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Environment influences morphology and development for *in situ* and *ex situ* populations of the black-footed ferret (*Mustela nigripes*)

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Abstract

For selected species, conservation breeding has become integrated into recovery plans, most often through the production of offspring for reintroduction into nature. As these programs increase in size and scope, it is imperative that conservation managers retain the biological integrity of the species. This study investigated the causes of morphological changes that are known to occur in black-footed ferrets (*Mustela nigripes*) maintained *ex situ*. In a previous study, ferrets maintained in captivity were 5–10% smaller in body size than pre-captive, *in situ* animals. In the present study, the authors compared nine morphological characters among *ex situ* animals and their *in situ* descendants. Within the *ex situ* population, cage types were compared to determine whether housing influenced morphometry. Black-footed ferrets born to reintroduced individuals quickly returned to their pre-captive size suggesting that a diminutive morphology *ex situ* did not have a genetic basis. Furthermore, cage type affected overall body size and shape; ulnas and tibias were as much as 9% shorter for *ex situ* animals. The authors hypothesise that small cage size and environmental homogeneity inhibit the mechanical stimuli necessary for long bone development. These findings have ramifications for *ex situ* managers who need to create artificial captive settings that promote natural physical development. In the absence of such an environment, 'unnatural' morphologies can result that may contribute to poor fitness or perhaps even domestication.

INTRODUCTION

Conservation breeding is a powerful tool for preserving endangered species (Ebenhard, 1995). Candidates for this drastic conservation measure are taxa whose *in situ* populations are in eminent risk of extinction due to anthropogenic disturbance (Snyder *et al*., 1996). Typically, some or all of the remaining individuals of a species are taken into captivity with the goal of reinforcing small isolated *in situ* populations or reintroducing animals to previously extirpated regions once a sufficient number of animals have been produced in captivity (Soulé *et al*., 1986). Because healthy, self-sustaining reintroduced populations are the ultimate goal of conservation breeding (Ebenhard, 1995), it is imperative to understand how the captive environment affects reintroduced individuals. Very little scientific information is available on the influence of captivity on the behavioural, physiological and morphological fitness of individuals destined for reintroduction.

Conservation breeding is not a panacea; serious risks to species' viability exist both *in situ* and *ex situ* (Snyder *et al*., 1996). For example, some taxa such as the giant panda (*Ailuropoda melanoleuca*) are difficult to breed in captivity and self-sustaining *ex situ* populations have, consequently, been difficult to establish (Wildt *et al*., 2003), which creates the potential to diminish the global population of a species (Conway, 1986). Because animals in the *ex situ* environment are often at higher densities than *in situ*, the risk of disease epidemics (e.g. cheetahs (*Acinonyx jubatus*) and feline infectious peritonitis: Evermann *et al*., 1983) and other catastrophic events are also a significant threat to *ex situ* populations (Jacobson, 1993). Other species, such as California condors (*Gymnogyps californianus*) are difficult to reintroduce back in the wild,

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because *ex situ* animals are naïve to potential threats (Meretsky *et al*., 2000).

Some risks have a genetic basis. All species are subject to the loss of genetic diversity as successive generations are produced in captivity (Lande & Barrowclough, 1987; Lacy, 1994). Although conservation-breeding programs focus on retaining the maximal genetic variation, incremental loss each generation is inevitable (Ballou & Foose, 1996). The loss of rare alleles and increase in homozygosity may lead to a decrease in fitness (inbreeding depression), further inhibiting breeding efforts in captivity (Ralls & Ballou, 1983) or decreasing the chance for success in the wild.

Another genetic risk associated with captive breeding is the domestication of wildlife due to unintentional selection. Heritable changes to morphology, behaviour and physiology have all been documented in captive wildlife, yet these adaptations to the captive environment are seldom beneficial in the wild (Frankham *et al*., 1986). Morphological domestication includes shortened facial features (Zeuner, 1963), decreased brain size (Kruska, 1996) and smaller body size (Frankham *et al*., 1986). These changes can occur in a few generations in spite of efforts to minimise such forces, because the intensity of unintentional selection can be very strong. For example, estimated selection intensity was 10–20 times greater for captive tigers (*Panthera tigris altaica*) than for an idealised free-ranging population (Flesness & Cronquist-Jones, 1987), which would create ample opportunity for rapid morphological change.

