond, *M. eversmanii* – filled triangle, *M. putorius* – empty triangle.

The female group provides results that are slightly different than the results noted in the males. The biggest difference in the females group as compared to the male group is that there is no overlap between the *M. eversmanii* and wild group (Figure 3.2). These groups however, appear separated by a smaller measure than the wild group to the modern captive groups, particularly the recovery phase I group. Again, the *M. putorius* group is extremely distinctive from the other groups. One interesting observation is that the early captive group entirely encompasses the wild group, with the two groups sharing near identical positions on the canonical plot. In regards to both canonical 1 and 2 taken

individually, the wild group is located between the *M. evermannii* group and the modern captive groups.

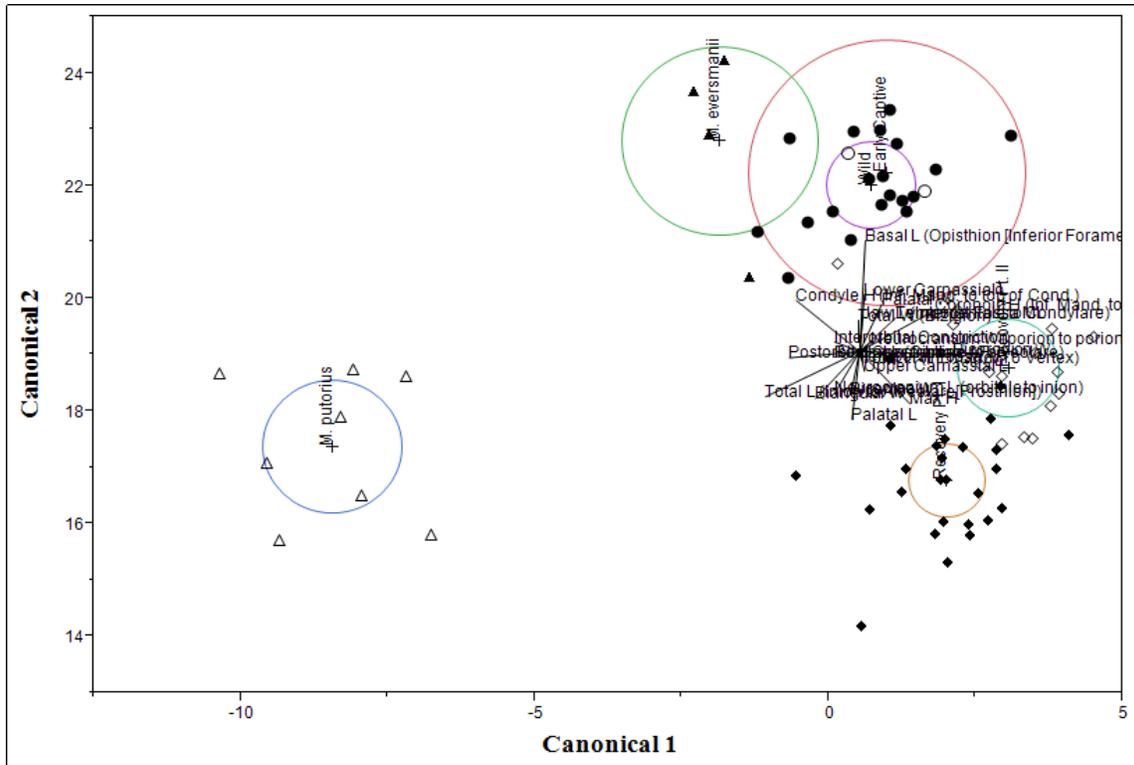


Figure 3.2. Canonical plot of discriminant analysis for females for all four black-footed ferret groups and the two comparative outgroup species *M. evermannii* and *M. putorius*. Markers for the groups are as such, wild group - filled circle, early captive – empty circle, recovery phase I – filled diamond, recovery phase II – empty diamond, *M. evermannii* – filled triangle, *M. putorius* – empty triangle.

