VARIATION IN THE SKULL MORPHOLOGY OF CAPTIVE MAMMALS: A GEOMETRIC MORPHOMETRIC AND META-ANALYTIC APPROACH

A Dissertation

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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August 2020

Major Subject: Ecology and Evolutionary Biology

ABSTRACT

Morphological changes in the crania of captive mammals compared to their wild counterparts have been documented for over a century. Although these morphological differences are potentially deleterious, their exact trends, mechanisms, and consequences are poorly understood. Using meta-analytic and geometric morphometric techniques, this dissertation addresses whether captive mammals display predictably distinct morphology compared to wild populations, whether those differences are impacted by ecological covariates, and the probable mechanisms behind these changes. This dissertation finds significant differences in cranial morphology between captive and wild populations of mammals. The magnitude and directionality of these changes vary with taxonomy and diet, where carnivory specialists differentiate most consistently. This suggests that changes in cranial morphology cannot be generalized for all captive mammal species. Likewise, captive and reintroduced wolves display significantly different cranial morphology compared to the wild population. Captive individuals display greater morphological variation than wild populations, suggesting the influence of relaxed selection. Reintroduced individuals aligned more closely with the captive population but display much less morphological variation, suggesting the effects of genetic drift and the reapplication of natural selection pressures that occur in the wild. Several additional canid species also display similar cranial changes in captivity. Hypercarnivorous species often display the greatest changes with shorter and wider skulls. Although captive diets have recently improved, morphological changes have continued to occur among recent captive specimens suggesting that either captive diets still need improvement or that morphological changes occurring in captivity may be unrelated to diet. Regardless of carnivory type, many canid species shifted in a similar morphological direction away from their wild counterparts, displaying deeper mandibles and broader crania. Zoos may represent the last hope for the long-term survival of threatened and endangered species especially given that habitat destruction is projected to continue and intensify in the future. By understanding the nature of the morphological changes occurring in captivity, we may be able to alter captive management practices to prevent these changes in future generations and preserve the conservation value of captive animals.

ACKNOWLEDGEMENTS

I am extremely grateful to a number of people who helped to make this dissertation possible. First, I would like to thank my committee chairs, Dr. Light and Dr. Lawing, as well as my committee members, Dr. Linderholm and Dr. Athrey, for their guidance, support, and contributions to this research.

I would also like to thank my friends, colleagues, faculty, and staff in the Ecology and Evolutionary Biology program at Texas A&M University for their help and encouragement. I would especially like to thank all of the Light and Lawing Lab members from the past several years. In particular, my sincerest gratitude goes out to Dr. Adrian Castallanos for his help with R-coding. I would also like to thank Grace Vielleux and Emma Dohnalik, who accompanied me to Washington, D.C. and helped to photograph specimens at the Smithsonian.

I am also extremely grateful to all of the museum collection staff and curators who enabled this research by allowing me to photograph specimens in their facilities and in many cases helped to field questions and even locate specimens. In particular, I would like to thank Adrienne Raniszewski, Jon Dunnum, and Joe Cook at the Museum of Southwestern Biology at the University of New Mexico; Barb Lundrigan and Laura Abraczinskas of the Michigan State University Museum; Cody Thompson of the Museum of Natural History at the University of Michigan; Bruce Patterson at the Field Museum; Jeff Stephenson of the Denver Museum of Nature and Science; Gary Shugart of the Slater Museum of Natural History at the University of Puget Sound: Jon Nations

and Jacob Esselstyn of the Louisiana State University Museum of Natural Science; Jacqueline Miller of the Royal Ontario Museum; Brandi Coyner of the Sam Noble Museum of the University of Oklahoma; Jim Dines of the Natural History Museum of Los Angeles County; Jonathan Marcot of the Donald R. Dickey Bird and Mammal Collection of the University of California Los Angeles; Darrin Lunde and John Ososky of the Smithsonian National Museum of Natural History; Bob Timm of the Biodiversity Institute of the University of Kansas; Eleanor Hoeger of the American Museum of Natural History; Allison Bronson of the Humboldt State University Natural History Museum; and Martha Velez of the California Academy of Sciences.

I would also like to thank my Mom and Dad and my brother Luke, for their lifetime of love and support.

Finally, I want to thank my husband and best friend, Dr. Jason Martina, for his patience and unyielding support. Jason has spent countless hours talking about research and encouraging me even when the road seemed impossible. I cannot imagine a better partner.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a dissertation committee consisting of Dr. Lawing (co-advisor) and Dr. Light (co-advisor) of the Department of Ecology and Conservation Biology, and Dr. Linderholm of the Department of Anthropology, and Dr. Athrey of the Department of Poultry Science.

Heritability analyses in Chapter IV were conducted by Professor Riley of the Department of Animal Science.

All other work for the dissertation was completed by the student under the advisement of and in collaboration with Dr. Lawing and Dr. Light (Department of Ecology and Conservation Biology).

Funding Sources

Graduate study was supported by a Graduate Fellowship from the Office of Graduate and Professional Studies (OGAPS) at Texas A&M University.

This work was made possible in part by the Ecology & Evolutionary Biology Program at Texas A&M University (EEB Research Award), the American Society of Mammalogists (Grants-In-Aid of Research), and the Natural History Museum of Los Angeles County (Student Collections Study Award). Its contents are solely the responsibility of the author and do not necessarily represent the official views of these funding sources.

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CHAPTER I

INTRODUCTION

Overview

In 1904 President Theodore Roosevelt was presented with an adult male lion as a gift from Emperor Menelik of the former Abyssinia (Heller, 1913; Hollister, 1917; Howell, 1925; Erickson et al., 2004). Upon its receipt, this animal spent the remaining years of its life in the National Zoologic Park in Washington, D.C. (Heller, 1913; Hollister, 1917). After the animal passed away in 1906 it was designated as a type specimen representing a new subspecies of African lion known as *Felis panthera roosevelti* (Heller, 1913; Hollister, 1917). This distinction was based on several unique phenotypic qualities displayed by the specimen including thick, widely bowed zygomatic arches, enlarged mandibular condyles, reduced tooth size, and an overall enlarged body size (Heller, 1913; Howell, 1925). Further investigation into these anomalous traits revealed that these differences reflected morphological changes that occur consistently among captive lions and were not reflective of any particular wild population (Hollister, 1917). Since that time, a variety of studies have been conducted on captive animals representing multiple taxa and often recovering similar findings (e.g., Antonelli, 2015; Curtis et al., 2018; Groves, 1966; Groves, 1982; Hartstone-Rose et al., 2014; Hollister, 1917; McPhee, 2004; O'Regan, 2001; O'Regan & Kitchener, 2005; Selvey, 2018; Van Velzen, 1967; Wisely et al., 2002; Wolfgramm, 1894). Even so, the trends associated with morphological differentiation among captive populations compared to their wild counterparts are poorly understood, a point which is particularly important given the incredible value that captive animals represent to conservation efforts.

Regardless of intentionality, humans have a long history of modifying animal phenotypes. In fact, the start of human ‘civilization’ is arguably defined by its alteration and domestication of other species (Diamond, 2002; Fuller & Stevens, 2019; Larson & Fuller, 2014; Zeder et al., 2006). Through domestication, humans have modified animals for millennia (Larson & Fuller, 2014; Zeder et al., 2006; Zeder, 2012), selecting for traits intentionally (e.g., enhanced dairy production among cattle; Francis, 2015; Rauw et al., 1998) and unintentionally (e.g., curly tails among domestic canids; Ruvinsky & Sampson, 2001; Trut, 1999). Captivity has also been associated with a suite of unusual traits including behavioral, physiological, and morphological shifts away from wild populations (O’Regan & Kitchener, 2005; Pelletier et al., 2009). Such changes may cause the loss of essential functional traits associated with behavior and morphology, which may be deleterious to the organism if a reintroduction were ever attempted (Bremner-Harrison et al., 2004; McPhee, 2004; McPhee & Carlstead, 2010; Pelletier et al., 2009; Wisely et al., 2005). Such changes, away from the morphology and behavior of wild populations, work counter to the goals of conservation-based captive facilities like modern zoos that seek to maintain healthy, captive animals as proxies for their wild counterparts (Conway, 1995; Lacy, 2013; Patrick et al., 2007; Rudnick & Lacy, 2008; Willoughby et al., 2015).

Modern Zoos

As habitat losses continue to decimate wild animal populations, zoos have become increasingly instrumental in conservation efforts (Conde et al., 2011; Martin et al., 2014; West & Dickie, 2007). Zoos offer a safe environment for the breeding and maintenance of rare or endangered species, some of which may be extinct in the wild and currently exist solely in captivity (Alroy, 2015; Witzenberger & Hochkirch, 2011). Exotic animals have been exhibited in private menageries since at least 2,500 B.C.E. (Fa et al., 2011; Hosey et al., 2009; Zimmerman et al., 2007). However, public zoological gardens did not begin to emerge until the late 18th century. These facilities quickly became popular destinations for entertainment and recreation and began to open worldwide throughout the 19th century (Hosey et al., 2009; Kisling, 2001). However, an increased interest and understanding of animal welfare and impending species endangerment caused a dramatic shift in the imperatives of those facilities, from living cabinets of curiosities to modern zoos focused on education and conservation (Hosey et al., 2009; Kisling, 2001). These goals are overseen and maintained by several accrediting organizations worldwide. In North America, the Association of Zoos and Aquariums (AZA) accredits captive facilities that offer above-average nutrition, enrichment, and enclosure quality and display a strict adherence to the conservation and education goals that drive the organization (Butler, 2001; Conde et al., 2011; Hutchins et al., 2003; Patrick & Caplow, 2018).

The modern zoo is an important hub for *in situ* and *ex situ* conservation and houses roughly one in seven threatened species (Conde et al., 2011; Hutchins et al., 2003; Patrick & Caplow, 2018). Zoos may not always prioritize at-risk species, opting in some cases to house easily displayed, highly charismatic, large-bodied, or particularly attractive animals (Alroy, 2015; Balmford et al., 2011; Fa et al., 2014; Frynta et al., 2013). Zoos also increasingly house fewer total animals in an effort to improve the welfare of each individual resident (Alroy, 2015). Regardless, zoos continue to have a positive impact on conservation, where preferentially displaying charismatic species actually improves the overall commitment to conservation reported by zoo visitors (Skibins et al. 2013). AZA-accredited facilities participate in collaborative captive breeding programs such as Species Survival Plans (SSP), which helps preserve endangered and threatened species through careful breeding recommendations (Ballou et al., 2010; Hutchins & Wiese, 1991; Songasasen & Rodden, 2010). These efforts have been somewhat hampered by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) regulations monitoring the inflow of endangered species into captivity, effectively reducing gene flow with wild populations (Glatston, 2011; Pelletier et al., 2009; Vehrs, 2001). In spite of this, many see zoos as the last bastion for species recovery (Conde et al., 2011; Glatston, 2011), even though the conservation-value of captive-bred animals has not been thoroughly examined.

The Ark Paradigm and Animal Reintroductions

The Ark Paradigm suggests that species preservation may be best ensured by maintaining animal populations in captivity until they can be safely reintroduced into the wild (Bowkett, 2009; Tudge, 1991). This concept gained wide acceptance during the 1980's and 1990's and has had renewed enthusiasm as a means of preventing large-scale extinctions (Bowkett, 2009; Hutchins et al., 2003). In practice, this feat could require the care of captive populations for up to 200 years or more prior to release, given that reintroductions are typically not attempted until threats that originally endangered the species are neutralized (Frankham, 2008; Soulé et al., 1986; Woodworth et al., 2002). This can be a particularly disconcerting issue given that captive populations may become differentiated and cease to be appropriate proxies for their wild conspecifics (Frankham, 2008; Håkansson & Jensen, 2005; Shepherdson, 1994; Snyder et al., 1996; Williams & Hoffman, 2009; Woodworth et al., 2002).

While the Ark Paradigm has been met with considerable criticism, zoos have facilitated several large-scale, high-expense reintroduction programs with mixed, often limited, success (Conde et al., 2011; Woodworth et al., 2002). Success typically refers to a situation in which a reintroduced population is self-sustaining, and independent of human support (Beck et al., 1994). While a base-line population size of 500 individuals is also often used as a measure of reintroduction success, this threshold is generally unrealistic for large carnivores. An alternative measure has been proposed for these species in which a reintroduction is said to be successful if recruitment has exceeded adult mortality over a three-year period, although specific time-lines vary based on life history traits of individual species (Beck et al., 1994; Fischer & Lindenmayer, 2000; Hayward et al., 2007). There are varying assessments regarding the success of these programs and varying definitions of success based on species ecology (Beck et al., 1994; Fischer & Lindenmayer, 2000; Hayward et al., 2007). Assessments evaluating reintroduction programs estimate 11% to 53% of programs are successful, but the overall consensus is that reintroductions typically fail more than they succeed (Beck et al., 1994; Fischer & Lindenmayer, 2000; Frankham, 2008; Matthews et al., 2005; Wolf et al., 1996). In addition to the substantial logistical coordination required, reintroducing a zoo-bred animal has the potential to negatively impact already fragile wild populations by introducing disease or deleterious traits from inbred captive populations (Lindburgh, 1992; Roots, 2007). Captive-bred animals may also show different mating preferences including inter-species mating (delBarco-Trillo et al., 2009) and in some cases preferential assortative mating with other captive-bred individuals (Slade et al., 2014). Instead of reintroductions, animal translocations are often a preferable alternative, where wild animals are collected from one location and moved to a depauperate area (Fischer & Lindenmayer, 2000; Smeeton & Weagle, 2000; Snyder et al., 1996; Wilson & Stanley Price, 1994). Translocated wild animals frequently perform better than reintroduced populations (Smeeton & Weagle, 2000; Snyder et al., 1996; Wilson & Stanley Price, 1994); however, this method may not always be possible for critically endangered species whose wild population cannot be fragmented any further (Smeeton & Weagle, 2000; Wilson & Stanley Price, 1994) or species that are extinct in the wild (Reading & Miller, 2001; Spalton et al., 1999).

Given the massive financial investment, extensive coordination, and number of worker-hours required to release a captive animal, there is no shortage of critical evaluations regarding the failures of reintroduction programs (Beck et al., 1994; Hutchins et al., 2003; Lindburgh, 1992; Snyder et al., 1996). It has frequently been noted that morphological and behavioral changes in captive populations may severely limit reintroduction success (Bryant & Reed, 1999; Edwards, 2014; Frankham et al., 1986; Håkansson & Jensen 2008; McPhee & Silverman, 2004; McPhee, 2004). One topic that is consistently examined is whether the behavior of captive populations is fundamentally changed in a way that makes reintroduction failure more likely (Bremner-Harrison et al., 2004; McPhee, 2003; McPhee & Silverman, 2004; Silva & Azevedo, 2013). Fewer studies have examined the breadth and retention of morphological variation arising in captive populations (e.g., Wisely et al., 2005) and the resulting functional and evolutionary consequences. Such information may help implicate the mechanisms that underlie morphological changes in captive populations given that variation arising from phenotypic plasticity (i.e., the ability for one genotype to produce different phenotypes under different environmental conditions) in captive populations would be eliminated from a reintroduced population within the first wild-born generation (Wisely et al., 2005).

Zoo Animals in Natural History Collections

Modern zoos and natural history museums share a similar goal of education and conservation (Kitchener, 1997; Miller et al., 2004). By preserving biological specimens, natural history collections are often important research centers in the fields of wildlife ecology, systematics, and morphology (Kitchener, 1997; Lister et al., 2011; Winkler, 2004). In this midst of rapidly declining biodiversity, such collections may represent the last vestiges of many biological materials (Krishtalka & Humphrey, 2000; Miller et al., 2004; Winkler, 2004). Zoos also preserve species, some of which may be extinct in the wild (Alroy, 2015; Witzenberger & Hochkirch, 2011). These institutions may have different roles, but zoos and natural history collections often complement each other and share a common purpose of education and conservation (Kitchener, 1997; Spartaco & Kitchener, 2007).

