Using canine width to determine age in the Black-footed Ferret *Mustela nigripes*

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Abstract

The Black-footed Ferret *Mustela nigripes*, a carnivore indigenous to North America's Great Plains, provides an example of species management that uses intensive population monitoring. Age class determination, however, is difficult because juveniles are adult-size at the time of dispersal in the wild. Our objective was to evaluate the use of body mass and canine width in aging Black-footed Ferrets. We measured known-aged captive-bred individuals and validated the findings in an intensively monitored free-ranging population. Body mass could not be used to distinguish between juvenile and adult wild females, but could be used in wild and captive males, and in captive females. Canine width can be used to distinguish between juvenile and adult animals within sex. Canine width was similar between wild and captive individuals; therefore, results were grouped. For males, mean (± SE) canine width was smaller for juveniles (n = 40, 3.38 ± 0.04 mm, 95% confidence interval [CI] = 3.31–3.45 mm) than for adults (n = 33, 4.13 ± 0.06 mm, 95% CI = 4.01–4.24 mm). Similarly for females, canine width was smaller for juveniles (n = 49, 3.18 ± 0.04 mm, 95% CI = 3.11–3.25 mm) than for adults (n = 47, 3.66 ± 0.03 mm, 95% CI = 3.60–3.73 mm). Canine width changes with age apparently through recession of the gum-line and exposure of the tooth root. Therefore, visualisation of the canine tooth root may be a reliable indication of adulthood. Body mass may be inconsistent in the wild because of high variation in food availability. Canine width can allow age determination in the field, which will assist with the assessment of population dynamics of free-ranging Black-footed Ferrets and the success of recovery efforts.

Keywords: aging technique, body mass, Endangered, morphometry, Mustelidae, population management

Introduction

Monitoring is an essential component of successful wildlife management, yet effective surveillance can be challenging. For populations that are rare or otherwise at risk of extinction, detailed population information often helps management decisions (Kleiman 1989, Armstrong & Seddon 2008). In particular, a population's age structure provides clues about population growth and stability; yet this information can be challenging to collect (Bingham & Purchase 2003).

Animals can be aged with techniques that use cementum layers, tooth wear, gum recession and radiographs of the tooth's pulp cavity. Cementum, the calcified tissue that surrounds the dentine, is deposited seasonally, forming layers that vary based on nutritional intake (Morris 1978, Wittwer-Backofen *et al.* 2004). These layers have been used to age various carnivores, including canids (Arctic Fox *Alopex lagopus*, Bradley & Prins 1981; Red Fox *Vulpes vulpes*, Cavallini & Santini 1995) and mustelids (Sea Otter *Enhydra lutris*, Ryazanov & Klevazal 1991; Eurasian Otter *Lutra lutra*, Hauer *et al.* 2002). The technique's major disadvantages are: 1) a tooth must be removed from the animal (Johnson *et al.* 1987); 2) interpreting layers can be subject to observer bias (Bodkin *et al.* 1997); and 3) juveniles may be difficult to distinguish from adults (e.g. Fisher *Martes pennanti*, Strickland *et al.* 1982). Tooth wear can be used as an index of age in wild animals such as the Puma *Puma concolor* (Gay & Best 1996), wild Reindeer *Rangifer tarandus* (Klevazal & Sokolov 2004) and Eurasian Wild Pig *Sus scrofa* (Nahlik & Sandor 2003), but varying abrasiveness of diets could wear down teeth at different rates. Gum-line recession has been used to age the Puma (Laundre *et al.* 2000). Radiographs of teeth, which show the narrowing of the pulp cavity with age, have been used to age carnivores, including Eurasian Pine Marten *Martes martes*, Stone Marten *M. foina*, American Marten *M. americana* and Fisher (Johnson *et al.*

1987, Helldin 1997, Bingham & Purchase 2003). Measurements of the pulp width have been used to age carnivore carcasses, but the modified technique for live specimens requires a portable x-ray machine for fieldwork, and radiographic images can often be blurred by the mandibular bone, which may cause errors in the interpretation of radiographs (Helldin 1997).