When comparing cranial measurements to periodontal disease in captive black-footed ferrets, results suggest that there is a correlation between cranial shape and periodontal disease, but not cranial size and periodontal disease (Figure 3.3, Table 3.5, and Table 3.6). There were no discernable differences among any of the three possible maximum periodontal scores in terms of PC 1, a measure for overall size. Likewise, an orthogonal regression for periodontal mean scores and PC 1 found no significant correlation between the two variables. Contrary to PC 1, PC 2 did result in discrepancies

when observing both periodontal max scores and periodontal mean scores (Figure 3.3, Table 3.5, and Table 3.6). Ferrets with a max periodontal score of 2 were significantly different from ferrets with a max score of 1 or 0. In regards to periodontal mean, PC 2 value decreased significantly as mean increased with an absolute value correlation of 0.438.

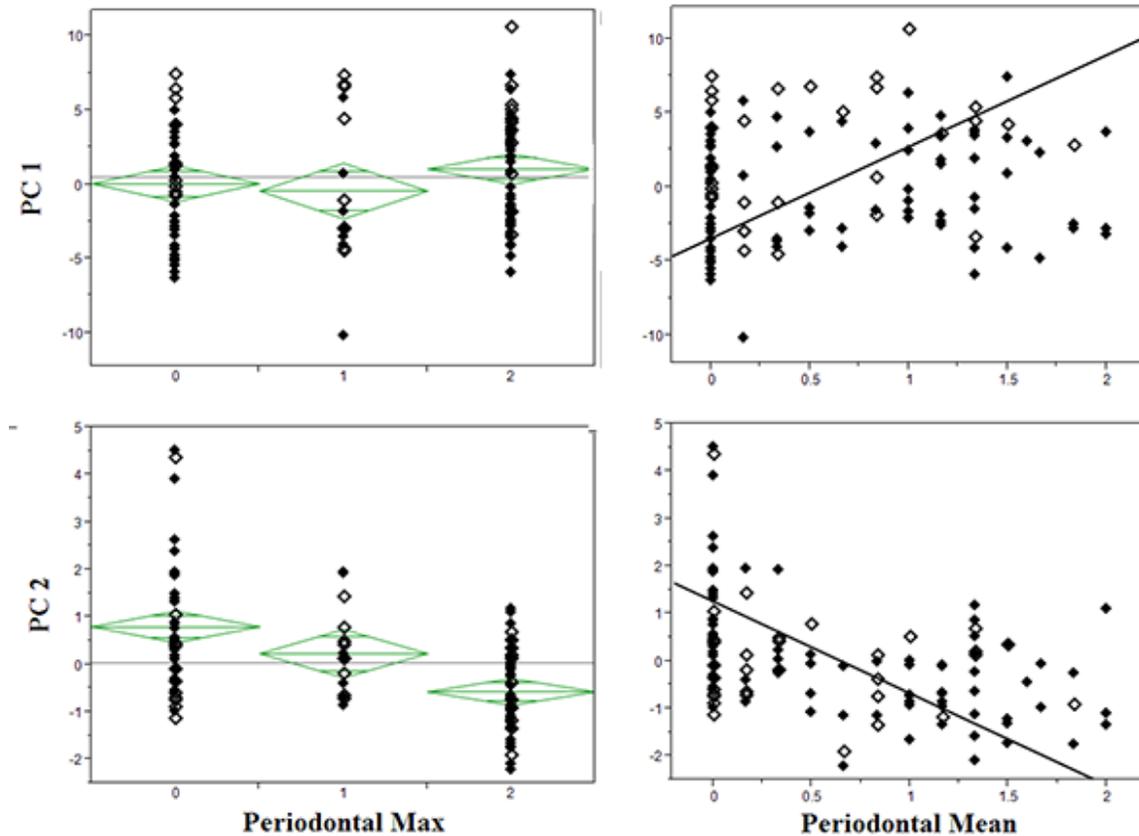


Figure 3.3. PC 1 and PC 2 as factors of periodontal max and periodontal mean scores among recovery phase I and phase II black-footed ferrets.

Table 3.5. Connecting letters and means report for the three possible periodontal max scores for both PC 1 and PC 2.