Few studies have verified phenotypic change as a result of conservation breeding. A study of museum specimens, however, documented morphological differences between historical populations of *in situ* black-footed ferrets (*Mustela nigripes*) collected prior to captive breeding and black-footed ferrets maintained in captivity (Wisely, Ososky & Buskirk, 2002*a*). The study suggested that skull size decreased and skull shape changed once this species was brought into captivity, but whether the causes were environmental or genetic was undeterminable. In this paper, we compared morphology between captive black-footed ferrets and their *in situ* descendants to determine the basis of these changes. If morphometric changes were heritable, we predict that *in situ* individuals would remain the same size and shape as *ex situ* individuals; however, if morphometeric changes were due to environmental factors, *in situ* individuals would return to the same size and shape as pre-captive ancestors. Because *ex situ* individuals were raised in two types of enclosures, we examined whether enclosure size affected morphology of *ex situ* animals.

The history and status of the black-footed ferret has been reviewed elsewhere (Seal *et al*., 1989; Clark, 1994; Dobson & Lyles, 2000). Briefly, all individuals comprising the black-footed ferret recovery program are descendants of 8–11 founders (the unknown paternity of some wild-caught litters created some uncertainty as to the exact number) and have been bred in captivity for 18 years (7.4 generations with 2.3 years generation time: Wisely, McDonald & Buskirk, 2003). Genetic variability is limited (Wisely *et al*., 2002*b*) and inbreeding has increased over time (Wisely *et al*., 2003), but the rate of loss has remained below the goal set by the American Zoo and Aquarium Association's Species Survival Plan (SSP) due, in part, to the mean kinship strategy used for genetic management (Wisely *et al*., 2003). Medium-sized carnivores comprise 13% of all SSP species subject to conservation breeding (see http://www.aza.org/ConScience/ConScienceSSPList/) and many of these captive populations are small with vulnerably low amounts of genetic diversity. The black-footed ferret provides a model for understanding the implications of *ex situ* conservation and the possible ramifications to *in situ* recovery.

METHODS

Animal handling

We measured nine morphological characters (defined in Table 1, below) on 211 black-footed ferrets ($n = 77$ juveniles, $n = 134$ adults) that were living in captivity ($n = 129$) or in the wild ($n = 82$) between September 2002 and October 2003. For the health and safety of anaesthetised animals, we chose morphological traits that minimised their manipulation. To increase measurement accuracy, we chose morphological traits that minimised the amount of soft tissue measured. Each animal was handled to induce and maintain anaesthesia by inhalation of isoflurane gas. Morphological characters were measured on each animal's right side by RMS using digital calipers ($\pm$ 0.02 mm, Mitutuyo Corporation, Japan). Because we wanted to minimise the duration of animal handling, measurements were not repeated. We excluded individuals from analysis when morphological structures (exclusively dental traits) were missing or damaged ($n = 41$).

Ex situ animals were measured during routine health examinations; these animals were part of a captive-breeding/reintroduction program. Data on juvenile black-footed ferrets were exclusively collected from the National Zoological Park's Conservation & Research Center (CRC, Front Royal, Virginia). Data on adults were collected from six captive-breeding facilities (National Black-Footed Ferret Conservation Center, Wheatland, Wyoming; Cheyenne Mountain Zoo, Colorado Springs, Colorado; Phoenix Zoo, Phoenix, Arizona; Louisville Zoo, Louisville, Kentucky; Toronto Zoo, Toronto, Ontario; and CRC, Front Royal, Virginia). Animals were raised in two types of enclosures. At CRC, enclosures were $6 \times 3 \times 3.6$ m or $6 \times 6 \times 3.6$ m pens that included a nest box on a mulch covered floor. Some pens were outdoors and others were indoors with skylights supplemented with artificial light. All other facilities held ferrets in smaller enclosures ($1.2 \times 2.4 \times 0.9$ m) with two nest boxes connected by a flexible tube.

We evaluated *in situ* black-footed ferrets during autumn or spring monitoring surveys at the Conata Basin/Badlands reintroduction site in the Buffalo Gap National Grasslands, South Dakota. All *in situ* animals measured for this study were born in the wild. We

chose to compare this *in situ* population to the *ex situ* population because it was a large, self-sustaining population ($n > 200$) that had genetic diversity equivalent to that of the *ex situ* population (Wisely *et al*., 2003). Because genetic diversity was equivalent between *in situ* and *ex situ* populations, we could exclude differences in genetic diversity, overdominance, inbreeding or inbreeding depression as a possible cause for differences in body size. Founders of the Conata Basin population were representative of the captive-breeding population (Wisely *et al*., 2003).