Given the unique nature of zoo animals, museum specimens from these collections could be useful for a variety of research questions (Barrios-Quiroz et al., 2012; Behringer et al., 2018; Hlusko & Mahaney, 2007; Kohn & Lubach, 2019; Spartaco & Kitchener, 2007). After all, captive animals typically represent a highly controlled population and frequently have corresponding data regarding the animal's lineage, diet, and complete medical history (Hlusko & Mahaney, 2007; Spartaco & Kitchener, 2007). Captive animals are frequently rare, endangered, or possibly extinct-in-the-wild and collecting additional specimens from that species may be either impossible or impractical (Barrios-Quiroz et al., 2012; Kohn & Lubach, 2019). The inclusion of captive specimens in natural history collections provides a unique opportunity for a myriad of research fields. Thus, it is paramount to understand whether captive populations are good representations of their wild counterparts. The use of captive animals in biological research has long been a scientific taboo given the evidence that at least some populations may be morphologically and genetically distinct from wild populations (Drumheller et al., 2016; Hartstone-Rose et al., 2014; Spartaco & Kitchener, 2007). However, the breadth, depth and nature of those differences are poorly understood and in need of further investigation.

Morphological Variation among Captive Mammals

Captive mammals may display a variety of morphological differences compared to wild populations. These differences have included changes in the sizes and shapes of cranial elements (e.g., wider crania among African lions, *Panthera leo*; Hartstone-Rose et al., 2014; Hollister, 1917; O'Regan, 2001), locomotor-related traits (e.g., changes in limb bone sizes among captive chimpanzees, *Pan troglodytes*; Lewton, 2017), internal organ sizes (e.g., brain sizes among bank voles, *Myodes glareolus*; Runzheimer, 1969; kidney and spleen masses among captive house mice, *Mus musculus*; Courtney Jones et al., 2018), and overall body size (e.g., increased body size among common marmosets, *Callithrix jacchus*; Araújo et al., 2000). Not all studies on morphological variation in captive populations have recovered significant differences (Bello-Hellegouarch et al., 2013; Courtney-Jones et al., 2018; Serna-Lagunes et al., 2010). It remains unclear why species develop their particular morphological changes in captivity and why certain species do not appear to display morphological differences in captivity at all, suggesting the need for further evaluation.

It has been suggested that the changes that arise in captivity may be akin to the early stages of domestication (Guay et al., 2012; Håkansson & Jensen 2005; Wisely et al., 2005). Domestication describes a mutualistic relationship that may be formed between humans and a population of animals, where humans exert substantial control over critical aspects of animal feeding, reproduction, and life style (Galibert et al., 2011; Zeder, 2012; Zeder, 2015). These changes may be accompanied by a difference in phenotype, including changes in body size, brain size, coat color, and cranial shape, among other traits (Lord et al., 2019; Trut et al., 2009; Wilkins et al., 2014). Although this process requires multiple generations and includes several different pathways, it may begin when a population is brought into captivity (Larson & Burger, 2013; Mason et al., 2013; Zeder, 2012). The primary hallmarks of domestication include docile and human-affiliative behaviors (Lord et al., 2019; Trut et al., 2009; Zeder et al., 2006), changes which are adaptive and heritable and may arise quickly under strong artificial selection pressure (Driscoll et al., 2009; Topál et al., 2005; Trut et al., 2009; Zeder, 2012). Superficially similar plastic changes in behavior can arise within an individual's lifetime due to taming (e.g., a hand-reared opossum may be tame, but it is not a domestic species), although unlike domestication, these changes are not generally heritable (Driscoll et al., 2009; Galibert et al., 2011). Whether the changes arising among captive populations of exotic species represent the early stages of domestication remains unclear.

Changes in body size are often associated with captive animals compared to their wild counterparts (Araújo et al., 2000; Crossley & Del mar Miguélez, 2001; Leigh, 1994; Terranova & Coffman, 1997). Given the quality and abundance of food that may be available to captive animals, obesity is a frequent concern (Dierenfeld, 1997; Oftedal & Allen, 1996; O'Regan & Kitchener, 2005). However, beyond excess body weight, other measures of body size increase among certain populations of captive mammals include increases to body length and overall skull size (Baranowski et al., 2013; Crossley & Del mar Miguélez, 2001; Groves, 1982). Interestingly, the opposite trend is also apparent among other species, where larger measurements of body and skull size may be recorded within the wild population (Fredrickson & Hedrick, 2002; Groves, 1982; Laikre & Ryman, 1991; Wisely et al., 2002).

Variation in cranial morphology among captive mammals has been a recurrent topic of research for over a century (e.g., Hollister, 1917; Wolfgramm, 1894). In many cases, changes appeared to be quite similar across fairly diverse taxa. For instance, African lions (*Panthera leo*), Amur tigers (*P. tigris*; Hartstone-Rose et al., 2014; Hollister, 1917; O'Regan, 2001), coyotes (*Canis latrans*; Curtis et al., 2018), Indian rhinos (*Rhinoceros unicornis*; Groves, 1982), and chinchillas (*Chinchilla laniger*; Crossley & del Mar Miguélez, 2001) have all displayed comparatively wide skulls in captivity. Likewise, other features such as skull length (Crossley & del Mar Miguélez, 2001; Groves, 1982; Van Velzen, 1967) and mandibular length and height (Hartstone-

Rose et al., 2014; Kamaluddin et al., 2019; Van Velzen, 1967; Wolfgramm, 1894) are also frequently implicated as predictably differentiated in captivity. However, the specific traits and the degree of the changes varies considerably between individual species, where certain species do not appear to display cranial differences in captivity (e.g., house mice, *Mus musculus*; Courtney-Jones et al., 2018), while others tend to show significant differences (e.g., lions and tigers, *Panthera leo* and P. *tigris*, respectively; Hartstone-Rose et al., 2014; Hollister, 1917; O'Regan, 2001).

Changes in cranial morphology are particularly concerning given the functional importance associated with an animal's bite force and by extension, their dietary niche (Meloro et al., 2017; Van Valkenburgh, 1989; Wroe et al., 2007). Cranial and mandibular shapes largely reflect the size and shape of the temporalis and masseter muscles (Figures I-1 & I-2), two of the primary muscles involved in mastication (Antonelli, 2015; Elbroch, 2006; Penrose, 2018). The masseter muscle attaches to the skull from the zygomatic arch to the mandibular masseteric fossa (Antonelli, 2015; Elbroch, 2006; Penrose, 2018; Figure I-1; Figure I-2). This powerful muscle is arranged in layers and is instrumental in the opening and closing of the jaw (Elbroch, 2006; Penrose, 2018). Similarly, the temporalis muscle attaches on the braincase, through the zygomatic arch, to the coronoid process of the mandible (Antonelli, 2015; Elbroch, 2006; Penrose, 2018). The relative spread of the zygomatic arch is highly indicative of cranial musculature and functionality, where a wider zygomatic arch implies the presence of enhanced musculature and a stronger bite force often associated with carnivores and gnawing rodents (Elbroch; 2006).

Carnivores are among some of the most charismatic and common zoo inhabitants (Albert et al., 2018; Fa et al., 2014). These species are frequently divided into three diet subcategories representing the amount of vertebrate prey they consume, where a hypercarnivorous diet includes roughly 70% vertebrate prey, a mesocarnivorous diet includes 50-70% vertebrate prey, and a hypocarnivorous diet includes less than 30% vertebrate prey (Damasceno et al., 2013; Van Valkenburgh, 1989; Van Valkenburgh, 2007). These dietary preferences typically correspond to an animal's bite force, where hypercarnivorous species have the greatest bite force and hypocarnivorous species have the lowest bite force (Figure I-3; Damasceno et al., 2013; Slater et al., 2009; Wroe et al., 2005). Dietary preferences are also generally reflected by an animal's cranial morphology, where a hypercarnivorous species may have wide, robust skulls, with broad zygomatic arches and deep jaws (Figure I-3; Damasceno et al., 2013; Grossnickle, 2020; Slater et al., 2009; Van Valkenburgh, 2007) and species that consume smaller prey or have more generalist diets may display a longer rostrum and a narrower jaw (Slater et al., 2009; Figure I-3).

Figure I-1: Temporalis (pink) and masseter (teal) muscles of the skull. The temporalis muscle attaches from the braincase through the zygomatic arch and attaches to the upper portion of the coronoid process of the mandible. The masseter muscle attaches from the mandibular masseteric fossa to the zygomatic arch (Elbroch, 2006). Domestic dog (*Canis lupus domesticus*) cranium and mandible, MSB specimen 40048.

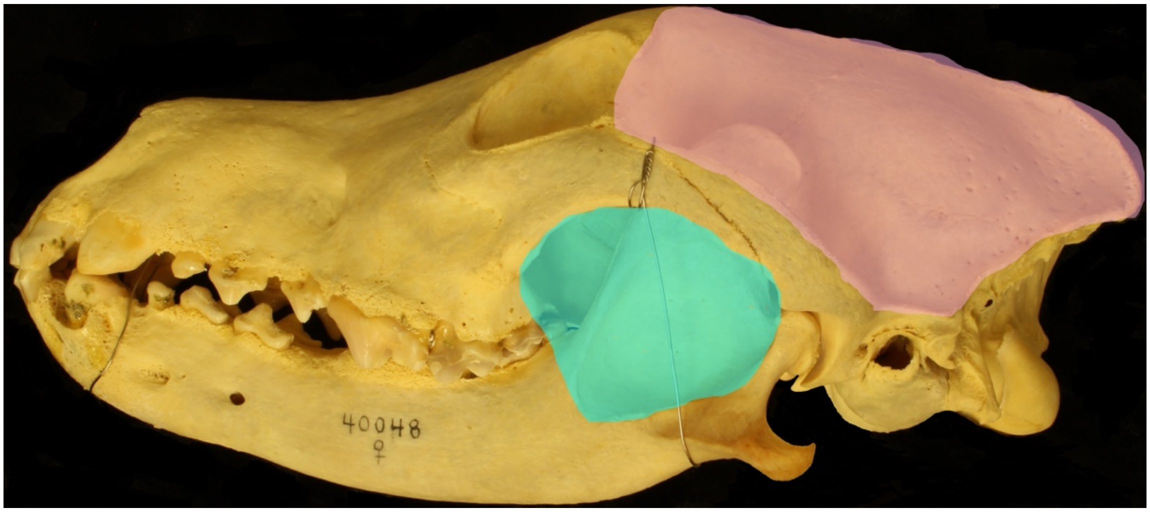


Figure I-2: Anatomical regions associated with the masseter and temporalis muscles. A. Cranium, B. Mandible. Mexican wolf (*Canis lupus baileyi*) cranium and mandible, MSB specimen 65673.

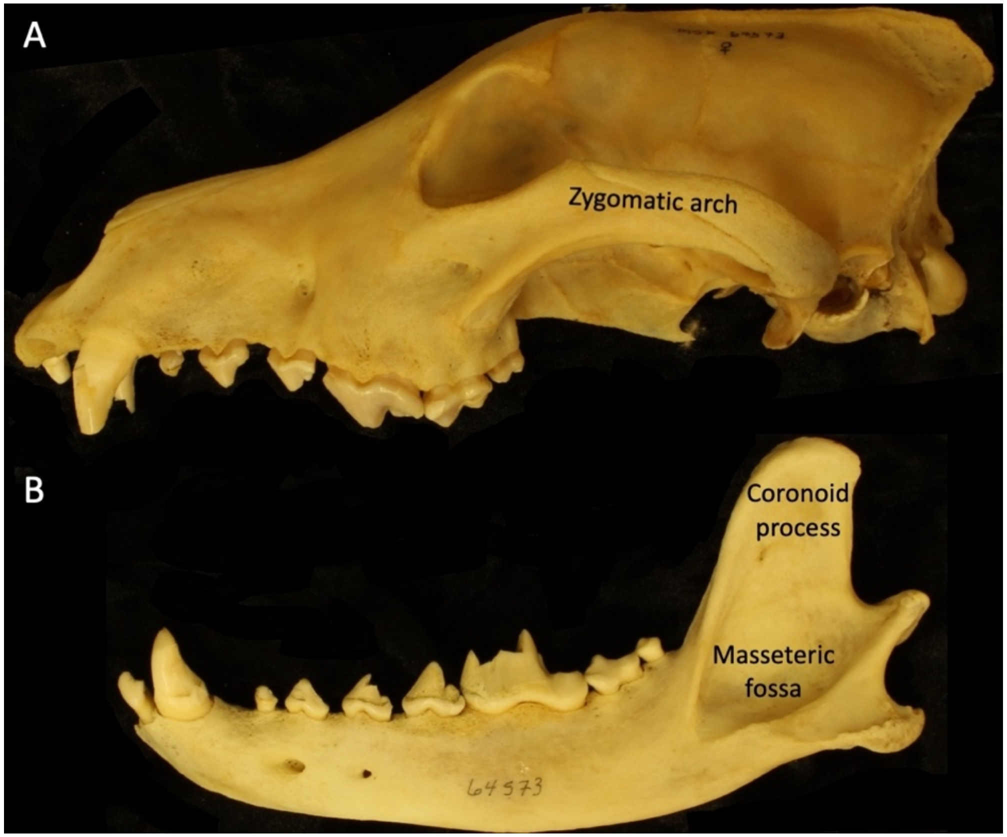
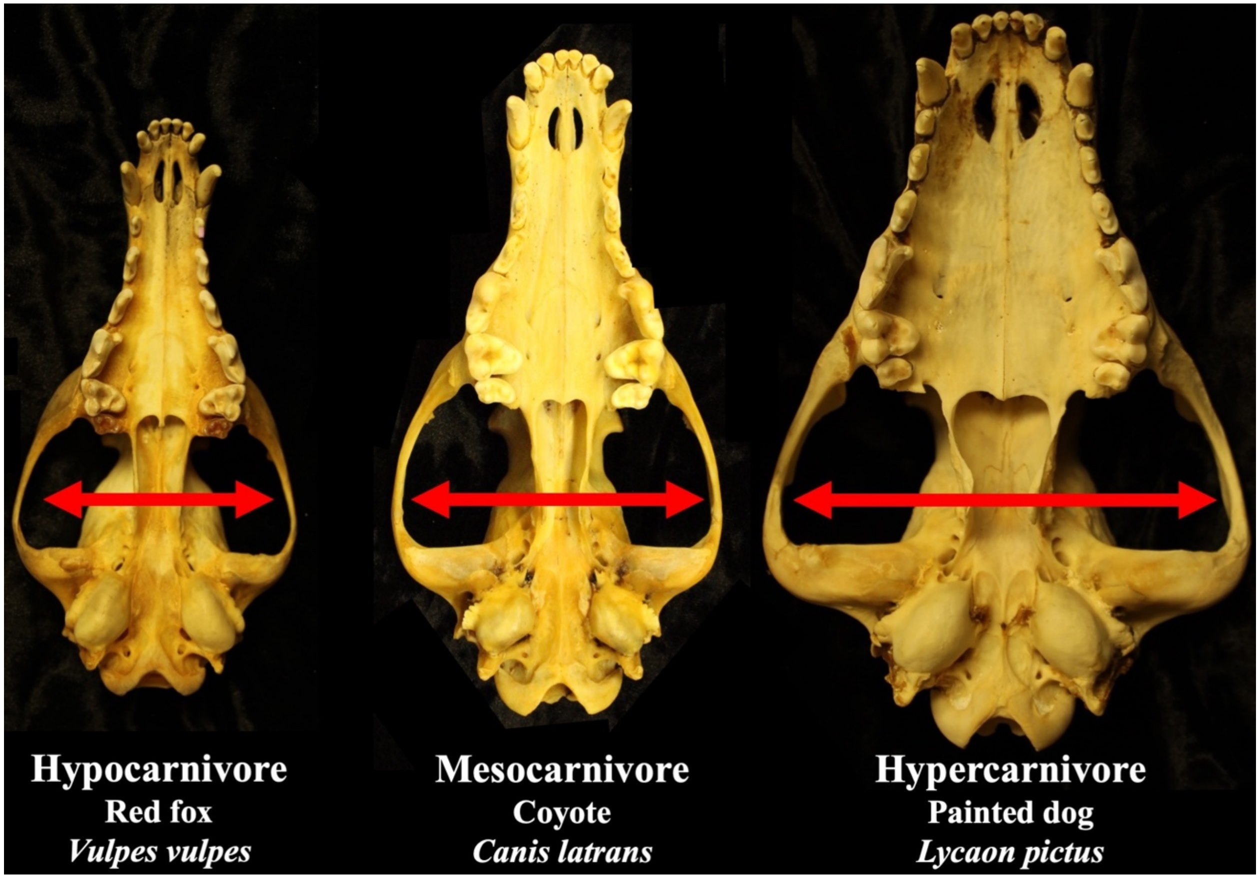


Figure I-3: Relative zygomatic breadth (indicated by the red arrows) of differing carnivory types. Hypocarnivores have the narrowest zygomatic breadth and the weakest bite force, whereas hypercarnivores typically have the widest zygomatic breadth and the strongest bite force. Mesocarnivore zygomatic arches and bite forces are between those of hypo- and hypercarnivores.