Periodontal Max	0	1	2
PC 1	A (0.064)	A (-0.430)	A (1.056)
PC 2	A (0.802)	A (0.238)	B (-0.568)

*Table 3.6. Orthogonal regression variables based on periodontal mean (X-axis) and principal component score (Y-axis).*

<b>Periodontal Mean</b>	<b>Slope</b>	<b>Intercept</b>	<b>[Correlation]</b>	<b>Lower CL*</b>	<b>Upper CL*</b>
<b>PC 1</b>	6.174	-3.421	0.103	N/A	N/A
<b>PC 2</b>	-1.939	1.264	0.438	-2.913	-1.29

## CHAPTER 4

### DISCUSSION

#### *Captivity and Cranial Morphology*

The results supported our main hypothesis that captivity would drive changes to the cranial morphology of black-footed ferrets. This is evidenced by the 12 of 23, and 15 of 23 measurements in males and females, respectively, that were significantly different in captive ferrets from wild ferrets. Perhaps the most interesting piece of data from this analysis though, was not that the specimens were different but rather what cranial features were driving the difference. Similar research by Hartstone-Rose et al. (2014) showed that in large felids, zygomatic arch width was the greatest cause for variation between captive and wild specimens. Anatomically, the zygomatic arch breadth makes sense when considering mastication because the masseter muscle originates partly on the zygomatic arch. Diets in captivity, though very similar to a natural diet in terms of nutrition, tend to lack the same structural components as a wild diet, usually being softer and less obdurate. As a result, less force is needed to chew food and the masseter muscle does not need to develop to such an extent as it would in the wild. The zygomatic arch breadth was significantly different in our study, but did not have as strong effect on dividing the wild and captive specimens as measures such as post-orbital constriction and condyle height. This is likely due to functional differences between felids and mustelids. As reported by Ewer (1973), the masseter tends not to play as critical a role in mustelids as it does in other carnivores. Thus, cranial measurements regarding areas affected by the

masseter (i.e. zygomatic arch breadth), will likely behave differently when comparing subsets of mustelid specimens as opposed to felid specimens.

Post-orbital constriction is one of the most noticeable cranial features distinguishing black-footed ferrets from other closely related mustelids (Coues, 1877). Observations noted that the constriction was extremely narrow as opposed to other mustelid species, and our personal observations confirmed this. Post-orbital constriction has long been studied in hominins because of its implication in both mastication and brain development. A narrower post-orbital constriction limits the growth of the frontal cortex while allowing for stronger mastication because of increased temporalis origin, and a wider post-orbital constriction allows for more frontal cortex development but may limit mastication abilities (Coues, 1877). With this in mind, it may initially appear as though the narrower post-orbital constriction in captives allowed them greater masticatory power. However, PC 1 data from females and various other cranial measurements in males suggests that captives were simply smaller overall, including post-orbital constriction. Although it cannot be entirely ruled out that the narrower post-orbital constriction in captives was from increased masticatory use, its influence in driving PC 2 differences is more likely an artifact of the generally smaller size of captive ferrets. Interestingly, it has been hypothesized previously that the captive black-footed ferrets had smaller brains so this also remains a possible explanation (Wisely et al., 2002b).

Condyle height was another cranial metric that largely influenced cranial shape in both male and female ferrets. Condyle height, which measures from the tip of the condylare to the inferior of the mandible, is generally believed to increase masticatory

force as it increases (Vinyard et al., 2001). Thus, the current findings are concurrent with our hypothesis that the unnaturally soft diet fed to captive ferrets likely influenced their cranial morphology. As condyle height increases, it presents more surface area for the attachment of both the masseters and the medial pterygoids. The shortening of the condyle height in captive ferrets likely arose due to poor masticatory muscle development during growth; previous research has also defended the hypothesis that diet and lifestyle can influence the cranial development in mustelids (Lee and Mill, 2004). Another possible explanation for this is simply that there was no competitive pressure on the captive animals. Since one of the main goals of the captive breeding program was to simply produce a high number of offspring, many birthed ferrets were maintained and possibly bred that would not have survived under wild circumstances. This could account for the smaller condyle height in captives, and a number of other measurements that were significantly smaller in captives. If this were the case, more naturalistic skull measurements would be observed in future generations as the ferrets are reintroduced to the wild.