In sexually dimorphic species, like mustelids, body weight and skeletal measures may be used to distinguish between the sexes (Moors 1984). Canine width has been used successfully to sex Sea Otter (Dayan *et al.* 1989a, Ryazanov & Maminov 1996), Virginia Opossum *Didelphis virginiana* (Patterson & Mead 2009), Bobcat *Lynx rufus* (Williams *et al.* 2011), European Badger *Meles meles* (Johnson & Macdonald 2001), American Marten (Belant *et al.* 2011), Pine Marten in Ireland, Stoat *Mustela erminea*, American Mink *M. vison* (Dayan & Simberloff 1994), Long-tailed Weasel *M. frenata* and Least Weasel *M. nivalis* (Dayan *et al.* 1989b); however, most often canine teeth are removed from the animal to do this, which may be too invasive, especially for carnivores. Recently, field methods have been established to age Galapagos Sea-lions *Zalophus wollebaeki* using both canine measurements and body mass (Jeglinski *et al.* 2010).

Wild populations at elevated risk of extirpation are often intensively monitored, yet managers must balance collection of the maximum amount of information using techniques not overly intrusive. The Black-footed Ferret *Mustela nigripes* provides an example of intensively monitored populations of conservation concern (Biggins *et al.* 1997). This species is endemic to North America, with its former range including the Great Plains from Canada to northern Mexico. Black-footed Ferrets are obligate predators of prairie-dogs *Cynomys* and, currently, about 830 Black-footed Ferrets live in the wild in a maximum of four self-sustaining populations (Gober 2009, Jachowski & Lockhart 2009). More recently, some of the reintroduction sites are requiring intensive disease management (Matchett *et al.*

2010). Site managers monitor Black-footed Ferret populations intensively, to determine population size and trends and to estimate the number of captive individuals needed to maintain a viable population (Biggins *et al.* 1993).

Black-footed Ferret monitoring involves spotlight surveys in the fall during juvenile dispersal (Biggins *et al.* 2006). Animals are captured and unmarked animals are tagged with passive integrative transponder chips (Avid Identification Systems, INC., Norco, CA, U.S.A.; see Fagerstone & Johns 1987, Stoneberg 1996). Age determination is difficult because Black-footed Ferrets are adult-sized by 95–100 days of age (Vargas & Anderson 1996) and during fall surveys, juveniles are approximately four months of age (Miller *et al.* 1996). For a species at risk of extinction, like the Black-footed Ferret, an accurate method of age determination would assist managers in estimating recruitment into the population, success of the reintroduction site, and ultimately, success of the recovery effort. Demographic data can: 1) predict the ability of animal populations to withstand perturbation, 2) suggest which segment of the population is most vulnerable to catastrophic events, and 3) suggest which segments contribute the most to population growth (Bingham & Purchase 2003). Accurate aging of individuals may provide reintroduction site managers with a useful tool to assess the age distribution of the wild populations.

Black-footed Ferrets are short-lived carnivores with an average generation length of < 2.3 years in the wild (Wisely *et al.* 2003); therefore, it is important to monitor juvenile survival and recruitment, reflecting this age class's role in the population growth rate (Grenier *et al.* 2007). Because both body weight and canine width have been successful at distinguishing between sexes in mustelids, our goal was to determine if these techniques could be applied to age Black-footed Ferrets. Specifically, our objective was to determine if measurements of body mass and canine width vary between sex, age class (juvenile versus adult) and site-types (captive versus wild) in known-aged Black-footed Ferrets.

Methods

Captive Black-footed Ferrets
The captive-born Black-footed Ferrets (n = 82) used in this study were part of a breeding and reintroduction programme and were anaesthetised for routine health examinations in the fall from 2002 to 2004. These Black-footed Ferrets were maintained at one breeding facility, the Smithsonian Conservation Biology Institute near Front Royal, Virginia, U.S.A. Individuals were maintained in enclosures 3.6 m wide × 6.0 m long × 4.0 m high, with a mulch substrate and nest-boxes filled with Alpha Dry® substrate (Shepherd Specialty Paper, Watertown, TN, U.S.A.). Lighting was provided both naturally (by skylights) and artificially (via fluorescent illumination) with a minimum of 25 foot-candles at the cage/enclosure base, regulated by automatic timers set to turn on artificial lights 15 min before sunrise and turn them off 15 min after sunset. Black-footed Ferrets were fed a Toronto Carnivore Diet (Milliken Meat Products; Scarborough, Ontario, Canada). Fresh water was provided *ad libitum*.