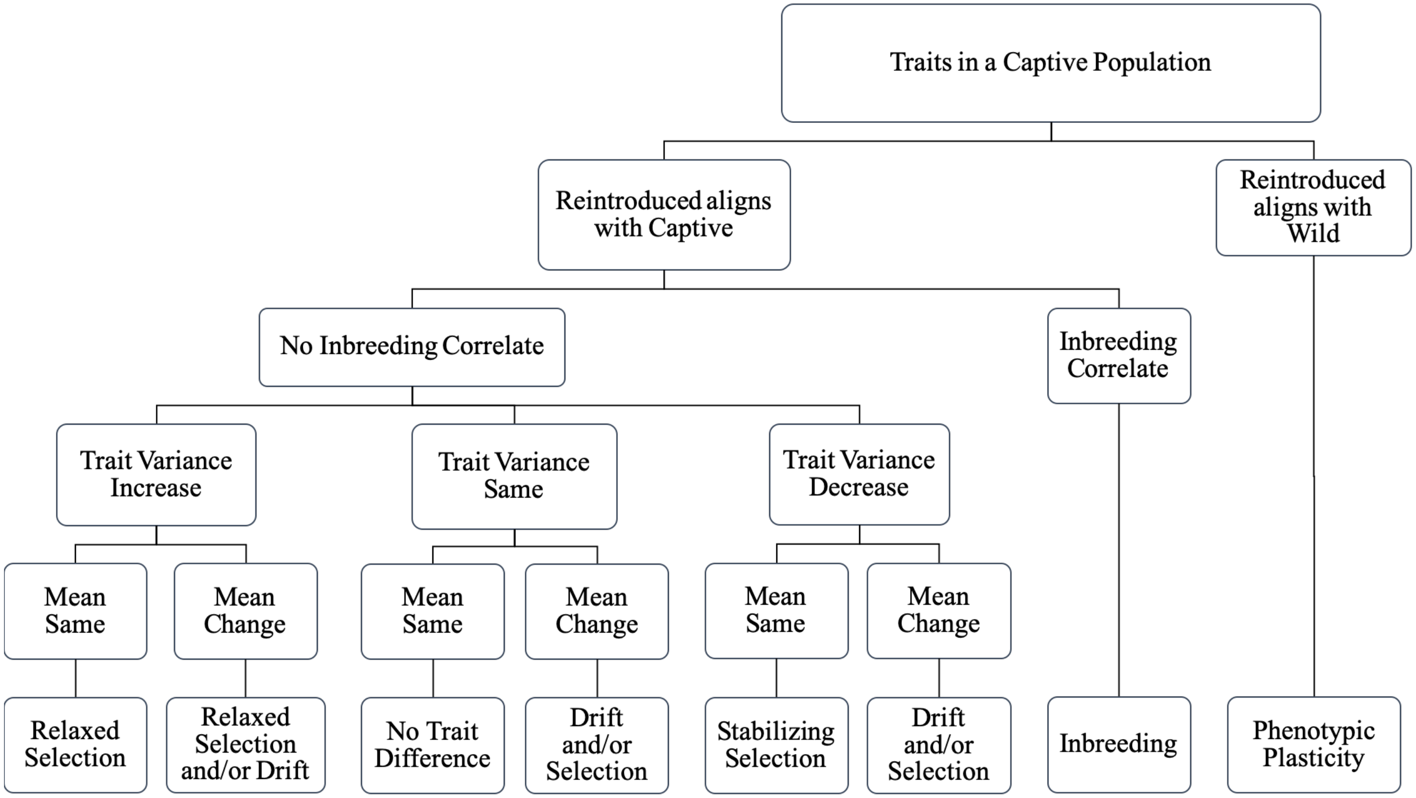
Mechanisms of Change

When small populations become genetically isolated and exposed to new environments, substantial morphological changes may arise within the population relatively quickly (Conner & Hartl, 2004; Lacy, 1987; Lande, 1986; Millien, 2006; Pergams & Lacy, 2008). Such effects have been documented within urban and island populations of mammals, leading to changes in body size and cranial morphology (e.g., Clegg et al., 2002; Pergams & Ashley, 2001; Pergams & Lacy, 2008; Jojic et al., 2017; Yom-Tov, 2003; Yom-Tov et al., 2013). Captive animals are similarly maintained in relatively small, isolated populations and experience novel environments, which may make them prone to morphological changes (Frankham et al., 1986; Pelletier et al., 2009; Williams & Hoffman; Woodworth et al., 2002) due to natural selection, genetic drift, factors related to inbreeding, or phenotypic plasticity (Figure I-4; Frankham et al., 1986; Lacy, 1987; Lynch & O'Hely, 2001; Pelletier et al., 2009; Schulte-Hostedde & Mastromonaco, 2015; Williams & Hoffman, 2009; Willoughby et al., 2015). However, these mechanisms are not mutually exclusive; captive populations may respond to multiple mechanisms of change and different traits may respond to different mechanisms (Figure I-4; Conner & Hartl, 2004; McPhee & Carlstead, 2010).

Many of the selection pressures that exist in wild environments are relaxed in captivity and may lead to a greater degree of trait variance among captive populations (McPhee & McPhee, 2012). Unlike wild populations, for example, captive animals that lack the ability to evade predators or capture prey will not suffer fitness consequences given that such traits are generally not necessary in captivity (Bryant & Reed, 1999; Håkansson & Jensen 2008; Lacy, 1987; Lynch & O'Hely, 2001; Williams & Hoffman, 2009). Captive animals also have limited control of their mate choice, potentially neutralizing the consequences of sexual selection, and in the most extreme cases, where females are artificially inseminated, sexual selection pressure is removed altogether (Courtney-Jones et al., 2018; Lacy, 1987; Schulte-Hostedde & Mastromonaco, 2015; Van den Berghe et al., 2012; Williams & Hoffman, 2009). The relaxation of sexual selection among captive mink (*Neovison vison*), for example, has been associated with a loss of cranial sexual dimorphism (Lynch & Hayden, 1995).

Captive environments may also exert unique pressures whereby animals that respond poorly to captivity may suffer fitness consequences due to increased mortality (e.g., injuries resulting from fear behaviors, a failure to eat or drink, hypertension related ailments) or decreased reproductive success (e.g., behavioral and hormonal abnormalities; Barnes et al., 2002; Bremmner-Harrison et al., 2004; Mason et al., 2013; Terio et al., 2004; Willoughby et al., 2015). Although conservation-based captive facilities avoid artificial selection for specific traits, a form of unintentional selection may occur whereby the most human-tolerant animals have the highest fitness (Bremner-Harrison et al., 2004; Frankham et al., 1986; Shepherdson, 1994; Snyder et al., 1996). For instance, small captive felids that interact most frequently with their animal keeping staff have the highest reproductive success, which may suggest selection for human-affiliation if the trait is heritable (Mellen, 1991; Shepherdson, 1994).

Figure I-4: Probable mechanisms driving morphological change for populations in captivity and the key predictors. This figure is not meant to inform the absolute causes of morphological changes in captive populations, but may be a useful indicator of probable mechanisms that can be attributed to observed changes in variation across generations between captive and wild populations. While multiple mechanisms can be responsible for the changes observed in captive populations, this figure is designed to help implicate the most likely mechanisms, which are not necessarily intended to be mutually exclusive.



Although AZA-accredited institutions design breeding recommendations to maintain genetic diversity, captive populations are unavoidably plagued by small population sizes and reduced genetic diversity that is not representative of the wild population, potentially resulting in the effects of inbreeding or genetic drift (Athrey et al., 2018; Ballou et al., 2010; Lacy, 1987; Lynch & O'Hely, 2001; Williams & Hoffman, 2009; Willoughby et al., 2015). Among captive populations, inbreeding has been associated with a reduced lifespan and body size, as well as increased internal deformities and morphological differentiation; however, relatedness coefficients are typically closely monitored to avoid these effects in zoos (Ballou et al., 2010; Wayne et al., 1986; Willoughby et al., 2015). Genetic drift has been similarly associated with changes in cranial and mandibular size and shape among captive mammal populations (McPhee, 2004). Changes that occur in small captive populations are frequently attributed to drift, particularly given that the founding members of such populations typically supply a disproportionate amount of genetic material to future generations, potentially leading to rapid, random changes in the captive population (Athrey et al., 2018; Ballou et al., 2010; Lacy, 1987; Puzachenko & Korablev 2016; Schulte-Hostedde & Mastromonaco, 2015). Unfortunately, threatened and endangered species may be particularly prone to these effects given the likelihood that their wild populations underwent a bottleneck prior to forming their captive population (Kraaijevelt-Smit et al., 2006).

The most frequently implicated cause of morphological change in captivity is associated with phenotypic plasticity. Phenotypically plastic traits, especially those associated with the skull (size, bones, or muscles; Figure I-1), can show rapid change due to dietary differences in captivity (Antonelli, 2015; Curtis et al., 2018; Fitch & Fagan, 1982; Hartstone-Rose et al., 2014; Hollister, 1917; O'Regan & Kitchener, 2005). Several studies have documented corresponding cranial changes in direct association with diet texture (Corruccini & Beecher, 1982; Lieberman et al., 2004). These trends are related to Wolff's Law, which states that the shape of living bones is formed, in part, due to the repeated application of mechanical stressors, such as muscles (Wolff, 1892). The shape of highly muscularized anatomical areas is related to the repeated application of force (Wolff, 1892). In fact, as tissue, bones can exhibit plasticity throughout an organism's lifetime in response to these effects, where adult animals may experience a form of bone 'remodeling' provided a strong enough mechanical force or the sudden absence of such force (Anderson et al., 2014; Curtis et al., 2018; Ehrlich & Lanyon, 2002; Hall & Witten, 2019; Jones et al., 1977; Pead et al., 1988). For example, Jones et al. (1977) found significant differences between the dominant and non-dominant arms of professional tennis players, where the heightened musculature of their serving arm led to distinct differences in the thickness of the humerus bones of each individual.

Given the extreme abrasive qualities of certain diets, plastic cranial changes may be most acutely experienced in captivity by species that consume particularly tough materials such as bones, bamboo, or large-bodied animals (Curtis et al., 2018; Hartstone-Rose et al., 2014). Improved captive husbandry may reduce the cranial differentiation associated with captivity. Over the past several decades, the nutrition and diet of captive animals has become a central focus in zoo animal husbandry (Dierenfeld, 1997; Oftedal

& Allen, 1996; Vosburgh et al., 1982). Although captive carnivoran diets have historically consisted of soft dog or cat food, the enhanced efforts to improve nutrition and provide diets with appropriate abrasive qualities has led to an overall improvement in captive conditions (Bond & Lindburg, 1990; Haberstroh et al., 1984; Kapoor et al., 2016; Dierenfeld, 1997; Oftedal & Allen, 1996). In fact, it has been suggested that these improvements have potentially reduced the plastic cranial changes associated with captivity, although it has been argued that these improvements apply primarily to nutrition and that captive diet texture has not been adequately addressed (Kapoor et al., 2016; Spartaco & Kitchener, 2007).

Dissertation Research

This dissertation is broadly designed to examine the impacts of captive environments on morphological variation of canids and other mammalian species to assess some of the standing hypotheses in the field. I employ a meta-analysis to evaluate trends in studies that test differences in morphology between captive and wild populations by examining the literature and quantitatively assessing reported effect sizes (Chapter II). The results of the meta-analysis establish the commonality, directionality, and magnitude of cranial differentiation in captivity across mammalian taxa. This study also assesses the hypothesized predictor variables (including trophic level, dietary breadth, and home range size) associated with those changes to establish the species most at risk of cranial changes in captivity. Similarly, the examination of multiple different canid species establishes the prevalence and nature of the morphological changes that occur in captivity across Family Canidae and tests the role of carnivory type in the morphological variation among captive populations and their wild counterparts (Chapter III). Although isolated studies have examined the impacts of captivity for a single species or several closely related species, none have taken a larger approach to encapsulate the overall changes across species groups. Likewise, by examining the morphological trends of wild, captive, and reintroduced Mexican wolves, this dissertation helps to illuminate the mechanisms that drive changes in captive populations and the role of heritability, inbreeding, and phenotypic plasticity in those shifts (Chapter IV). In doing so, this dissertation tests several long-standing hypotheses associated with the morphology of captive mammals and helps to resolve the nature of these trends. Specific hypotheses are addressed below.

Chapter II

The morphology of captive specimens has been examined for over a century across several different species and although trends appear to arise in these studies, it is difficult to ascertain whether the changes represent predictable morphological shifts in captive populations. To assess these questions, Chapter II of my dissertation employs a meta-analysis examining the published literature on cranial differentiation among captive mammals. The objectives of this study are: 1. Address whether captive mammals show predictably distinct cranial morphology, with specific examinations of the significance, magnitude, and directionality associated with these trends. 2. Assess ecological covariates to develop a better understanding of whether we can predict which species will display the greatest morphological differentiation in captivity. In particular, this study examines trophic level, dietary breadth, and home range size of species with their changes in captive populations to understand which attributes may make species more prone to a phenotypic response.

Chapter III

The family Canidae in the mammalian order Carnivora is composed of 39 extant species of dogs, wolves, foxes, coyotes, and jackals, including hypo-, meso-, and hypercarnivorous species, with correspondingly diverse cranial morphology (Burgin et al., 2018; Damasceno et al., 2013; Fleming et al., 2017; Meloro et al., 2015; Slater et al., 2009). Cranial morphotypes typical of a species may become expanded and modified due to urbanization and selective breeding. For example, red foxes (*Vulpes vulpes*) residing in or near human-modified habitats, such as urban centers or agricultural fields, develop distinct cranial forms (Jojic et al., 2017; Simonsen et al. 2003; Yom-Tov, 2003; Yom-Tov et al., 2003; Yom-Tov et al., 2007; Yom-Tov et al., 2013). Artificial selection has also been highly effective at exposing novel cranial morphology among domestic dogs, whose occupation within cranial morphospace is much more extensive than the rest of Order Carnivora combined (Drake & Klingenberg, 2010). Similarly, Russian farm-fox experiments revealed unique cranial morphology (among a variety of other phenotypic differences) associated with *V. vulpes* bred for human-affiliative behaviors (Trut, 1999; Trut et al., 2009). This suggests that in addition to displaying a great deal of diversity, canid cranial morphology is also capable of exploring new regions of morphospace when presented with unique conditions.