Results including the outgroup species indicate that not only were there significant differences between the modern captive black-footed ferrets and the wild black-footed ferrets, but that these differences were in some ways more pronounced than differences noted between congeners. When regarding these results, it is particularly important to note the relative closeness and even overlap (in the male specimens) of the wild group and the *M. eversmanii* group for the multivariate analysis (Figure 3.1, Figure 3.2). This is particularly important when compared to the distance existing between the wild group and the two modern captive groups. What this analysis demonstrates, is that

based on cranial metrics, the skulls of the wild and modern captive groups are actually more distinguishable than the skulls of the wild and *M. evermanni* groups. These observations help add perspective on truly how severe the cranial changes are between captive and wild specimens. In this case, captivity can be seen as a greater driving force for variation in cranial morphology than speciation, results that are not only of interest to the black-footed ferret, but also in the broad fields of conservational biology and zoology.

#### *Possible Effects of Diet on Cranial Morphology*

Perhaps some of the most intriguing results come from the comparison of the two modern captive groups, where significant differences in a number of measurements are apparent in both males and females. These results are certainly the strongest from this experiment in pinpointing diet as the main cause of cranial morphological changes, as the change in diet was perhaps the only major change in between the groups aside from time. But time does present an interesting alternative to diet in terms of driving cranial variation, particularly in these animals as they recover from a significant bottleneck. However, previous research has found that captive specimens after 1990 did not suffer from inbreeding depressions which could have been a major issue in cranial morphology (Wisely et al., 2002b). This is not to say that improvements in genetic diversity did not contribute to the improvement of cranial measurements in the ferrets, but just that it is likely not the only, or even the strongest, driving factor.

Another point of interest from the comparison of all four groups in males was that the early captives and wild specimens did not differ on any cranial measurements or principal components. While this information by itself is useful, it becomes increasingly

pertinent when compared with the modern captive groups, which did in fact vary with the wild group on a number of measurements and PCs. For this disparity among early captives and modern captives, two main possibilities arise. The first possibility is that a lack of dietary regulations among early captives may have actually helped captive ferrets maintain natural cranial morphologic shape. A majority of these specimens were accessioned from 1880 – 1930, likely before captivity centers strongly regulated nutrition and feeding practices. As such, zoos may have actually fed ferrets a diet more consistent with the textures and mechanical properties of the ferrets' natural diet. The other conceivable explanation for this phenomenon arises from the fact that most of the wild ferrets were accessioned around the same time as the early captive specimens – late 19<sup>th</sup> century and early 20<sup>th</sup> century. It is then entirely likely that arriving at the brink of extinction simply brought about drastic physical changes to the species during the 20<sup>th</sup> century. By breeding the species from a single population of only 7 individuals, evolutionary factors such as genetic drift or lack of genetic diversity would have been amplified resulting in ferrets that were significantly different than their ancestors. Not only time, but location may have driven this difference. The modern captive specimens were all descendant from Wyoming black-footed ferrets, whereas the wild black-footed ferrets included specimens from a more broad range where changes in diet (i.e. different species of prairie dog) could have been causing different characteristics in cranial morphology to arise. This is one setback that we simply cannot overcome due to the nature of this species. Studying a species that experienced near extinction provides excellent insight into that species and evolutionary biology as a whole, but it does also

present unique complications - in this case the lack of modern wild specimens as a comparison for the modern captives.

Another factor that we have considered for the current study, is the possible effects that general stress in captivity can have on animal health, including cranial morphology. Indeed, there is no shortage of research to deduce that indicators of stress are usually more prevalent in captive animals than in wild animals (Bayazit, 2009; Burgener et al., 2008; Morgan and Tromborg, 2007; Terio et al., 2004) and that this increased levels of stress can negatively impact the overall health of captive animals (Baker et al., 1998; Constable et al., 1998; Kilbourn et al., 2003). As such, it must be clarified that while the present study certainly aimed to focus on diet as the main cause of morphological changes, we recognize that other factors may too be involved in the differences discovered between captive and wild black-footed ferrets. It should thus be reiterated that the main hypothesis of this study was simply that a difference between captive and wild specimens exists, and indicting diet as the main cause is a more specific objective of the overarching study. Yet, based on the results of this study and strong supporting evidenced recorded in previous studies (Hartstone-Rose et al., 2014; Lieberman et al., 2004; O'Regan and Kitchener, 2005), we recognize other factors likely play a role in varying cranial morphology, but still consider diet as the primary cause for differences among captive and wild specimens.