Wild-born Black-footed Ferrets
Wild Black-footed Ferrets (n = 87) were all wild-born and were being trapped during fall 2002 monitoring surveys at the Conata

Basin reintroduction site within the Buffalo Gap National Grasslands (43°46'N, 102°14'W) in south-west South Dakota, U.S.A. The Conata Basin encompasses 22,267 ha of mixed grass prairie with more than 5,290 ha of Black-tailed Prairie-dogs *Cynomys ludovicianus* in three sub-complexes: Agate (1,483 ha), Sage Creek (3,142 ha) and Heck Table (665 ha). Black-footed Ferret reintroduction occurred in 1996–1999. Sixty or more litters were documented annually from 2000 to 2008 and the site is considered self-sustaining (Jachowski & Lockhart 2009) but requires intensive disease management (dusting for fleas to reduce the incidence of Sylvatic plague *Yersinia pestis*). Trapping and immobilisation protocols followed Sheets (1972) and Kreeger (1998). Briefly, animals were cage-trapped at night and returned to the same location following examination and recovery from anaesthesia (usually within 1 hr of capture). Trapping and handling techniques followed guidelines of the Animal Care and Use Committee (1998) of the American Society of Mammalogists.

Animal handling
We measured the body mass and canine width on live, anaesthetised captive-born (n = 82; 31 males and 51 females) and wild-born (n = 87; 42 males and 45 females) Black-footed Ferrets. Each animal was handled to induce and maintain anaesthesia by inhalation of isoflurane gas (Kreeger 1998). Body mass was measured on a digital scale in grams (± 0.1 g). Maxillary canine width was measured on the right side by RMS using digital calipers (± 0.02 mm, Mitutuyo Corporation, Aurora, IL, U.S.A.) for all animals in this study. Canine width was measured as the width from the anterior to posterior edge of the tooth at the gum-line. Measurements were excluded when morphological structures were missing or damaged. Measurements were taken along with other body measurements and procedures; therefore, measurements were not repeated, thereby minimising duration of animal handling.

Data analysis
Data were analysed using Sigma Stat version 3.0 (SPSS Inc., Chicago, IL, U.S.A.). A Kolmogorov–Smirnov test was used to test for normality and the Levene median test for equal variance assumption testing. We used a two-way analysis of variance (ANOVA) to test for differences among age classes (juvenile versus adult animals); data also were partitioned by sex because Black-footed Ferrets are sexually dimorphic (Anderson *et al.* 1986). *A posteriori* comparisons of means between age classes were performed using the Tukey test. For non-normal data, Kruskal–Wallis one-way ANOVA was used with Dunn's pairwise multiple comparison procedure. Values are presented as mean ± 1 standard error (SE). For all analyses, $P < 0.05$ was considered significant.

We classified animals less than six months old as juveniles and those of six months and older as adults. We knew exact birth dates for captive Black-footed Ferrets, but not for wild animals. At the time of the fall survey (16–23 September 2002), the estimated age of wild-born juveniles was 105 ± 30 days, based on a 1 June date of birth (Biggins *et al.* 2006). Vargas & Anderson (1996) reported that females and males reached 95% of their adult body mass by 105 and 126 days of age, respectively, and permanent canines for both sexes were fully erupted around 63 days of age. With intensive fall surveying efforts, biologists in Conata Basin captured 203 Black-footed Ferrets from fall 2002

to fall 2003 (TML, unpublished data). The ages of wild-born Black-footed Ferrets are well documented because all trapped animals are tagged with transponder chips. Live-trapping of Black-footed Ferrets typically begins in early September when juveniles begin dispersal (Miller *et al.* 1996).

Results

Comparison of body mass between age, sex and site-type
Overall, mean body mass was less ($F_{1,152} = 27.03$; $P < 0.001$) for females (n = 93, 739.2 ± 12.0 g, range = 372–988 g) than for males (n = 63, 931.1 ± 25.6 g, range = 386–1,454 g). Wild-born male Black-footed Ferrets were heavier ($F_{1,59} = 10.22$; $P = 0.002$) than captive males (Table 1). Wild male juveniles weighed less ($H_1 = 13.39$; $P < 0.001$) than adults. For captive males, juveniles weighed less ($H_1 = 7.23$; $P = 0.007$; Table 1) than adults. For females, captive individuals were heavier ($H_1 = 8.69$; $P = 0.003$; Table 1) than wild ones. Within captive females, juveniles weighed less ($H_1 = 4.99$; $P = 0.025$) than adults; however, body mass of both age classes of wild females seemed similar ($P > 0.05$; Table 1).