Chapter III of this dissertation uses geometric morphometric techniques to examine the morphological variation in captive populations of 15 different canid species representing diverse dietary preferences. In particular, the objectives of Chapter III are: 1. Assess the overall morphology of captive canid populations, many of which have not been examined in prior studies, to note any differentiation between captive and wild populations within individual species and document the nature of those morphological changes. 2. Assess the commonality and directionality of these trends across species in an effort to develop a better understanding of the functional impacts of the morphology of captive animals. 3. Assess captive changes in terms of the carnivory type, where hypercarnivores are expected to display the largest magnitude of change. 4. Assess whether the morphological differentiation of captive and wild specimens has remained consistent over the years the animals were captive. Based on improved husbandry practices, captive and wild population differentiation is expected to be reduced among more recent specimens. By examining these questions, this study broadly helps to determine whether variation in captive and wild populations is consistently differentiated across canid species and the ways in which carnivory type may influence those changes.

Chapter IV

Few species have received the high level of intensive captive breeding and restoration efforts as the Mexican wolf (*Canis lupus baileyi*). By 1976, wild populations of these wolves were nearly extinct due to eradication attempts (Hedrick & Fredrickson, 2010; Kalinowski et al., 1999; Lynch, 2005). However, captive breeding efforts led to the successful reintroduction of these animals to portions of their wild range starting in 1998 (Greely, 2018; Hedrick & Fredrickson, 2010; Kalinowski et al., 1999). The well-recorded pedigree of the captive and reintroduced populations along with the preservation of individual specimens has provided a unique opportunity to examine some of the standing hypotheses in captive animal research using Mexican wolves as an emerging model system.

Using geometric morphometric techniques on cranial and mandibular specimens, Chapter IV of this dissertation documents the morphological differences between the captive, reintroduced, and historical wild populations of Mexican wolves. In doing so, this study examines: 1. Whether there is a significant difference between the wild and captive individuals of this species and the number of captive generations required for these differences to arise in captivity, if at all. 2. Whether the reintroduced individuals align more closely with the wild population, as expected if the changes are due purely to phenotypic plasticity, or if they align more closely with the captive individuals as expected due to genetic drift or selection pressures occurring in captivity. 3. Examine the number of reintroduced generations the captive traits are retained. 4. Document the nature of the cranial changes, whether they are related primarily to shape or size, whether they are more acutely experienced among males or females, and whether they are translated into a change in the magnitude of sexual dimorphism for the species. By examining the captive, reintroduced, and wild populations of Mexican wolves, this study helps to define the changes that may occur in captivity, the potential mechanisms behind these shifts, and highlights the ways in which morphological variation in captive populations may impact conservation initiatives.

Summary

Zoos can act as genetic reservoirs for rare and endangered species; however, loss of genetic diversity and other evolutionary changes among captive populations could hinder their contribution to conservation. With increasing habitat losses, a higher burden is placed on such institutions, requiring additional captive generations before a species might be reintroduced, potentially resulting in an accumulation of morphological changes in captive populations, which may increase the likelihood of reintroduction failure (Frankham, 2008; Håkansson & Jensen, 2005; Snyder et al., 1996; Williams & Hoffman, 2009; Woodworth et al., 2002). Although most captive animals will never be considered for reintroduction, those that are represent a massive investment of resources (Frankham, 2008; Hutchins et al., 2003). For example, the total cost of the Mexican wolf recovery and reintroduction plan is estimated at over $178 million by 2043, when the species is expected to stabilize (US Fish and Wildlife Service, 2017). In staggering contrast to the Herculean effort involved in a reintroduction, success can be exceedingly limited, with some estimates suggesting a roughly 11% success rate (Beck et al., 1994; Frankham, 2008). Causes of these failures have been primarily linked to inappropriate behavior (e.g., boldness toward humans, inability to avoid danger, differences in foraging behaviors; Bremner-Harrison et al., 2004; Conde et al., 2011; Grueber et al., 2017; Lei et al., 2015; Stoinski et al., 2003). While morphology may change in captive populations (O'Regan & Kitchener, 2005) and such changes are strongly associated with functionality (Christiansen & Wroe, 2007; Meloro et al., 2017; Wroe et al., 2007), these topics are rarely studied in the context of reintroduction success. By examining morphological shifts more closely and understanding the long-term impacts of captivity, we may be able to mitigate these effects in the future and increase the potential for reintroduction successes. Captive animals often represent the last hope for a species. By understanding whether they fundamentally change in captivity, the mechanisms that drive those changes, and their impacts, we can better inform husbandry, conservation, and future morphological investigations.

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CHAPTER II

CRANIAL MORPHOLOGY OF CAPTIVE ANIMALS: A META-ANALYSIS OF MAMMAL SPECIES

Introduction

The size and shape of skulls of captive mammals may differ from wild populations (Curtis et al., 2018; Hartstone-Rose et al., 2014; O'Regan & Kitchener, 2005). Differences have included changes in cranial length and width (Hollister, 1917; Hartstone-Rose et al., 2014), sagittal crest height (Duckler, 1998), and mandibular morphology (Kamaluddin et al., 2019). However, the nature of the cranial differences that arise in captivity have remained unclear. Examining animals housed in zoos provides a unique opportunity to explore cranial differences between wild and captive mammal populations. While zoo animals are often maintained in an effort to preserve species as faithful representatives of their wild counterparts, morphological changes occurring in captivity could work against that goal (Conway, 1995; Lacy, 2013; Patrick et al., 2007; Rudnick & Lacy, 2008; Willoughby et al., 2015). Although previous literature has assessed the differences in cranial morphology of captive mammals compared to wild populations, it is unclear whether there is a common effect associated with the directionality or magnitude of the changes occurring among the traits that have been studied.

The morphological differentiation of captive populations from their wild counterparts may be the result of inbreeding or evolutionary processes (e.g., genetic drift, selection) or phenotypic plasticity (defined as the ability of a single genotype to display multiple, environmentally driven phenotypes), or some combination of these factors (DeWitt & Scheiner, 2004; Frankham, 2008; Lacy, 1987; Hall & Witten, 2019; Lynch & O'Hely, 2001; Pelletier et al., 2009; Schulte-Hostedde & Mastromonaco, 2015; Whitman & Agrawal, 2009; Williams & Hoffman, 2009; Woodworth et al., 2002). For example, correlations have been noted between a decrease in body size and inbreeding among captive wolves (Hedrick & Frederickson, 2010; Ibanez et al., 2011; Laikre & Ryman, 1991). Molecular signatures of inbreeding and genetic drift have been noted among white-footed mice (*Peromyscus leucopus*) maintained in captivity (Willoughby et al. 2015; Willoughby et al., 2017). Lynch & Hayden (1995) suggested the cranial changes they observed among farmed American mink (*Mustela vison*) were largely the result of differing selection pressures. Abnormal skull morphology of several captive mammals, including coyotes (*Canis latrans*; Curtis et al., 2018), African lions (*Panthera leo*; Hartstone-Rose et al., 2014), and Japanese macaques (*Macaca fuscata*; Kamaluddin et al., 2019) have all largely been attributed to phenotypic plasticity. In captivity, unusual phenotypes may be expressed as a plastic response to environmental factors related to novel diet textures (Curtis et al., 2014; Hollister, 1917), nutrient availability (Leigh, 1994; Terranova & Coffman, 1997), or any other factors unique to the captive environment.

Cranial responses to a captive environment may be explained by differences in muscle usage, which may impact osteological traits (Cheronet et al., 2016; Hollister, 1917; Wisely et al., 2005; Wolff, 1891). A soft diet requires less musculature and therefore less mechanical stress is applied to the cranial bones, potentially resulting in a bone whose difference is greater than the variance of the mean morphotype (Cheronet et al., 2016; Hollister, 1917; Wisely et al., 2005). Reduced mechanical constraint is also associated with reduced covariation between internal and external cranial morphology (Curtis et al. 2018). Therefore, a species whose wild diet is composed of particularly tough items may be more prone to a morphological response given that captive diets are frequently softer than what the animal might consume in the wild (Curtis et al., 2018; Hollister, 1917; Kapoor et al., 2016).

In addition to diet texture, the shape of cranial bones may also be influenced by other factors related to captivity, such as stereotypic behaviors. Stereotypies are repetitive behaviors that serve no obvious function (Mason, 1991); however, they may impact morphology due to the frequent, abnormal muscle usage involved in their performance (Duckler, 1998; Hartstone-Rose et al., 2014; O'Regan & Kitchener, 2005; Skibiel et al., 2007). Stereotypies can include normal behaviors performed to the point of self-destruction (e.g., licking, grooming, rubbing) as well as head swinging, bar-biting, and pacing (Mason, 1991; Mason, 2010; Mason et al., 2013; McPhee, 2002). Once incorporated into an animal's behavioral repertoire, stereotypies are performed repeatedly and with great frequency, potentially impacting morphology (Carlstead, 1991; Crast et al., 2014; Mason, 1991; Shepherdson et al., 2013). Stereotypic overgrooming, for example, has been correlated with changes in the cranial morphology of captive tigers, where captive individuals display a flattened sagittal crest associated with the heightened muscle usage involved in incessant grooming behaviors (Duckler, 1998). Although stereotypies are quite common among zoo animals, they are rare in wild populations (Mason, 1991; Mason, 2010). These behaviors tend to be most common among captive animals with large wild home ranges (Clubb et al., 2009; Kroshko et al., 2016; Mason & Veasey, 2010) and those with highly specialized diets or food acquisition behaviors (Lyons et al., 1997; Mason, 1991; McPhee, 2002).

The degree to which morphology differs in captivity compared to wild populations may vary between species. For example, while African lions tend to show rather drastic, reliable morphological changes associated with an increase in zygomatic breadth (Hartstone-Rose, 2014; Hollister, 1917; Zuccarelli), house mice (*Mus musculus*) show little morphological change in captivity (Courtney Jones et al., 2018). Even closely related taxa may differ in the degree of change that they exhibit once in captivity (Groves, 1982; Kamaluddin et al., 2019; Sargusty et al., 2014) possibly due to species ecology where certain traits may predispose species to a specific captive response. The likelihood of morphological changes occurring in captivity may increase when an animal’s habitat is difficult to replicate, leading to heightened stress behaviors or when diets are difficult to accommodate (Clubb & Mason, 2003; Curtis et al., 2018; Kroshko et al., 2016). It has been suggested that hypercarnivory (a diet that consists of roughly 70% vertebrate prey; Damasceno, et al., 2013; Van Valkenburgh, 2007), for instance, may predispose species to more extreme morphological differentiation in captivity

(Curtis et al., 2018; Hartstone-Rose et al., 2014) because skull shape is strongly linked to dietary function among wild carnivores (Slater et al., 2009; Van Valkenburgh, 2007) and diet in captivity may be drastically different than it is in the wild (Kapoor et al., 2016). Similarly, species that consume large prey have comparatively round skulls, where bowed zygomatic arches and heightened sagittal crests enable enhanced musculature and increased jaw strength (Elbroch, 2006; Slater et al., 2009; Van Valkenburgh, 2007). Thus, if appropriate diets are not provided, then differentiation in cranial morphology may occur in captivity (Corruccini & Beecher, 1982; Curtis et al., 2018; Hollister, 1917; Hartstone-Rose et al., 2014).

While the effects of captivity are a recurrent theme in morphological research, the design of these studies vary, making it difficult to draw substantive and comprehensive conclusions about the nature of morphological changes occurring in captivity. The literature is generally limited to case studies of single species, several closely related species, or computational models predicting phenotypic trajectories. Here, I use a meta-analytic approach examining effects reported in the existing literature to identify whether there are identifiable trends to help identify characteristics of species at the greatest risk of morphological change in captive populations. I address whether captive mammals show similar changes in cranial morphology, with particular attention on the directionality and magnitude of changes across taxa as well as notable co-variates associated with species ecology. The magnitude and directionality of morphological changes among captive populations are expected to vary based on species ecology, where the largest morphological changes are expected to occur among species whose diets and habitats are particularly difficult to accommodate in captivity (e.g., large home range size, carnivorous, narrow dietary breadth) given that they may be responding to similar captive stressors; these species are also expected to display similar morphological changes (e.g., wider zygomatic breadth). By examining these hypotheses and developing a more comprehensive understanding of morphological changes that occur in captivity, these effects may be addressed with updated husbandry practices to help ensure the long-term success of captive populations.

Methods

Literature Search and Meta-analysis Study Design

I conducted phylogenetic meta-analyses to examine differences in cranial morphology of captive mammals compared to their wild counterparts using previously published literature. I focused on three traits: skull length and skull width (traits associated with size) and the ratio of skull length-to-width (a trait associated with shape). These three traits are important because they are intimately linked to cranial size and functionality (Ellis et al., 2009; Law et al., 2018). I conducted an exhaustive search of the literature using search functions in Web of Science and Google Scholar. Searches were conducted using the key terms, ‘zoo’ or ‘captive’, ‘mammal’ or ‘animal’, and ‘morphology’ or ‘size’ and were completed in December 2019. Additional studies were located by searching the reference sections of literature on the topic. Literature searches were refined to only including studies which, 1) provided comparative size and/or shape data of captive and wild mammals, 2) assessed non-domesticated captive species (as described in Mason et al., 2013, following the species listed in Larson & Burger, 2013 and Zeder et al., 2012), and 3) assessed captive populations that had not experienced intentional artificial selection. Captive facilities included zoos, laboratories, or other breeding centers. Animals that were bred for specific traits (e.g., farm populations bred for size, laboratory colonies bred for particular attributes) were excluded from these analyses. I downloaded data associated with the publications and when relevant data were unavailable with the publication, I contacted the corresponding author of the study with a request to share available data. If data could not be obtained, these studies were removed from analyses (n = 2).

Search results revealed 506 potentially relevant publications, of which 13 met the complete search criteria (see criteria above) and included all applicable data required for inclusion in at least one of the size or shape-related traits (Table II-1). Given that the methods used in most studies (caliper measurements) have not improved considerably over the past century, the dates of the studies included in these analyses ranged from 1894 to 2018. In total, these publications included 59 comparative relationships between wild and captive populations (19 shape and 40 size-related variables), across 17 species, representing five mammalian orders. The most well-represented groups included primates (n = 5) and carnivorans (n = 5), although this analysis also included ungulates (n = 4), rodents (n = 2), and a marsupial (Table II-1). Different studies included in these meta-analyses reported varying shape and size measures, so each meta-analysis included a slightly different collection of taxa based on what was reported in each publication (Table II-1).

In total, my study consisted of nine meta-analyses (as described below). These included analyses of size (skull length and width) and shape (skull length-to-width) to examine the magnitude and directionality of changes. For each assessment of cranial size and shape, data were analyzed with and without outlying species and with an absolute value applied to the standard effect sizes (a standardized statistic that encodes quantitative data from multiple studies into a common form; Lipsey & Wilson, 2001). Applying an absolute value can inflate model significance and create artificially narrow confidence intervals, therefore results associated with such analyses should be interpreted cautiously (Morrissey, 2016; Paulus et al., 2013). In spite of this understanding, I chose to analyze these data with an absolute value because the direction of change in traits appeared inconsistent, yet there were many trait-species combinations that had no 95% confidence overlap with zero indicating change may be indicative of populations in captivity. Analyzing the absolute value of an effect allows for an overall test of change. Black-footed ferrets (*Mustela nigripes*) were frequent directional outliers (see below); thus, meta-analyses were conducted with and without this species. Each of these analyses were conducted as independent models with and without the inclusion of ecological covariates (Table V-1). Covariates may influence the degree of morphological differentiation between captive populations and their wild counterparts. All analyses followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses

Table II-1: Studies and species included in each meta-analysis. Meta-analyses include skull length (L), skull width (W), and the ratio of skull length-to-width (L:W). For full citations, please see the reference section.