#### *Cranial Morphology and Oral Health*

Further evidence that the captive diet could be affecting cranial morphology is the link discovered between oral health and cranial morphology. For this analysis, only

modern captive specimens were used, as only using captive specimens helped reduce any confounding effects between captivity and wild. Captive specimens were chosen as the analyzed group in place of wilds because they displayed a much broader range of oral health scores than the wilds. Interestingly, results found that while periodontal disease seemed to be driving a change in cranial morphology shape, noted for both periodontal max and periodontal mean, there was not a significant relationship between periodontal disease and cranial size, as represented by PC 1. This is concurrent with expected results in that we don't expect oral health to be worse in larger ferrets simply because they are larger. However, correlation between oral health and cranial shape makes sense when considering diet, since previous research has shown that diet is a likely cause for both higher oral health problems (Antonelli et al., 2016; Kapoor et al., in review) and abnormal cranial shape (Hartstone-Rose et al., 2014). Therefore, while this link may not be a truly direct link, it appears that both worsening of oral health and changes in cranial morphology are driven by a like factor.

### *Future Directions*

While this current study has provided valuable information on this exciting species, continued research would not only further benefit the black-footed ferret but could also increasing our understanding of other mammalian species. While observing the skulls of these specimens is useful in understanding the actions of mastication and how these could relate to cranial morphology, a look at live specimens could improve these observations and provide details previously unstudied. Future studies would look into the exact mechanisms of mastication in these animals as well as other behaviors that

could be effecting cranial morphology in captive specimens. For example, personal observations from John Ososky, someone who has worked intimately with these animals, found that male ferrets were prone to gnawing on enclosures, and so further observations of these actions and possible other actions seen in captive specimens not typical of wild specimens could present further explanations for the variation noted between captives and wilds.

The present observations of a relationship between oral health and cranial morphology, though exciting and relevant, are still rather preliminary and further investigation into this relationship could help improve the current understanding of cranial morphology. There are a number of factors that can drive cranial morphology, oral health appearing to be one of them to some degree. A study that is entirely devoted to observing this relationship could deduce just exactly to what extent oral health, and specifically periodontal disease, is driving cranial changes or even defects. This is a study that could also likely be easily applied to a range of mammals and even humans.

As previously mentioned, one of the difficulties of the current study was the lack of modern wild specimens. In order to better study a relationship with less of a temporal gap between wild and captive specimens, a duplicate study with the inclusion of modern, reintroduced specimens could be beneficial. Because modern specimens are all descendent of former captive specimens, we would not expect genetics to play a large role in varying the specimens. Thus, comparisons of captives to the new wild specimens would not only allow us to see how reintroduction has helped to improve or worsen cranial metrics compared to historical wild specimens, but would also allow a more controlled observation between wild and captive specimens.

### *Broad Impacts*

The present study has been productive in both expanding previously held knowledge and also ascertaining new results that push the understanding of cranial morphology and oral health. Wisely et al. (2002b) were the first to document cranial changes from captivity in the black-footed ferret. The current study furthered these findings with the inclusion of sixteen new cranial measurements as well as comparative outspecies groups to improve the knowledge of exactly in what ways these skulls were varying. In addition, captive ferrets were further divided into subgroups based on diet that allow us to observe the effects each specific diet had on black-footed ferrets and if there were adverse effects to changing the diet in 2000. This study found incredible results suggesting not only that diet can effect cranial morphology, but that the latter diet, preferred for its nutritional advantages, may actually have also improved animals' health in terms of cranial morphology, though not quite to a wild standards. Also, for the first time, correlation was found between periodontal disease and cranial morphology, providing greater understanding for the systemic effects of periodontal disease and the intertwined relationship between oral health and cranial function.

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