Comparison of canine width between age, sex and site-type
Overall, males (n = 73, 3.72 ± 0.05 mm, range = 2.99–4.63 mm) had wider ($H_1 = 11.66$; $P < 0.001$) canine teeth than females (n = 96, 3.42 ± 0.03 mm, range = 2.74–4.23 mm). Within sex, site-type (wild vs. captive) did not seem to influence canine width ($P > 0.05$); therefore, these data were combined. For males, juveniles (n = 40, mean, 3.38 ± 0.04 mm; range = 2.99–3.82 mm, 95% CI = 3.31–3.45 mm) had a smaller canine width ($F_{1,69} = 109.56$; $P < 0.001$) than adults (n = 33, mean, 4.13 ± 0.06 mm; range = 3.47–4.63 mm, 95% CI = 4.01–4.24 mm). For females, juveniles (n = 49, mean, 3.18 ± 0.04 mm; range = 2.74–3.60 mm, 95% CI = 3.11–3.25 mm) had a smaller canine width ($F_{1,92} = 106.41$; $P < 0.001$) than adults (n = 47, mean, 3.66 ± 0.03 mm; range = 3.12–4.23 mm, 95% CI = 3.60–3.73 mm). Additionally, one distinguishing difference between juvenile and adult Black-footed Ferrets in both sexes was the recession of the gum-line exposing more of the canine tooth root in adults, which may be the reason for the widening of the canine with age and may be used to confirm age classes (Fig. 1).

Discussion

Aging Black-footed Ferrets can be difficult due to a rapid growth rate (adult size by about four months of age; Vargas &

Table 1. Mean (± SE) and range body mass (g) of male and female Black-footed Ferrets *Mustela nigripes* in different age classes and site-types.

Sex	Age class*	Site-type	Mean (g)	Range (g)
Male	Juvenile (*n* = 24)	Wild	928.2 ± 36.3	659–1,454
	Adult (*n* = 13)	Wild	1,103.9± 31.7	965–1,330
	Juvenile (*n* = 16)	Captive	762.4 ± 49.6	386–998
	Adult (*n* = 10)	Captive	983.4 ± 48.4	722–1,223
Female	Juvenile (*n* = 26)	Wild	730.8 ± 15.7	520–874
	Adult (*n* = 19)	Wild	715.1 ± 12.6	592–797
	Juvenile (*n* = 22)	Captive	684.5 ± 38.8	372–933
	Adult (*n* = 26)	Captive	811.6 ± 12.7	716–988

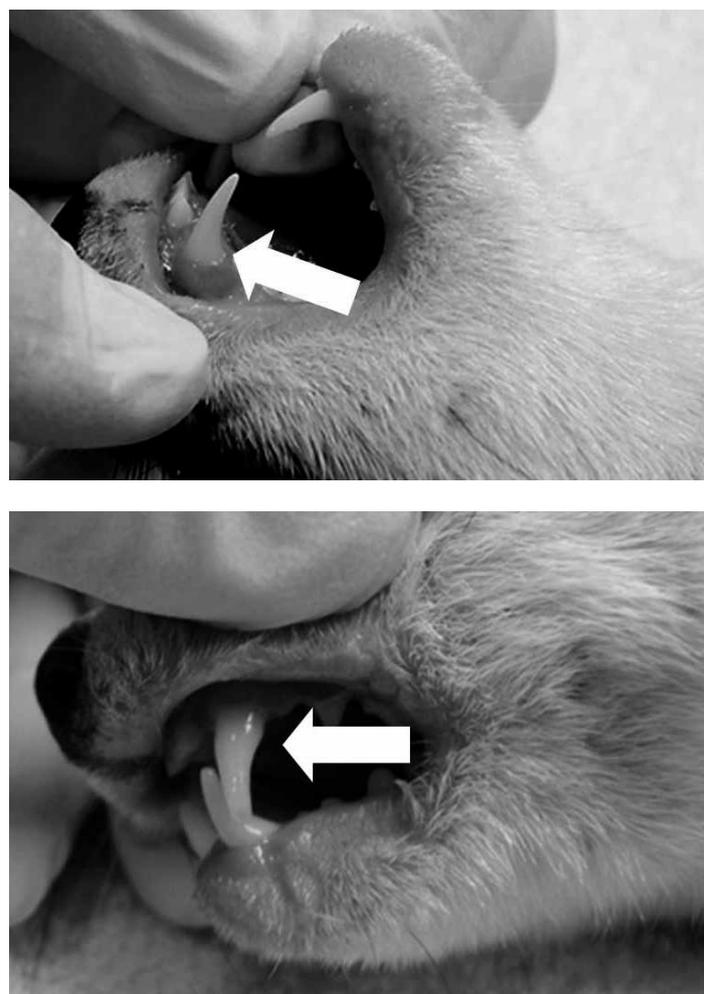*Number of individuals within age class in parentheses