The Conata Basin/Badlands population was established in 1996, then supplemented with *ex situ* individuals from 1997 to 1999 and exponential population growth was documented through 2003 (Conservation Breeding Specialist Group, 2003). Trapping and immobilisation protocols for the black-footed ferret are well established (Sheets, 1972; Kreeger *et al*., 1998). Animals were cage-trapped at night and returned to the same trap location following examination and recovery from anaesthesia. All trapping was authorised, coordinated and conducted by the U.S. Fish and Wildlife Service's Black-Footed Ferret Recovery Team as part of routine population monitoring for reintroduction sites.

Statistical Analysis

We conducted separate analyses for juvenile and adult animals. All captive-reared juveniles used in this study came from one location, CRC, and were measured between September 2002 and October 2003. At the time of the autumn *in situ* survey (16–23 September, 2002) we assumed the age of wild-born juveniles to be 75–135 (mean = 105) days, based on an average estimated June 1st date of birth (T. Livieri, unpublished data). We compared these wild-born individuals to captive individuals that were 104.0 ± 5.7 (mean $\pm$ SE) days old for females and 109.3 ± 4.5 days old for males. Although Vargas & Anderson (1996) reported that females reached 95% of their adult body mass by 105 days of age and males reached 95% of their adult body mass by 126 days of age, our pilot studies revealed that morphologically, individuals <135 days of age were smaller than the average adult size. Thus, *ex situ* animals were considered to be adults when >135 days old. Extensive monitoring since the year of the first release included marking animals, which helped us confirm the juvenile status of small, unmarked animals. We assumed all *in situ* animals to be adults at the time of the spring *in situ* survey (24–30 March 2003). Two individuals that were captured in the autumn survey were recaptured in the spring; autumn and spring measurements were used in separate analyses of juvenile and adult body size.

We tested for normality of the distribution of data for all nine morphological characters using the Kolmogorov–Schmirnov test; we corrected for experiment-wise error due to multiple univariate testing with a sequential Bonferroni adjustment (Rice, 1989). We excluded the distance from the second to the fourth mandibular premolars (P_2 to P_4) from adult analyses because many wild adults had missing or damaged P_2 teeth. Overall differences in morphological characters between *ex situ* and *in situ* animals were compared using a multivariate analysis of variance (MANOVA) with sex and location as fixed factors. For adults, location was divided into three treatments: large *ex situ* enclosures at CRC, small *ex situ* enclosures at all other breeding facilities and South Dakota where animals were born *in situ*. We used Tukey's Honestly Significant Difference to test for post-hoc differences between locations.

To test the hypothesis that *ex situ* animals were a different shape and size than *in situ* animals, we reduced the variables to two principal components. Other morphological studies (e.g. Fleischer & Murphy, 1992; Komers & Komers, 1992) including one for this species (Wisely *et al*., 2002*a*) inferred the first principal component to represent body size and the second principal component to be consistent with body shape. We analysed the first two principal components using a MANOVA with sex and location as fixed factors. We conducted separate analyses for juveniles and adults. All tests were conducted at $\alpha = 0.05$.

RESULTS

Of the nine morphological characters only tibia length was not normally distributed. Visual inspection of the distribution of values revealed a bimodal distribution influenced by sexual dimorphism. When each sex was tested for normality separately, the distribution was not significantly different from normal (male: Kolmogorov–Schmirnov $Z = 0.84$, $P = 0.5$; female: $Z = 0.61$, $P = 0.9$). This measure was included, untransformed, in further analyses.

In all multivariate analyses, males were significantly larger than females; test results are not presented because black-footed ferrets are known to be sexually dimorphic in body size (Anderson *et al*., 1986). In juveniles, four characters were significantly different for animals born at CRC and wild-born individuals (Table 1). Tail length, ulna and tibia length were significantly smaller in the *ex situ* group, however the distance between mandibular canines was significantly larger in this group (Table 1).