Study	Order	Family	Species	L	W	L:W
Aeschbach et al., 2016	Rodentia	Caviidae	*Hydrochoerus hydrochaeris*			X
Antonelli, 2015	Carnivora	Mustelidae	*Mustela nigripes*		X	X
Curtis et al., 2018	Carnivora	Canidae	*Canis latrans*	X	X	X
Groves, 1966	Perissodactyla	Equidae	*Equus asinus,* *Equus hemionus*	X	X	X
Groves, 1982	Perissodactyla	Rhinocerotidae	*Dicerorhinus sumatrensis,* *Rhinoceros unicornis*	X	X	X
Guay et al., 2012	Dasyuromorpha	Dasyuridae	*Sminthopsis macroura*	X		
Hartstone-Rose et al., 2014	Carnivora	Felidae	*Panthera leo,* *Panthera tigris*	X	X	X
McPhee, 2004	Rodentia	Cricetidae	*Peromyscus polionotus*			X
Selvey, 2018	Primates	Lemuridae	*Lemur catta*		X	

Table II-1 Continued

Study	Order	Family	Species	L	W	L:W
Turner et al., 2016	Primates	Cercopithecidae	*Chlorocebus aethiops*	X		
van Velzen, 1967	Primates	Hominidae	*Gorilla gorilla,* *Pan troglodytes,* *Pongo pygmaeus*	X	X	X
Wisely et al., 2002	Carnivora	Mustelidae	*Mustela nigripes*	X		
Wolfgramm, 1894	Carnivora	Canidae	*Canis lupus*	X	X	X

(PRISMA) statement guidelines, which provides recommendations for the inclusion of studies in meta-analyses, as well as parameters for data extraction (Moher et al., 2009).

Standard Effect Size

Data including sample size, mean, standard deviation, standard error, or t-statistics were extracted from each study and used to calculate a standard effect size for each variable (including skull length, skull width, and skull length-to-width; Table V-2). When no variance measures were provided (Groves, 1966; Groves, 1982), the prognostic method, a conservative estimate of missing variance terms, was applied to estimate missing standard deviations (Table V-2) using the sample size and variance data available in the other studies included in this dataset (see the following for a review of these methods: Ma et al., 2008; Ma et al., 2010). To calculate the corresponding standard effect size of shape ratios, pooled standard deviations were calculated based on 10,000 permutations using the sample size, mean, and standard deviation of both linear measures. To assess the effects across studies, Pearson's correlation coefficient (r) was calculated as a measure of the standard effect size (Cohen, 1988) and converted to Fisher's Z, a normality transformation typically applied to meta-analyses (Adams, 2008; Becker et al., 2018; Fisher, 1928; Rosenthal, 1986).

Both traditional (n = 11) and geometric morphometric (n = 2) studies were assessed in these analyses (Table V-2). Linear measures found in traditional morphometric studies were used to estimate specimen size. To derive shape variables from traditional morphometric studies, ratios of linear measures (e.g., skull length to skull width) were taken. Skull shapes were extracted from geometric morphometric studies using the principal component (PC) scores reported in the publications. The specific shape represented by each PC was determined from publication text and figures depicting morphology (e.g., thin-plate splines).

Phylogenetic Meta-Analysis

I pruned phylogenetic trees inferred by Upham et al. (2019) to species present in each of the nine meta-analyses using *picante* (Adams, 2008; Kembel et al., 2010). Pagel's λ was used to assess phylogenetic signal of standard effect size for each of the variables I evaluated (Freckleton et al., 2002, Pagel, 1994; Pagel, 1997) by assessing 2,000 randomly selected phylogenies from Upham et al. (2019) with *geiger* (Harmon et al., 2008). Pagel's λ results are reported as the mean and standard deviation of all iterations (Table V-3). These tests revealed that phylogenetic signal was present in the standard effect sizes and thus I needed to account for evolutionary non-independence in meta-analysis models (Freckleton et al., 2002; Garamszegi et al., 2012; Table V-3). Phylogenetic covariance was calculated for each phylogeny as described in Adams (2008).

Multi-variate phylogenetic meta-analyses were conducted with *Metafor* (Adams, 2008; Quintana, 2015; Viechtbauer, 2010). Studies included in my meta-analyses reported values for single sexes, individual values for each sex, or pooled samples of both sexes. Thus, I incorporated a random effect variable of sex in meta-analysis models, in addition to other typical random effects variables of study and species (Becker et al., 2018; Viechtbauer, 2010). The ‘species’ random effect accounted for the uneven inclusion of species in the analyses and the phylogenetic covariance was specified by the correlation matrix (Viechtbauer, 2010). The ‘study’ random effect accounted for the variation across individual studies. Each individual analysis was conducted using 2,000 randomly selected phylogenies from Upham et al. (2019) and reported as mean and standard deviations of those iterations. All analyses were conducted in R version 3.6.1 (R Core Team, 2019).

Due to the possibility of publication bias (the tendency for significant results to be disproportionally published; Borenstein et al., 2009; Quintana, 2015), I used funnel plots to display the distribution of standard effect sizes with corresponding variances. Asymmetry in a funnel plot is indicative of publication bias, whereas an unbiased sample will produce a relatively conical pattern of points. Egger’s regression (mixed-effect meta-regression model) was used to assess asymmetry in each funnel plot (Egger et al., 1997; Habeck et al., 2015; Murray et al., 2019; Sterne & Egger, 2005).

Life History

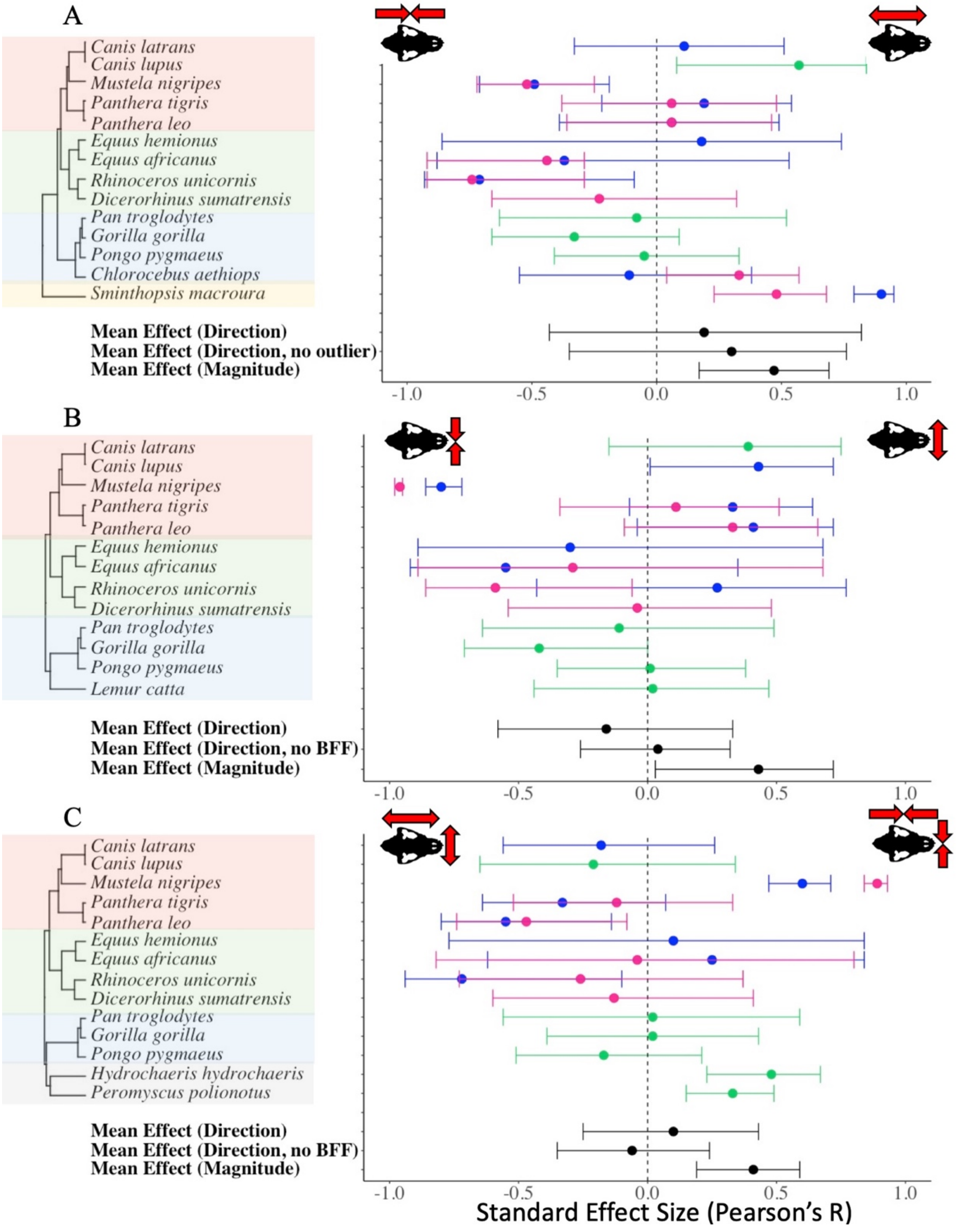
Because skull size and shape may be affected by ecological factors, I added the ecological covariates trophic level, dietary breadth, and home range size (assessed in wild populations) to evaluate the ways in which species ecology is associated with changes in morphology within captive populations (Table V-1). These ecological data were derived from the open-access PanTHERIA dataset, which is a publicly available compilation of ecological and life history traits for extant and recently extinct mammal species (Jones et al., 2009). Trophic levels included carnivorous, omnivorous, and herbivorous. Dietary breadth accounts for the number of dietary categories consumed by a species and ranges from one to eight in the PanTHERIA dataset. For these analyses, dietary breadth was parsed into three categories, including species that consume items from a single dietary category, those consuming two to three dietary categories, and those consuming four or more dietary categories. Lastly, home range sizes in the wild were recorded as the average area inhabited by a species (km^2); these values were converted to categorical variables (small, 0.01-5 km^2; medium, 11-30 km^2; and large, 55-160 km^2) based on the distribution of these values in this dataset. Home range sizes were estimated from additional sources when data were not available for a given species in PanTHERIA, including Asiatic wild asses (*Equus hemionus*; Giotto et al., 2015), vervet monkeys (*Chlorocebus aethiops*; Herzog et al., 2014), and stripe-faced dunnarts (*Sminthopsis macroura*) whose estimated home range size was inferred to be less than 0.1 km^2 based on the data available for other members of the genus in PanTHERIA.

Results

A strong phylogenetic signal was recovered in analyses of directionality assessing the effect sizes associated with skull length, skull width, and skull length-to-width (λ= 0.920, 0.791, and 0.876, respectively; Table V-3). A strong phylogenetic signal was also detected in directionality analyses conducted without black-footed ferrets (skull length λ=0.865; skull width λ=0.507; skull length-to-width λ=0.720; Table V-3). Thus, all analyses of directionality were conducted using phylogenetic comparative methods.

Black-footed ferrets were outliers in all analyses and displayed opposing trait values compared to the other carnivores in the dataset (Figure II-1). The majority of species (10 of 17) displayed distinct trait values in captive populations in at least one of the analyses (and in at least one of the sexes, if more than one sex was examined). When reported, similar morphological trends were typically displayed among both sexes within species, with some exceptions (e.g., female vervet monkeys, *Chlorocebus aethiops* displayed significantly longer crania in captive populations, although males did not; Figure II-1A). Skull length and width changes were often apparent among rodents and carnivores (including species in order Carnivora as well as the striped-faced dunnart, *S. macroura*, a carnivorous marsupial; Figure II-1). These changes were generally not detected among primates and were only apparent among female *C. aethiops* (Figure II-1). When changes were detected among carnivorous species, they were generally related to an elongation and widening of the skull (e.g., *Canis lupus, Panthera leo, S. macroura*), aside from the black-footed ferret, which displayed the opposite pattern with a shorter, narrower cranium in captivity (Figure II-1). Both rodents included in this study (*Hydrochoerus hydrochaeris* and *Peromyscus polionotus*) displayed a significant decrease in cranial length and width within the captive populations compared to their wild counterparts (Figure II-1C).

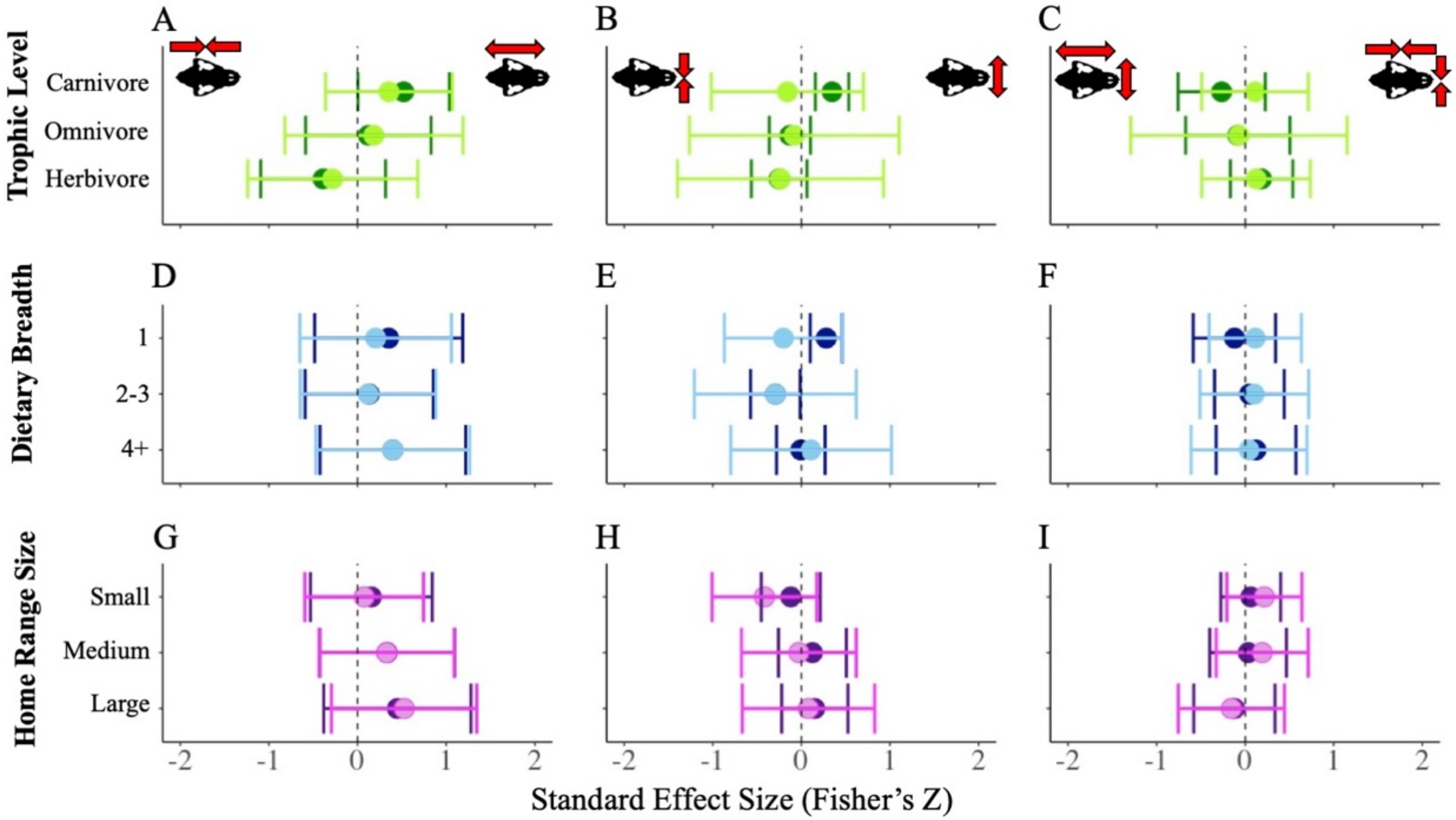
Figure II-1: Forest plots of each meta-analysis. A. skull length, B. skull width, C. skull length-to-width. The phylogeny from Upham et al. (2019) was pruned and shown to the left of each meta-analysis plot where the data point(s) for each species is shown to the right of the species name. Mammalian orders are indicated on the phylogeny, red represents Order Carnivora, green represents Order Perissodactyla, blue represents Order Primates, yellow represents Order Dasyurimorpha, and gray represents Order Rodentia. Additional taxonomic information is available in Table V-1. Colors within the plot indicate the sex of the specimens from each study, where magenta=females, blue=males, and green indicates a study that used a pooled sample of both sexes. In some cases, only one sex was present. Summary effect sizes for both directional analyses conducted with and without black footed ferrets, as well as analyses of magnitude are indicated in black. Red arrows on the skull illustrations indicate the morphology associated with the positive and negative effect sizes on each plot. Forest plot lines that do not cross the dotted zero line are associated with a significant effect (i.e. the effect is not zero).