Fig. 1. Juvenile (top; photo: Michael Forsberg) and adult (bottom; photo: Travis Livieri) Black-footed Ferret *Mustela nigripes* teeth. Arrows point to the receding gum-line in the adult exposing the tooth root, which can be used to distinguish between the age categories.

Anderson 1996) and weight fluctuations that can occur during the year depending on food availability. This study presents a method for classifying age of Black-footed Ferrets using canine width and recession of the gum-line exposing the tooth root. For both male and female Black-footed Ferrets, the width of the canine can distinguish between most juveniles and adults and was not influenced by site-type; however, there was some overlap between age classes. Therefore, the use of canine width as an aging technique may need to be corroborated with the obvious exposure of canine tooth root and/or other biological indicators, such as mammary development in females. Additionally, body mass may not be an accurate age classification technique in wild and captive Black-footed Ferrets. The large variation and overlap among age classes in body mass was probably due to variable food availability and nutrition. Additionally, in captivity, births may be more spread out across the spring months due to housing conditions.

Our body mass data were consistent with previous assessments of sexual dimorphism in Black-footed Ferrets: Anderson *et al*. (1986) studied Black-footed Ferret museum specimens and determined females on average were 68% of males' body weight, whilst Vargas & Anderson (1996) studied captive, live Black-footed Ferrets and found males weighing

approximately 25–30% more than females. We determined that there was a greater body mass dimorphism in wild animals (females were 73% of males' weight) than in captives (females were 89% of males' weight). Many mustelids are highly sexually dimorphic in body size (Moors 1980, 1984). This size difference may have evolved for sexual selection (Thom *et al.* 2004) or for niche separation (Darwin 1871), suggesting larger males would have the ability to catch larger prey and reducing intra-specific competition for food (Dayan *et al.* 1989a, Hedrick & Temeles 1989, Dayan & Simberloff 1996). Additionally, females are smaller, thus, reducing energy requirements which may allow for more energy to be used for reproduction (Moors 1980). Reduced sexual dimorphism in Black-footed Ferrets may be an effect of the captive environment (Wisely *et al.* 2005). In American Mink, captivity was determined to lessen sexual selection, reduce resource competition and select the larger specimens of both sexes for breeding within ranch populations (Lynch & Hayden 1995).

Previous studies have used morphometric features to sex animals. Anderson *et al.* (1986) were able to sex Black-footed Ferrets accurately using skull measurements 89.4% of the time. Our results demonstrate that the canine width of live, anaesthetised Black-footed Ferrets is also sexually dimorphic. Overall, male canines tended to be about 9.2% larger than female canines. Site-type did not influence canine width, consistent with previous studies (Wisely *et al.* 2005). Our study was designed to differentiate between adults and juveniles during fall monitoring surveys. Differences may change if measurements are taken at other times of year. In males, 21% adults (7 of 33) overlapped with juveniles and 38% (18 of 47) adult females overlapped with juveniles. For animals within the range of overlap, secondary attributes, such as mammary development and/or lactation in females, may help. A definite distinction between the age classes was the gum-line recession, exposing the canine tooth root. This root exposure caused the increasing width of the canine tooth; thus, it can be used to confirm the width measurements.

Conclusion

Determining the age of wild-born animals is important for identifying age-specific estimates of population parameters. Using canine width to age individuals has the potential to be applied to similar wild species, particularly if a captive population is available to validate parameters. Animals maintained in zoological institutions could be used as models for the wild counterparts, although mean canine width with an estimate of precision (e.g. 95% confidence intervals) would need to be established for both wild and captive populations. These results highlight the importance of validating data to determine the reliability of extending inferences from captive to wild populations. As the Black-footed Ferret recovery enters its thirtieth year after the rediscovery of the species (1981) and the twentieth year of returning it to the wild (1991), understanding the population dynamics and what factors are affecting population growth will lead to improved success of the programme.

Acknowledgements

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