In adults, we found no difference in tibia length between animals born at CRC or in the wild, but animals from captive-breeding facilities other than CRC had significantly shorter tibias (Table 2). Compared to the *in situ* group, ulna length was shorter for ferrets at all captive locations, including CRC, and upper canine width was smaller in CRC-housed animals compared to captive animals at other facilities or in the wild-born group (Table 2). We found a significant interaction effect between sex and location for long bone measurements (Fig. 1); the difference in size between males in the wild and males in captivity was greater than between females.

For the juvenile data set, PC1 had an eigenvalue of 5.4 and explained 61% of the variance. PC2 had an eigenvalue of 1.0 and explained 11% of the variance. Trends in eigenvector character loadings for PC1 and

Table 1. Mean ± SE (in mm) for nine morphometric measurements from 77 juvenile black-footed ferrets (*Mustela nigripes*)

	UCC	LCC[a]	IW	NP	P24	TA[b]	UCW	RL[c]	FA[d]
Females									
Captive ($n = 14$)	10.2 ± 0.08	7.1 ± 0.16	6.8 ± 0.05	10.5 ± 0.11	8.9 ± 0.11	124.8 ± 3.77	3.1 ± 0.08	59.1 ± 0.45	50.2 ± 0.42
Wild ($n = 24$)	10.1 ± 0.10	6.9 ± 0.08	6.8 ± 0.04	10.4 ± 0.08	8.8 ± 0.08	128.4 ± 1.63	3.1 ± 0.04	60.5 ± 0.39	51.8 ± 0.31
Males									
Captive ($n = 18$)	11.2 ± 0.02	8.0 ± 0.12	7.3 ± 0.07	11.7 ± 0.12	9.6 ± 0.12	128.5 ± 3.39	3.4 ± 0.07	64.2 ± 0.73	54.7 ± 0.59
Wild ($n = 21$)	11.1 ± 0.09	7.7 ± 0.11	7.3 ± 0.04	11.4 ± 0.11	9.6 ± 0.13	136.9 ± 1.50	3.4 ± 0.04	66.3 ± 0.46	55.9 ± 0.44

UCC, distance from medial edges of maxillary canines; LCC, distance from medial edges of mandibular canines; IW, distance from lateral edges of I^3s; NP, greatest distance between lateral edges of the nose pad; P24, distance from anterior edge of P_2 to posterior edge of P_4; TA, distance from the base of the tail to the end of the last vertebrae; UCW, distance from the anterior to the posterior edge of the maxillary canine; RL, tibia length; FA, ulna length. A MANOVA test for the effect of location (wild versus captive) was significant (Wilks' Lambda = 0.6, $F_{9,73} = 4.7$, $P < 0.001$).

[a] For effect of location: $F_{1,73} = 5.6$, $P = 0.02$.

[b] For effect of location (captive or wild): $F_{1,73} = 5.6$, $P < 0.001$.

[c] For effect of location: $F_{1,73} = 10.8$, $P = 0.002$.

[d] For effect of location: $F_{1,74} = 9.6$, $P = 0.03$.

Table 2. Mean ± SD (in mm) for eight morphological measurements from 134 adult black-footed ferrets

	UCC	LCC[a]	IW	NP	TA	UCW[b]	RL[c]	FA[d]
Females								
CRC captive ($n = 8$)	10.3 ± 0.07	7.2 ± 0.17	6.7 ± 0.07	10.6 ± 0.10	132.9 ± 2.69	3.4 ± 0.03	60.5 ± 0.49	51.5 ± 0.49
Other captive ($n = 14$)	10.1 ± 0.13	6.7 ± 0.16	6.8 ± 0.08	10.5 ± 0.08	125.8 ± 2.30	3.5 ± 0.05	59.4 ± 0.53	49.8 ± 0.35
Wild ($n = 20$)	10.0 ± 0.07	7.1 ± 0.11	6.8 ± 0.04	10.4 ± 0.11	129.4 ± 1.39	3.7 ± 0.04	61.0 ± 0.36	52.3 ± 0.38
Males								
CRC captive ($n = 22$)	11.2 ± 0.11	7.6 ± 0.19	7.3 ± 0.06	11.7 ± 0.15	132.4 ± 2.15	3.8 ± 0.06	65.6 ± 0.47	54.7 ± 0.75
Other captive ($n = 53$)	11.0 ± 0.08	7.3 ± 0.08	7.2 ± 0.03	11.7 ± 0.08	132.7 ± 1.13	4.0 ± 0.04	65.1 ± 0.30	53.7 ± 0.23
Wild ($n = 17$)	10.9 ± 0.12	7.5 ± 0.12	7.3 ± 0.05	11.9 ± 0.14	134.6 ± 1.9	4.2 ± 0.07	69.5 ± 0.44	59.3 ± 0.34

For description of morphometric measurements, see Table 1. A two-way MANOVA test with sex and location (wild versus captive) was significant (Wilks' Lambda = 0.4, $F_{16,242} = 10.4$, $P < 0.001$). Results of post-hoc univariate tests of the effect of location and the interaction effect of sex and location are given when significant ($\alpha < 0.05$).