A
Canis latrans
Canis lupus
Mustela nigripes
Panthera tigris
Panthera leo
Equus hemionus
Equus africanus
Rhinoceros unicornis
Dicerorhinus sumatrensis
Pan troglodytes
Gorilla gorilla
Pongo pygmaeus
Chlorocebus aethiops
Sminthopsis macroura
Mean Effect (Direction)
Mean Effect (Direction, no outlier)
Mean Effect (Magnitude)
-1.0
-0.5
0.0
0.5
1.0
B
Canis latrans
Canis lupus
Mustela nigripes
Panthera tigris
Panthera leo
Equus hemionus
Equus africanus
Rhinoceros unicornis
Dicerorhinus sumatrensis
Pan troglodytes
Gorilla gorilla
Pongo pygmaeus
Lemur catta
Mean Effect (Direction)
Mean Effect (Direction, no BFF)
Mean Effect (Magnitude)
-1.0
-0.5
0.0
0.5
1.0
C
Canis latrans
Canis lupus
Mustela nigripes
Panthera tigris
Panthera leo
Equus hemionus
Equus africanus
Rhinoceros unicornis
Dicerorhinus sumatrensis
Pan troglodytes
Gorilla gorilla
Pongo pygmaeus
Hydrochaeris hydrochaeris
Peromyscus polionotus
Mean Effect (Direction)
Mean Effect (Direction, no BFF)
Mean Effect (Magnitude)
-1.0
-0.5
0.0
0.5
1.0
Standard Effect Size (Pearson's R)

The inclusion of trophic level, dietary breadth, and home range size in the analyses of directionality (conducted without the black-footed ferret) suggested that certain ecological covariates were predictive of the morphological changes observed in captivity (Figure II-2). A significant difference in skull width was detected among carnivorous species ($r=0.347$) and dietary specialists (species that consume a single dietary item, $r=0.281$; Table II-2), where captive animals from these groups displayed comparatively wide skulls (Figures II-2B and II-2E). Species with moderate dietary breadths (consuming two to three dietary items) also displayed a significant morphological difference associated with skull width ($r=-0.292$; Table II-2), where captive individuals displayed a comparatively narrow cranium (Figure II-2E). Analyses of directionality conducted with the black-footed ferret did not detect differences associated with any of the size or shape traits.

Analyses of the magnitude of change similarly recovered a strong phylogenetic signal for skull length, skull width, and skull length-to-width ($\lambda= 0.692$, 0.696, and 0.414 respectively; Table II-2, Figure II-1). Meta-analysis models of the magnitude of change detected a difference for skull length ($r = 0.542$, $p < 0.05$), skull width ($r = 0.498$, $p < 0.05$), and skull length-to-width ($r = 0.434$, $p < 0.05$; Table II-2). In ecological literature, a standard effect size (r) greater than 0.1 is generally interpreted as a small effect, values between 0.3 and 0.5 are interpreted as medium effects, and values above 0.5 are considered strong effects (Garamszegi et al, 2012; Moller & Jennions, 2002).

Figure II-2: Analysis of directionality and ecological covariates. A-C. trophic level, D-F. dietary breadth, G-I. relative home range size. Columns indicate analyses of skull length, skull width, or skull length-to-width as indicated. Color saturation indicates analyses conducted with or without black-footed ferrets, where lighter colors include all species and darker colors indicate analyses conducted without the ferrets. Red arrows on the skull illustrations indicate the morphology associated with the positive and negative effect sizes on each plot. Forest plot lines that do not cross the dotted zero line are associated with a significant effect.



Analyses of magnitude revealed effects associated with the carnivorous trophic level (skull length, r=0.542, $p < 0.05$; skull width, r=0.737, $p < 0.05$; skull length-to-width, r=0.566, $p < 0.05$; Table II-2; Figure II-3A-C), the narrowest dietary breadth (skull length, r=0.375, $p < 0.05$; skull width, r=0.684, $p < 0.05$; skull length-to-width, r=0.496, $p < 0.05$; Table II-2; Figure II-3D-F), and the smallest home range size (skull length, r=0.638, $p < 0.05$; skull width, r=0.650, $p < 0.05$; skull length-to-width, r=0.568, $p < 0.05$; Table II-2; Figure II-3G-I). Magnitude analyses of skull length also revealed effects associated with herbivory (r=0.716, $p < 0.05$; Table II-2; Figure II-3A) and a moderate dietary breadth (r=0.731, $p < 0.05$; Table II-2; Figure II-3D).

Publication bias was not evident in most analyses; however, publication bias was detected in skull length analyses ($P<0.05$*) and in skull length-to-width analyses conducted with the black-footed ferrets ($P<0.05$*; Figure V-1). Publication bias has the potential to over-inflate the significance of meta-analytic models potentially leading to Type 1 errors, given that studies that recover significant results may be more likely to be published (Borm et al., 2009; Lipsey & Wilson, 2001). However, neither of the meta-analyses for which publication bias was detected recovered significant results in the meta-analyses models (Figure V-1; Table II-2), suggesting that publication bias was not an issue in this data set.

Table II-2: Results for all meta-analyses, r represents the summary effect of the model, SE represents the standard error, Z represents the Z-Statistic of the model, and LB and UB represent the lower and upper bounds, respectively, of the confidence interval. Meta-analyses assessing directionality (with and without the outlying black-footed ferret) and magnitude on skull length, skull width, and skull length-to-width (L:W). This table reports the full model results as well as the results associated with individual ecological covariates (trophic level, dietary breadth, and home range size; Table V-1). The trophic level is divided into carnivores (carn), omnivores (omn), and herbivores (herb), the dietary breadth is divided into species that consume a single dietary category (1), those that consume 2 to 3 categories (2-3), and those that consume four or more dietary categories (4+), and the home range sizes are divided into small (sm), medium (md), and large (lg) categories. Results report the mean value from 2,000 randomly selected phylogenic trees from Upham et al. (2019).

			r	SE	Z	LB	UB
Skull Length	Full Model		0.27	0.41	0.66	-0.53	1.06
(Directionality)	Trophic Level	Carn	0.41	0.49	0.82	-0.56	1.37
		Omn	0.29	0.65	0.45	-0.98	1.56
		Herb	-0.17	0.61	-0.27	-1.36	1.03
	Dietary Breadth	1	0.38	0.56	0.68	-0.72	1.47
		2-3	0.17	0.50	0.35	-0.80	1.14
		4+	0.49	0.54	0.91	-0.56	1.54
	Home Range	Sm	0.17	0.43	0.39	-0.67	1.00
		Md	0.49	0.47	1.06	-0.42	1.41
		Lg	0.69	0.49	1.40	-0.27	1.64
Skull Length	Full Model		0.32	0.37	0.88	-0.39	1.04
(Directionality w/o ferrets)	Trophic Level	Carn	0.55	0.31	1.77	-0.06	1.15
		Omn	0.20	0.41	0.50	-0.59	0.10

Table II-2 Continued			r	SE	Z	LB	UB
		Herb	-0.31	0.41	-0.76	-1.12	0.50
	Dietary Breadth	1	0.57	0.51	1.12	-0.43	1.57
		2-3	0.18	0.44	0.41	-0.67	1.03
		4+	0.49	0.48	1.03	-0.45	1.42
	Home Range	Sm	0.23	0.43	0.53	-0.62	1.08
		Md	0.49	0.47	1.05	-0.43	1.41
		Lg	0.65	0.51	1.27	-0.35	1.66
Skull Length	Full Model		0.54	0.20	**2.67****	0.14	0.94
(Magnitude)	Trophic Level	Carn	0.60	0.27	**2.19***	0.06	1.13
		Omn	0.37	0.36	1.03	-0.34	1.08
		Herb	0.72	0.36	**1.99***	0.01	1.42
	Dietary Breadth	1	0.38	0.17	**2.16***	0.03	0.72
		2-3	0.73	0.17	**4.26*****	0.40	1.07
		4+	0.23	0.20	1.48	-0.10	0.70
	Home Range	Sm	0.64	0.21	**2.98****	0.28	1.06
		Md	0.26	0.27	0.97	-0.27	0.80
		Lg	0.37	0.27	1.34	-0.17	0.90
Skull Width	Full Model		-0.16	0.25	-0.65	-0.66	0.33
(Directionality)	Trophic Level	Carn	-0.15	0.41	-0.36	-0.94	0.65
		Omn	-0.08	0.59	-0.13	-1.24	1.09

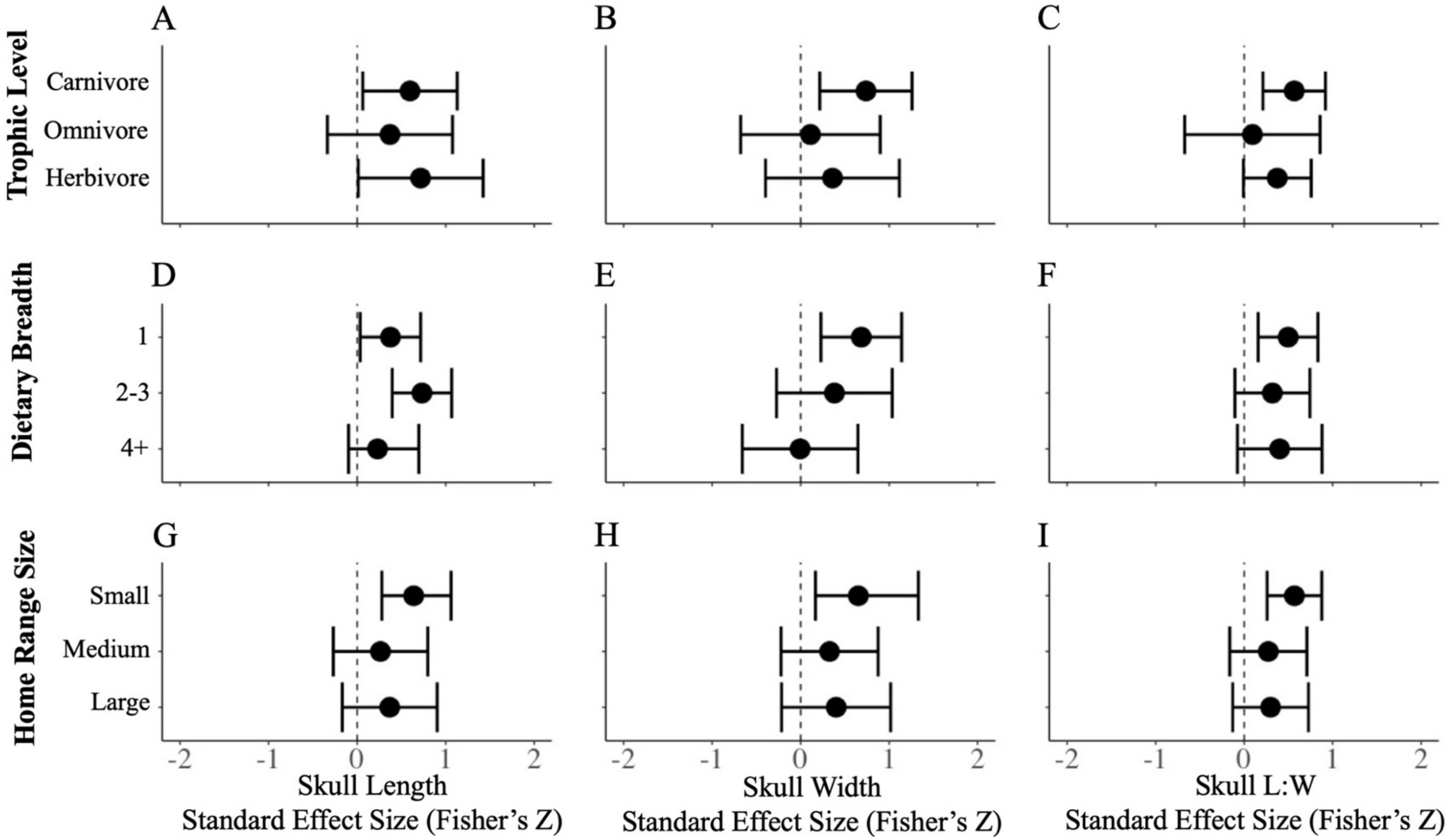
Table II-2 Continued			r	SE	Z	LB	UB
		Herb	-0.25	0.58	-0.43	-1.38	0.89
	Dietary Breadth	1	-0.20	0.34	-0.59	-0.87	0.47
		2-3	-0.29	0.47	-0.63	-1.21	0.62
		4+	0.11	0.46	0.24	-0.80	1.02
	Home Range	Sm	-0.41	0.29	-1.43	-0.97	0.15
		Md	-0.04	0.31	-0.13	-0.66	0.57
		Lg	0.07	0.36	0.18	-0.65	0.78
Skull Width (Directionality w/o ferrets)	Full Model		0.01	0.16	0.07	-0.30	0.32
	Trophic Level	Carn	0.35	0.10	**3.61*****	0.16	0.54
		Omn	-0.13	0.12	-1.08	-0.36	0.10
		Herb	-0.25	0.16	-1.56	-0.56	0.06
	Dietary Breadth	1	0.28	0.09	**3.04****	0.10	0.46
		2-3	-0.29	0.14	**-2.04***	-0.57	-0.01
		4+	5.3e-3	-0.14	-0.04	-0.28	0.27
	Home Range	Sm	-0.12	0.18	-0.64	-0.47	0.24
		Md	0.12	0.21	0.61	-0.28	0.53
		Lg	0.13	0.21	0.65	-0.27	0.54
Skull Width (Magnitude)	Full Model		0.50	0.20	**2.56***	0.12	0.88
	Trophic Level	Carn	0.74	0.27	**2.77****	0.22	1.26
		Omn	0.11	0.40	0.27	-0.68	0.90

Table II-2 Continued			r	SE	Z	LB	UB
		Herb	0.36	0.39	0.93	-0.40	1.12
	Dietary Breadth	1	0.68	0.24	**2.94****	0.23	1.14
		2-3	0.38	0.33	1.14	-0.27	1.03
		4+	-0.01	0.33	0.02	-0.66	0.65
	Home Range	Sm	0.65	0.25	**2.64****	0.17	1.13
		Md	0.33	0.28	1.66	-0.22	0.88
		Lg	0.40	0.31	1.27	-0.22	1.02
Skull L:W	Full Model		0.10	0.19	0.55	-0.26	0.47
(Directionality)	Trophic Level	Carn	0.12	0.31	0.40	-0.48	0.73
		Omn	-0.09	0.62	-0.14	-1.30	1.13
		Herb	0.11	0.32	0.34	-0.52	0.73
	Dietary Breadth	1	0.20	0.27	0.44	-0.41	0.65
		2-3	0.07	0.33	0.21	-0.58	0.72
		4+	0.04	0.35	0.11	-0.64	0.72
	Home Range	Sm	0.23	0.23	1.01	-0.21	0.68
		Md	0.19	0.27	0.68	-0.35	0.72
		Lg	-0.19	0.30	-0.63	-0.78	0.40
Skull L:W	Full Model		0.04	0.16	0.21	-0.28	0.35
(Directionality	Trophic Level	Carn	-0.21	0.27	-0.79	-0.75	0.32
w/o ferrets)		Omn	-0.10	0.31	-0.32	-0.72	0.51

Table II-2 Continued			r	SE	Z	LB	UB
		Herb	0.18	0.20	0.91	-0.21	0.56
	Dietary Breadth	1	-0.07	0.26	-0.26	-0.58	0.45
		2-3	0.04	0.21	0.17	-0.38	0.46
		4+	0.09	0.24	0.38	-0.38	0.57
	Home Range	Sm	0.04	0.19	0.20	-0.33	0.41
		Md	0.07	0.23	0.31	-0.39	0.53
		Lg	-0.07	0.25	-0.28	-0.56	0.42
Skull L:W	Full Model		0.43	0.13	**3.46*****	0.19	0.68
(Magnitude)	Trophic Level	Carn	0.57	0.18	**3.14****	0.21	0.92
		Omn	0.09	0.39	0.24	-0.67	0.86
		Herb	0.37	0.20	1.91	-0.01	0.76
	Dietary Breadth	1	0.50	0.17	**2.87****	0.16	0.83
		2-3	0.32	0.22	1.47	-0.11	0.74
		4+	0.40	0.24	1.64	-0.08	0.88
	Home Range	Sm	0.57	0.16	**3.61*****	0.26	0.88
		Md	0.27	0.22	1.22	-0.16	0.71
		Lg	0.30	0.22	1.36	-0.13	0.73

P-value significance: 0.01-0.05*, 0.001-0.01**, 0-0.001**

Figure II-3: Magnitude analyses of ecological covariates. A-C. trophic level, D-F. dietary breadth (including species that consume a single dietary category, two to three dietary categories, or four or more dietary categories), G-I. relative home range size. Columns indicate analyses of skull length, skull width, or skull length-to-width as indicated. These data imply the magnitude of the morphological changes but are not informative to the directionality of the changes (e.g., longer or shorter skull). Forest plot lines that do not cross the dotted zero line demonstrate a significant difference between captive and wild populations.