[a] Test of location: $F_{2,128} = 3.8$, $P = 0.03$.

[b] Test of location: $F_{2,128} = 13.0$, $P < 0.001$; Tukey's HSD: CRC versus OTHER + WILD.

[c] Test of location: $F_{2,128} = 24.2$, $P < 0.001$; Tukey's HSD: CRC + WILD versus OTHER; interaction of sex and location $F_{2,128} = 7.7$, $P = 0.001$.

[d] Test of location: $F_{2,128} = 41.3$, $P < 0.001$; Tukey's HSD: CRC + OTHER versus WILD; interaction of sex and location $F_{2,128} = 8.3$, $P < 0.001$.

PC2 for both adult and juvenile data sets were similar to those found by Wisely *et al*. (2002*a*) and thus we used the same logic in interpreting our results. For both adult and juvenile data, character loadings for all variables were positive and ≥ 0.5 (Fig. 2) for PC1, suggesting that PC1 was correlated with overall body size. Character loadings in the eigenvector of PC2 were both positive and negative; we interpreted this component to be indicative of body shape (Somers, 1986). Results of the MANOVA using PC scores were significant for the test of *ex situ* versus *in situ* juveniles (Wilks' Lambda = 0.8, $F_{2,72} = 7.3$, $P = 0.001$); post-hoc univariate tests indicated that only PC2 was significantly different between *ex situ* and *in situ* juveniles ($F_{1,73}$=14.9, $P < 0.001$). For the adult data, eigenvalues of PC1 and PC2 were 4.2 and 1.0 explaining 53% and 13% of the variation in the variables, respectively. For adults, we found significant differences in PC scores between location treatments (Wilks' Lambda = 0.73, $F_{2,127} = 11.1$, $P < 0.001$). Post-hoc tests revealed a difference in values of PC1 ($F_{2,128} = 12.5$, $P < 0.001$) and in PC2 ($F_{2,128} = 7.6$, $P = 0.001$: Fig. 3) between locations.

DISCUSSION

Environment or genetics?

Wisely *et al*. (2002*a*) previously reported that pre-captive populations of black-footed ferrets were larger and were differently shaped than *ex situ* animals based on skull morphology. They found no correlation between pedigree-based inbreeding and skull size suggesting that inbreeding depression was not the cause for small *ex situ* animals, but they could not further distinguish the cause of these morphological differences. The present study compared individuals raised in captivity to those born in the wild to

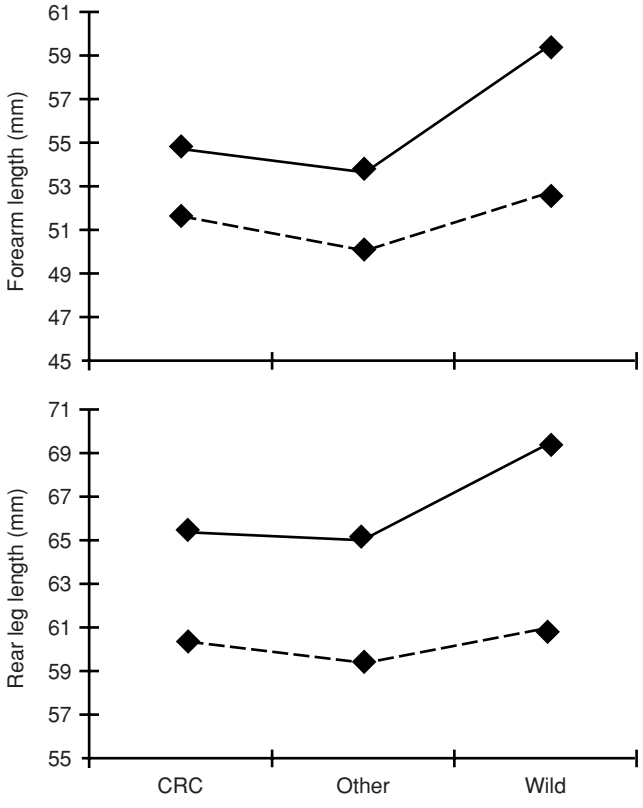

Fig. 1. Mean length (mm) of ulna and tibia of adult anaesthetised black-footed ferrets born and raised in two captive environments, large enclosures at the National Zoological Park's Conservation and Research Center (CRC) or small enclosures at other breeding facilities (Other) and animals born and raised in the wild (Wild). We found a significant interaction between sex (continuous line, male; broken line, female) and location treatment groups. Males were disproportionately smaller than females in captivity.

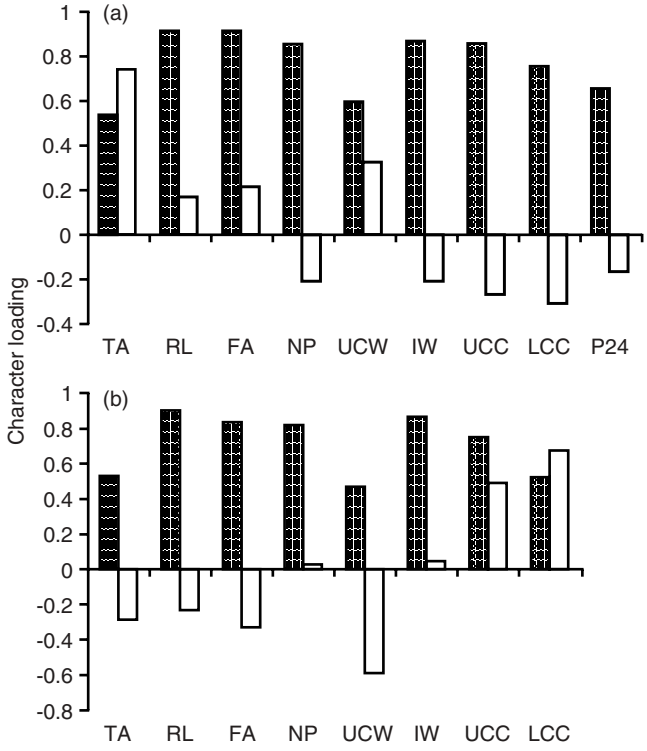

Fig. 2. Character loadings from a principal component analysis of nine morphometric measures of (a) juvenile and (b) adult black-footed ferrets. Character loadings of principal component one (PC1) are filled bars and loadings of principal component two (PC2) are open bars. We interpreted PC1 to represent overall body size and PC2 to represent body shape.

determine if these morphological changes were the result of the developmental environment and, therefore, not heritable or were the product of domestication resulting from unintentional selection. Our results confirm that environment induced the diminutive body size of *ex situ* black-footed ferrets. Principal component analysis suggested that overall body size was smaller and body shape differed for *ex situ* versus *in situ* adults (Fig. 3). While our measurements of live, anaesthetised animals were not directly comparable to those taken by Wisely *et al*. (2002*a*) on skulls of pre-captive *in situ* animals, we did find similarity in the magnitude of observed change in body size. Wisely *et al*. (2002*a*) concluded that *ex situ* animals were 5–10% smaller than pre-captive *in situ* individuals; the present study found that *ex situ* individuals ranged from 2–9% smaller than *in situ* descendants of the captive population. These findings suggest that wild-born members of reintroduced populations returned to pre-captive body size in the 3 years that elapsed between the final reintroduction and our study.

It is unlikely that these rapid morphological changes in the *in situ* population were a result of natural selection for increased body size. High survival and fecundity rates and concomitant exponential growth experienced by the Conata Basin/Badlands population would not promote a 'large body size genotype' differentially contributing to the next generation. In addition, captive animals that were reintroduced between 1996 and 1999 would have overwhelmed any differential success of these genotypes due to genetic swamping with *ex situ* individuals. Finally, the rapid return to pre-captive body size (3 years or 1–2 generations) suggests that the observed changes were not due to natural selection. Our results confirm that environmental factors, not heritable ones, influenced the observed difference in these characters between populations.