Discussion

Differing cranial shapes and sizes have frequently been reported among captive populations of mammals (O'Regan & Kitchener, 2005). The results of this study indicate that the nature of those changes varied between species, where certain species display longer, wider crania and other display shorter, narrower crania (Figure II-1). Analyses of magnitude detected moderate to strong effects. Although such analyses may create artificially narrow confidence intervals (Morrissey, 2016; Paulus et al., 2013), applying absolute values to standard effect sizes can help interpret whether there are any general effects of captivity given that such analyses neutralize directionality, particularly when extreme positive and negative values exist in the dataset. The application of the absolute values implied that although directionality of the morphological changes occurring in captivity varied between taxa, as we might expect, the overall magnitude of standard effect size was different from zero, suggesting that mammals do display size and shape changes in captivity, but that they do not all differ in the same way.

The differentiation of captive rodent and carnivore crania may be related to the well-developed temporalis muscles that these species possess (Elbroch, 2006). Temporalis muscles enhance jaw strength at the anterior portion of the skull, which translates to enhanced incisor gnawing strength among rodents and increased force distributed to the canine teeth of carnivores (Elbroch, 2006). The presence of an enlarged temporalis muscle requires a common set of morphological specializations including a wider zygomatic arch (i.e., a wider skull; Elbroch, 2006). Species that do not rely as heavily on anterior jaw strength, such as most herbivores and omnivores, typically display a narrower zygomatic breadth (i.e., a narrower skull; Elbroch, 2006). While heavy usage of the temporalis muscle helps to shape cranial morphology, the disuse that may occur in captivity can also influence cranial shape and may explain the morphological differences observed in this study (Curtis et al., 2018; Hartstone-Rose et al., 2014; Hollister, 1917; Kapoor et al., 2016). For example, although certain species (e.g., hypercarnivores) may be adapted to consuming tough materials (e.g., bones, bamboo, large-bodied animals), carnivorous diets provided to captive animals may be quite soft relative to what is consumed in the wild and may not require adequate mechanical force, potentially causing a change in cranial morphology (Curtis et al., 2018; Hartstone-Rose et al., 2014; Kapoor et al., 2016).

Dietary breadth and home range size was also linked to changes in morphology in captive animals. Captive animals with the most specialized dietary and habitat requirements are predicted to show heightened stereotypic behaviors, which can have a major impact on cranial muscle usage (Duckler, 1998; Hartstone-Rose et al., 2014; O'Regan & Kitchener, 2005; Skibiel et al., 2007). Although species with the largest home range sizes in the wild frequently display heightened stereotypies in captivity (Clubb et al., 2009; Kroshko et al., 2016; Mason & Veasey, 2010), the most distinct morphological shifts were associated with species that inhabit the smallest home range sizes. This may instead be associated with their trophic levels, given that the species that displayed the largest changes, including striped-faced dunnarts (*S. macroura*) and black-footed ferrets (*M. nigripes*), are carnivorous and also have some of the smallest home range sizes (both occupying less than one km^2; Table V-1; Jones et al., 2009).

Captive specimens can be useful in morphological research given that captive animals often have thoroughly recorded histories, exist in highly controlled environments, and may represent the largest accessible populations of rare or endangered species (Behringer et al., 2018; Bello-Hellegouarch et al., 2013; Spartaco & Kitchener, 2007). However, the results of this study suggest that certain species should be preferentially avoided in morphological research. In particular, certain carnivores and rodents may display distinct morphological shifts in captivity. However, captive primates and other omnivorous species appear to show negligible shifts in cranial size and shape. This supports the findings of Bello-Hellegouarch et al. (2013), whose geometric morphometric study of great ape scapula found similarly limited differences between wild and captive populations. This may suggest that researchers examining certain primate species, or anatomical regions unlikely to be impacted by captivity, need not avoid captive specimens in future morphological studies.

Although differences in the cranial morphology of captive mammals has long been recognized, the nature and commonality of those trends have been poorly understood. While trends seem to arise within certain groups of species, the findings of these meta-analyses suggest that although differences may occur in captivity, the nature and magnitude of those differences varies between species. While the direction of changes in captivity may differ considerably between species, the overall magnitude of these trends implies that morphological changes arise in captivity and that these trends deserve further investigation within individual species and higher taxonomic levels. The differences noted among captive populations, specifically carnivores and rodents, may suggest the need for different captive husbandry practices, including diets that more closely resemble what is consumed in the wild and increased access to gnawing enrichment. As captive facilities such as zoos become increasingly responsible for the long-term survival of threatened and endangered species (Conde et al., 2011; West & Dickie, 2007; Witzenberger & Hochkirch, 2011), developing an understanding of the morphological changes occurring in captivity will be essential to avoid these effects in the future.

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CHAPTER III

MORPHOLOGICAL CHANGES OCCURRING IN CAPTIVITY DISPROPORTIONALLY IMPACTS HYPERCARNIVOROUS CANIDS

Introduction

Captive populations of carnivores belonging to the order Carnivora often display changes in their cranial morphology compared to their wild counterparts (Antonelli, 2015; Curtis et al., 2018; Duckler, 1998; Hartstone-Rose et al., 2014; Hollister, 1917; Lynch & Hayden, 1995; O'Regan, 2001; Saragusty et al., 2014; Wisely et al., 2002; Wisely et al., 2005; Wolfgramm, 1894; Yamaguchi et al., 2009; Zuccarelli, 2004). Among African lions (*Panthera leo*), Amur tigers (*P. tigris*), and leopards (*P. pardus*), changes have been associated with differences in cranial proportions (Duckler, 1998; Hartstone-Rose et al., 2014; Hollister, 1917; O'Regan, 2001; Saragusty et al., 2014; Zuccarelli, 2004) and braincase volumes (Yamaguchi et al., 2009) of captive populations as compared to conspecifics in the wild. Similar changes in cranial size and shape have been documented among captive mustelids such as American mink (*Neovison vison*) and black-footed ferrets (*Mustela nigripes*; Antonelli, 2015; Lynch & Hayden, 1995; Wisely et al., 2002; Wisely et al., 2005). Likewise, the cranial morphology of captive canids has received some attention, with an increased palate length and zygomatic breadth first documented among captive gray wolves (*Canis lupus*) near the end of the 19th century (Wolfgramm, 1894). More recently, Curtis et al. (2018) revealed shifts in captive coyote

(*C. latrans*) crania involving palate length, zygomatic breadth, and sagittal crest height. Famously, captive silver foxes (*Vulpes vulpes*) selectively bred for human-affiliative behavior also display a variety of phenotypic changes in captivity including cranial changes in size and shape (Trut, 1999; Trut et al., 2009).

Morphological changes occurring in captivity may be in conflict with the conservation goals of Association of Zoos and Aquariums (AZA; Conway, 1995; Lacy, 2013; Patrick et al., 2007; Patrick & Caplow, 2018; Willoughby et al., 2015). Modern zoos invest incredible efforts to preserve threatened and endangered species and are active in reintroduction initiatives worldwide (Conway, 1995; Hutchins et al., 2003; Patrick et al., 2007; Patrick & Caplow, 2018). In this pursuit, zoos attempt to maintain captive animals as appropriate proxies of their wild population and limit their genetic, behavioral, and morphological differentiation (Frankham, 2008; Patrick et al., 2007; Willoughby et al., 2015; Woodworth et al., 2002). Changes occurring in captivity may be deleterious particularly among captively-bred reintroduced populations given that such changes may alter functionality and decouple internal and external cranial traits (Curtis et al., 2018; Pelletier et al., 2009; Wisely et al., 2005). Although captive populations are known to be morphologically distinct from their wild counterparts, limited comparative data is presently available (although see chapter II of this dissertation) regarding the prevalence of these changes.

Members of Family Canidae are frequently maintained in zoos and are also common targets of conservation initiatives (MacDonald & Sillero-Zubiri, 2004; Moehrenschlager & Somers, 2004). Canid cranial morphotypes are known to change based on selective breeding and diet availability (Drake & Klingenberg, 2010; Trut et al., 2009; Yom-Tov et al., 2003). Artificial selection is highly effective at altering cranial morphology of domestic dogs and silver foxes, resulting in cranial size and shape changes, as well as an overall wider variety of cranial morphotypes (Drake & Klingenberg, 2010; Morey, 1992; Trut et al., 2004). Although intentional artificial selection generally does not occur in zoos, captive animals are exposed to a novel environment in which many of the selection pressures wild populations experience are removed (Bryant & Reed, 1999; Lynch & O'Hely, 2001; Williams & Hoffman, 2009) and new selection pressures may be applied (Willoughby et al., 2015). Captive populations are also frequently plagued by small population sizes, with few founding members, which may lead to morphological changes resulting from inbreeding or genetic drift (Frankham, 2008; Lacy et al., 2013; McPhee, 2004; Willoughby et al., 2015; Woodworth et al., 2002). Unique captive environments may similarly lead to changes in cranial morphology associated with diet texture (Antonelli, 2015; Curtis et al., 2018; Hartstone-Rose et al., 2014; Hollister, 1917; O'Regan & Kitchener, 2005). In wild populations of *V. vulpes*, for example, differences in cranial size and shape have been documented based on the availability of anthropogenic foods (Jojic et al., 2017; Simonsen et al. 2003; Yom-Tov, 2003; Yom-Tov et al., 2003; Yom-Tov et al., 2007; Yom-Tov et al., 2013).

Canids have relatively diverse dietary preferences; while all canids are carnivorous, their diets may include varying proportions of vertebrates, invertebrates, and plant material (Damasceno et al., 2013; Fleming et al., 2017). Carnivorous diets can be divided into three subcategories including hypercarnivorous diets (consisting of roughly 70% vertebrate prey), mesocarnivorous diets (consisting of 50-70% vertebrate prey), and hypocarnivorous diets (including at least 70% non-vertebrate food sources) (Damasceno et al., 2013; Van Valkenburgh, 2007). Changes associated with captivity, particularly increased zygomatic width, are related to cranial musculature, which is enhanced among hypercarnivorous species given the relative toughness of their wild diets (Christiansen & Wroe, 2007; Curtis et al., 2018; Hartstone-Rose et al., 2014; O'Regan & Kitchener, 2005; Slater et al., 2009; Tseng & Flynn, 2015; Van Valkenburgh, 2007). For example, massive morphological shifts have been detected among several captive hypercarnivorous populations such as lions and tigers (Hartstone-Rose et al., 2014; Hollister, 1917; O'Regan, 2001; Saragusty et al., 2014; Yamaguchi et al., 2009; Zuccarelli, 2004). In general, there has been a research bias toward the study of captive hypercarnivores, to the exclusion of meso- and hypocarnivores.

Wild animals have been maintained in captivity for centuries, with varying levels of success (e.g., certain species survive and reproduce better than others in captivity), due in part to the provisioning of appropriate captive diets (Dierenfeld, 1997; Oftedal & Allen, 1997; Yang et al., 2007). Over the past several decades, captive diets have improved significantly owing largely to the increasingly conservation- and welfare-based goals of modern zoos and other husbandry-focused facilities (Dierenfeld, 1997; Oftedal & Allen, 1997). But these improvements took time to implement. Although the first public zoos began to open in the last 18th century (Lindholm, 2013), these facilities did not begin employing professional nutritionists until the mid-1970's. Since that time, it has become clear that the classic captive carnivoran diet, which often consisted of canned dog or cat food, lacked the appropriate abrasive qualities required to maintain proper dental health and may have had serious effects on cranial morphology (Dierenfeld, 1997; Fitch & Fagan 1982; Glatt et al., 2008; Kapoor et al., 2016; Oftedal & Allen, 1997; O'Regan & Kitchener, 2005). Although major strides have been made in an effort to provide captive carnivorans appropriate diets (Dierenfeld, 1997; Oftedal & Allen, 1997), provisioning hypercarnivores with appropriately tough materials (such as animal carcasses) can be a challenge for zoos given the relative difficult with obtaining those materials, the amount of preparation and cleaning required, and the risk of injury (e.g., broken teeth) or disease transmission (Glatt et al., 2008; Young, 1997). Thus, the enhanced musculature of the hypercarnivore morphotype (Christiansen & Wroe, 2007; Slater et al., 2009; Tseng & Flynn, 2015; Van Valkenburgh, 2007) and the relative difficulty associated with providing a 'tough' hypercarnivore diet in captivity could leave these animals more vulnerable to morphological changes in captive populations.

Here, I explore the cranial morphology of 15 species of canids with both captive and wild populations to systematically assess the nature of morphological changes occurring in captivity within a closely related group of carnivorans. I also assess the pervasiveness of changes across canid species and the degree to which morphological differentiation is related to diet. If hyper-, meso-, and hypocarnivorous canids show similar degrees of differentiation between captive and wild populations, it suggests diet toughness is not instrumental in morphological changes occurring in captivity, as implied by previous literature. However, if the magnitude of cranial changes is explained by carnivory type, it suggests the need for continued improvement to captive husbandry and additional research into hypercarnivore diets in captive populations. By documenting the changes in cranial morphology occurring in captivity, this study can help direct management of captive canid populations and promote long-term conservation success.