Morphological development

Adult black-footed ferrets had equivalent tail length regardless of source location (Table 2; TA), yet *ex situ* juvenile males had tails that were 6% shorter than age-matched juveniles *in situ*, suggesting delayed tail growth in the former group (Table 1; TA). The mechanism for this difference is poorly understood, but may be due to slower vertebral development for young ferrets raised in captivity. It appears, however, that tail vertebrae reach their full growth potential *ex situ*; adult tail lengths were equivalent among *in situ* and *ex situ* groups.

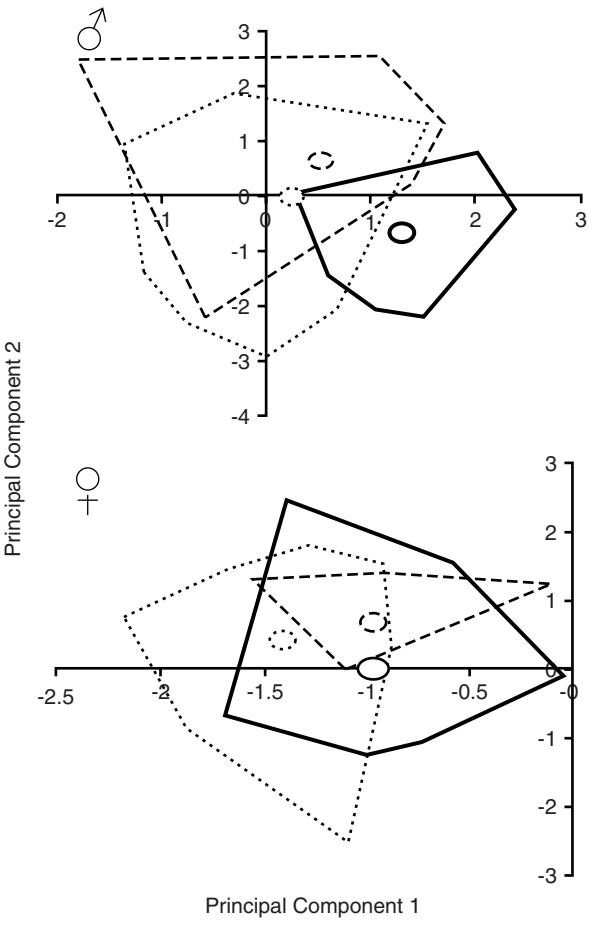

Fig. 3. Polygons encircling principal components one and two out of eight morphometric variables measured from adult black-footed ferrets raised at National Zoological Park's Conservation & Research Center (long broken line), all other captive breeding facilities (short broken line) and *in situ* (continuous line). Ovals represent the centroid of values for each location.

The most striking feature of our comparative assessment was the significantly shorter ulna and tibia in black-footed ferrets maintained *ex situ*, for both juveniles and adults. Limb length was 9% smaller for *ex situ* adult males than for *in situ* males (Fig. 1). This difference was less profound in animals raised in the larger enclosures of CRC. We interpret these results to mean that long bone development and growth is curtailed in *ex situ* animals and that enclosure size plays a role in the difference. Because we found differences in juvenile morphometry, we hypothesise that differential elongation begins prenatally or early (< 75 days) in postnatal development.

Many factors affect long-bone development including growth hormones that are regulated by the pituitary gland (Williams & Hughes, 1977) and induction of growth by mechanical stimuli (Lovejoy, Cohen & White, 1999). Differences in relative long bone length among domestic breeds of dogs are believed to be controlled by polygenic regulation of the concentration of growth hormones during development (Wayne, 1986). For black-footed ferrets it is possible that unknown environmental stimuli inhibit a growth factor responsible for prenatal or early postnatal long bone growth. A more parsimonious explanation involves differential mechanical stimuli in juvenile black-footed ferrets raised in different captive environments or born in the wild. Long bones develop from the epiphyseal growth plates at the proximate and distal ends (Hinchliffe & Johnson, 1980); cartilage cells at these growth plates transduce mechanical stimuli into extra-cellular matrix production (Lovejoy *et al*., 1999). These stimuli are believed to simultaneously build bone, yet prevent the epiphyses from ossifying prematurely (Marzke *et al*., 1996). The result is that bone architecture is modelled in response to the environment such that bone is built in areas subjected to stress and reabsorbed in areas without stress, a phenomenon known as Wolff's Law (Wolff, 1891; Salter, 1970). Experimental studies confirm these assertions; for example, immature rats whose legs were immobilised with casts, had shorter and thinner long bones (Steinberg & Trueta, 1981). For the black-footed ferret, there appears to be a direct relationship between bone length and enclosure size. Ulna length was the longest in wild-born animals, shorter in those raised in large enclosures and shortest in animals kept in small enclosures. Insufficient mechanical stimulation of the long bones of young ferrets raised in a small homogeneous space could explain the shorter limb length. *In situ*, juveniles were exposed to a more heterogeneous environment that allowed for more uninhibited movement and stimulated a greater degree of bone lengthening than in the *ex situ* environment. We hypothesise that these opportunities to experience multi-variant stimuli in an expansive space may be a necessary prerequisite in early postnatal development for proper limb development.

We found sexual dimorphic body size differences to be more pronounced in the wild; developmental differences between captive-reared and wild-born animals were greater for males (Fig. 1). A similar pattern of reduced dimorphism was found in ranch-raised American mink (*M. vison*) when compared to feral mink in Norway, Ireland and Britain (Lynch & Hayden, 1995). These authors attributed their findings to genetic mechanisms associated with selection. For ranch mink, an animal under extreme artificial selection, this is the most parsimonious explanation. For the reasons outlined above, we believe that size differences between *in situ* and *ex situ* black-footed ferrets were due to environmental factors, which probably affected the sexes differentially.

Implications for *in situ* and *ex situ* conservation

We have documented morphological distinctions between *ex situ* and *in situ* conspecifics caused by influences of the *ex situ* environment. Over the next several decades as more organisations use conservation breeding as a tool to save an increasing number of threatened taxa, it will be imperative that biologists understand the consequences of this conservation action. One of the biggest concerns for *ex situ* populations is that they remain representatives of their taxa (Snyder *et al*., 1996). As revealed by the present work, this concern is not a trivial mandate as animals

held *ex situ* are vulnerable to an array of perturbations. To date, most studies that have documented changes to *ex situ* populations have reported heritable changes that are the result of adaptation to the captive environment (e.g. Lewis & Thomas, 2001; Kruska & Sidorovich, 2003). Clearly, heritable changes to the phenotype that result in unintentional selection for morphological domestication should be a primary concern to conservation curators, yet non-heritable changes, that are the result of environmental factors, should not be overlooked. Phenotypically altered individuals may have lower survival or fecundity upon reintroduction than more representative individuals, which lowers the growth potential of reintroduced populations.

Survival and reproduction of black-footed ferrets with an altered phenotype demonstrates how easily development is altered to produce variant phenotypes in the captive environment. For this species, shorter limbs in captivity may or may not confer a fitness advantage, but genes that control the growth and ossification of cartilage cells are no longer under stabilising selection to maintain growth for a set time, which increases the opportunity for unintentional selection. To avoid possible genotypic change, we reiterate the importance of recommendations by Ballou & Foose (1996) that conservation breeding be maintained for as few generations and for as long a generation time as possible. For some species, such as the black-footed ferret whose habitat has nearly vanished, terminating the breeding program is not yet possible without incurring a serious risk of extinction. When conservation breeding is necessary for many generations, the effects of unintentional selection due to high density or small enclosures must be balanced with the positive effects of high juvenile production for reintroduction, as is the case for the black-footed ferret recovery program.

CONCLUSIONS

Morphological changes have occurred to black-footed ferrets *ex situ*; animals born in captivity are smaller and shaped differently than animals *in situ*. Most notably the fore- and hind-limbs of *ex situ* animals are shorter than animals *in situ*. These changes are environmentally induced and not heritable; nevertheless, selection for 'wild-type' phenotypes has been relaxed, setting the stage for the heritable morphological alteration of unintentional selection. Wolff's Law suggests that changes to limb length could be due to the lack of mechanical stimuli in the *ex situ* environment. We recommend that other conservation-breeding programs initiate comparative studies of long bone growth rates *in situ* and *ex situ* to determine if this phenomenon occurs in other endangered, medium-sized carnivores. Should morphological changes be found, recovery planners will need to balance the trade-off of increased enclosure size with the decreased numbers of animals available for reintroduction (assuming space limitations in captivity). For the black-footed ferret, a new breeding facility to be completed in 2005 will have large enclosures similar to CRC that will help to ameliorate this environmentally caused change in morphology.

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