Methods

Specimen Collection and Digitization

To examine the morphological differentiation of captive and wild canids, I assessed the crania and mandibles of 1,621 specimens from 15 canid species using 2D geometric morphometric techniques. Specimens were photographed from 17 museums across North America (Tables V-4 & V-5, Supplementary File 1). Only adult specimens were included in the analyses (confirmed by dental eruptions and cranial sutures). Specimens were identified as captive or wild based on museum record data. Captive individuals included only those specimens explicitly labeled either ‘captive’ or ‘zoo’. To avoid specimens that had undergone artificial selection, captive specimens whose records indicated that they originated from a fur farm (e.g., certain *Nycetereutes procyonoides, Vulpes lagopus,* and *V. vulpes* specimens) were removed from the dataset. Efforts were made to obtain a minimum of 10 captive and wild specimens for each species to develop an appropriate estimate of mean shape (Cardini et al., 2015). In total, this analysis included 425 captive and 1,196 wild specimens (Table III-1; Supplementary File 1). When possible, wild specimens from across the geographic range of a species were sampled for imaging and landmarking. Some species have larger geographic ranges (e.g., *V. vulpes, C. latrans*), which led to additional sampling to account for geographic variation. Given that Mexican wolves (*Canis lupus baileyi*) are the most divergent *C. lupus* subspecies in terms of both phenotype and genotype (Fredrickson & Hedrick, 2002; Hedrick & Fredrickson, 2010; Tomiya & Meachen, 2018), sampling of this species was limited to the geographic regions inhabited by the Mexican wolf subspecies.

Cranial elements were photographed in a ventral view to capture the breadth of shape variation associated with the toothrow, rostrum, and zygomatic arch. Mandibular elements were photographed in lateral view to capture shape of the toothrow, relative robustness of the mandibular body, and elongation of the mandibular ramus. Landmarks were applied to photographs of each element using tpsDIG2 (Rohlf, 2001). Landmark schemes were based on modified versions of the landmark schemes used in previous literature (Curth et al. 2017; Meloro et al., 2017; Prevosti et al., 2013) and were designed to highlight the tooth row, specifically the size, shape, and orientation of each tooth, as well as the relative length and width of the skull (Figure III-1; Table V-6). There were 22 landmarks in the cranial view and 19 landmarks in the mandibular view. These landmarks represent type 1 (strong histological support for homology) and type 2 (homology supported by geometric, not histological representation) landmarks as specified by Bookstein (1991). Broken specimens or those that were missing landmarks were removed from the analyses. Not all specimens had both crania and mandible available, so sample sizes varied between the views (Table III-1; Supplementary File 1). To remove non-biological differences in shape due to rotation, location, and scale, Procrustes Superimpostion (Lawing & Polly, 2010; Zelditch et al., 2012) was applied to raw landmark data using *geomorph* (Adams et al., 2020) in R 3.6.1 (R Core Team, 2017). Landmarks were ordinated to their principal components (PC) and derived PC axes were used as shape variables in all remaining analyses, as each PC axis represents a unique component of shape variation.

Data Analysis

To identify whether there are differences between captive and wild specimens within each species, I assessed PC1 and PC2 of the cranial and mandibular views for each of the 15 species in the dataset using either a Welch's Two Sample t-test or a non-parametric Wilcoxon Ranked Sum test, depending on the normality of the data associated with each species. Given the number of individual tests conducted, a Benjamini and Hochberg False Discovery Rate correction was applied to the p-values associated with each species using the *p.adjust* function in the base R *stats* package. This method helps guard against the possibility of a Type 1 error when multiple analyses are conducted on a dataset, but is also sensitive to Type 2 errors (Benjamini, 2010; Benjamini & Hochberg, 1995; Mohieddin & Ansari-Pour, 2019).

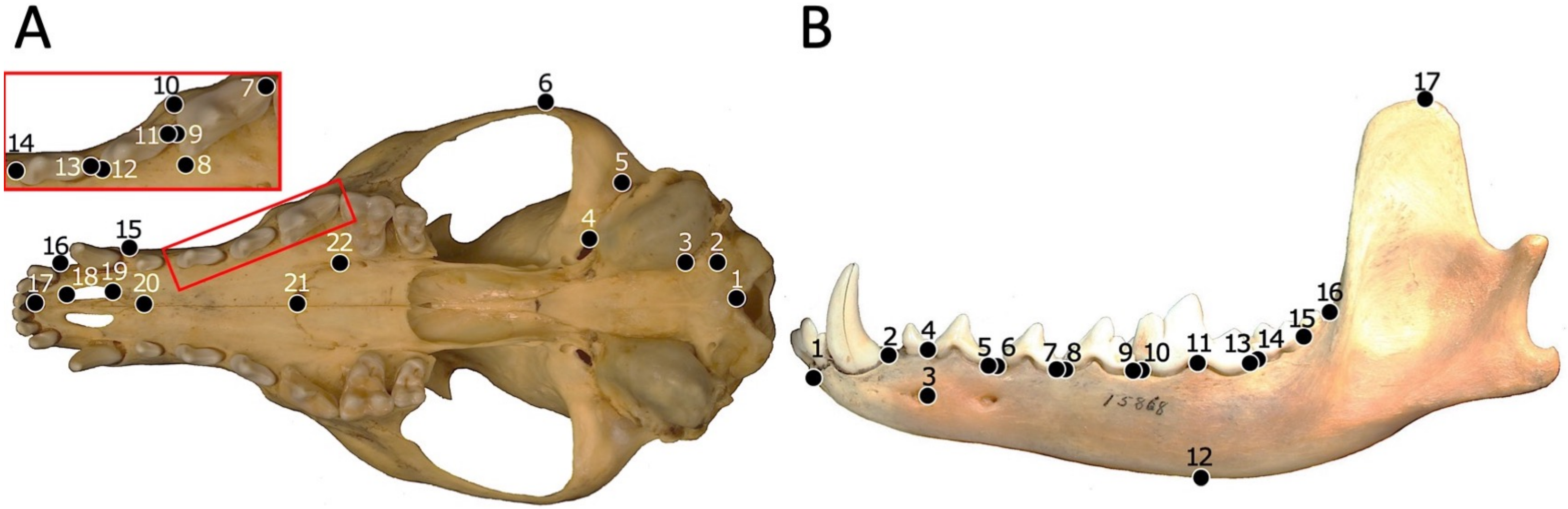
To assess the relationship between carnivory type and morphology, a phylogenetic ANOVA (pANOVA) was applied to PC1 and PC2 of the cranial and mandibular views. To conduct these analyses, difference in mean shape (for each PC) was calculated between wild and captive populations of each species. To account for the uncertainty in phylogenetic relationships, a distribution of F- and T- statistics were extracted from each pANOVA using 1,000 randomly sampled phylogenies from Upham et al. (2019). Results were based on the mean and standard deviation of that sample. Carnivory types included hyper-, meso-, and hypocarnivorous categories, which indicate the average amount of vertebrate prey found in a species diet, as assigned by Damasceno et al. (2013). In total, this dataset included 4 hypercarnivore, 4 mesocarnivore, and 7 hypocarnivore species. These analyses were conducted using the *phytools* package and were further assessed for pairwise relationships between categorical variables using a posthoc Holm test (Revel, 2012).

A Generalized Linear Mixed Model was used to assess the relationship between changes in morphology among captive canids of varying carnivory types and the dates of death in captivity, with species as a random effect. To examine this relationship, the difference between PC scores of individual captive specimens and the mean of the wild population of that species was calculated. The year of captivity was recorded based on the date of death provided in the museum record and ranged from 1884 to 2018. Specimens that did not have a corresponding date in their museum record were removed from this analysis. Given the limited records available for the captive population of *V. macrotis*, this species was not included in this analysis. Analyses were conducted using the *lme4* package (Bates et al., 2015).

Table III-1: Species and sample sizes (N) of captive and wild specimens included in this study. Cranial and mandibular views are partitioned to number of images from specimens in captive and wild populations. Carnivory types include hypercarnivorous (hyper), mesocarnivorous (meso), and hypocarnivorous (hypo) categories.

Type	Species	Abbreviation	Cranial Captive (N)	Cranial Wild (N)	Mandibular Captive (N)	Mandibular Wild (N)
Hyper	*Canis lupus*	C. lu	121	55	114	48
	Canis rufus	C. ru	67	51	64	49
	Lycaon pictus	L. pi	15	42	14	37
	Speothos venaticus	S. ve	22	7	22	6
Meso	*Atelocynus microtis*	A. mi	6	7	6	4
	Canis latrans	C. la	49	260	48	235
	Vulpes macrotis	V. ma	4	63	4	51
	Vulpes lagopus	V. la	20	83	19	73
Hypo	*Chrysocyon brachyurus*	C. br	17	12	15	11
	Nyctereutes procyonoides	N. pr	16	19	15	17
	Otocyon megalotis	O. me	13	35	11	30
	Urocyon cinereoargenteus	U. ci	17	221	15	200
	Vulpes velox	V. ve	16	26	16	24
	Vulpes vulpes	V. vu	18	296	18	270
	Vulpes zerda	V. ze	23	18	20	16

Figure III-1: Landmark schemes used for each canid specimen for geometric morphometric analysis, A. cranial view, B. mandibular view. See Table V-13 for full definitions of each landmark.



Results

Principal components analyses indicated several major axes of morphological variation across canids. The first two PC's from the cranial and mandibular views were retained for each of the analyses, both contributed at least 60% of the variance in PC scores (PC1 and 2 accounted for 43% and 21.4% of the variance in the cranial view and 42% and 19% of the variance in the mandibular view, respectively). In the cranial view, PC1 accounted for 42.7% of the morphological variation and primarily represented zygomatic breadth, tooth spacing, and tooth orientation (Figure III-2A; Figure III-3A). PC2 of the cranial view accounted for 21.5% of the variation and was associated the relative palate width, tooth row length, zygomatic breadth, and length of the post-dental anatomy (Figure III-2A; Figure III-3A). In the mandibular view, PC1 primarily represented the length of the tooth row and width of the mandibular ramus and accounted for 38.7% of the variation (Figure III-2B; Figure III-3B). PC2 of the mandibular view primarily represented the depth of the mandible relative to the height of the mandibular ramus, and the length of the toothrow and accounted for 17.8% of the morphological variation in the dataset (Figure III-2B; Figure III-3B).

Figure III-2: Principal component analysis results of captive and wild specimens of each PC axis with species differentiated by color, A. cranial view, B. mandibular view. Species abbreviations are defined in Table III-1.

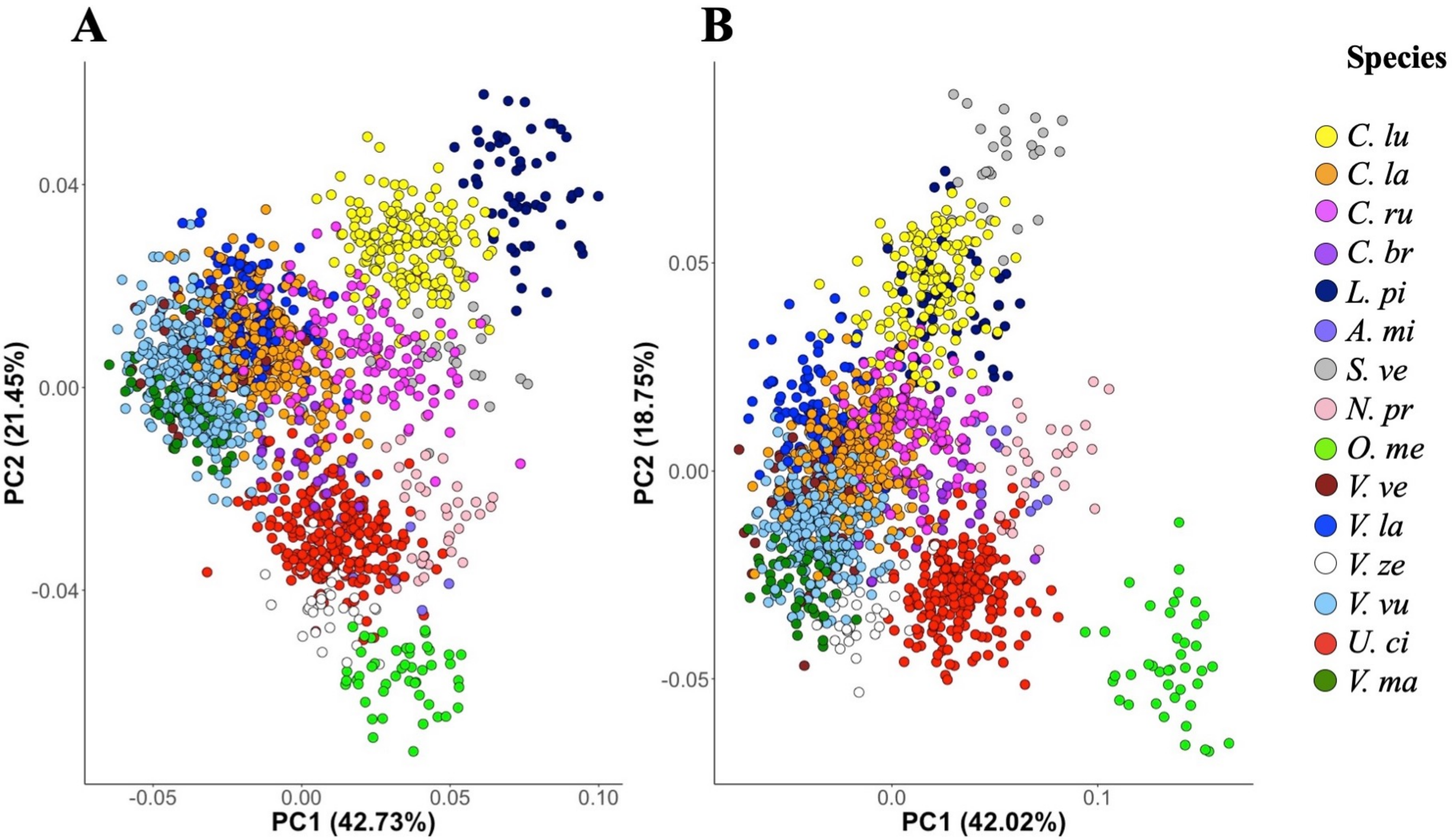
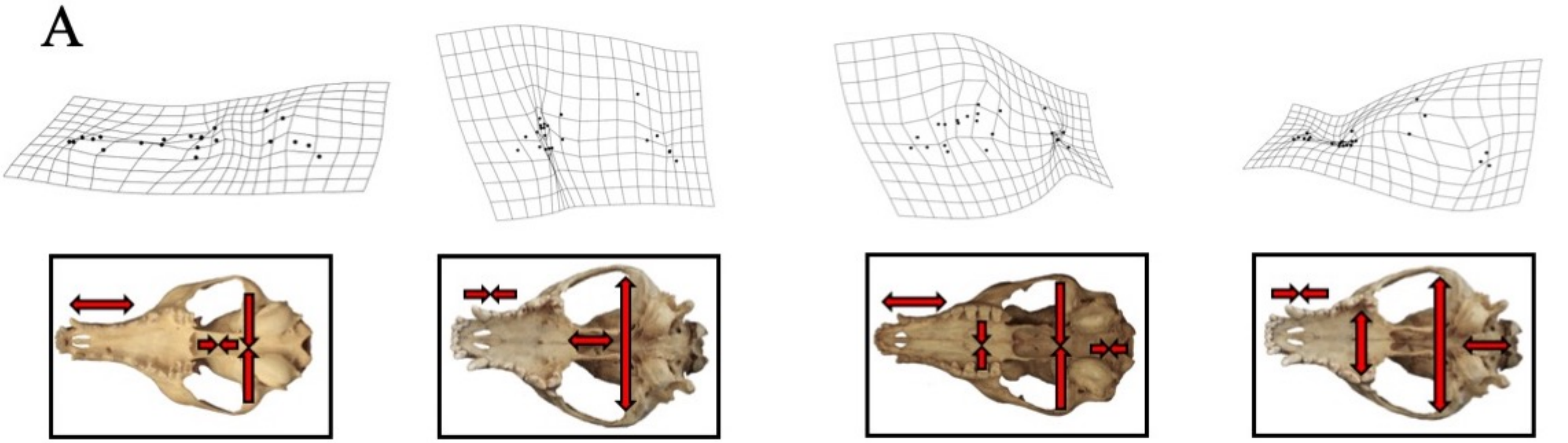
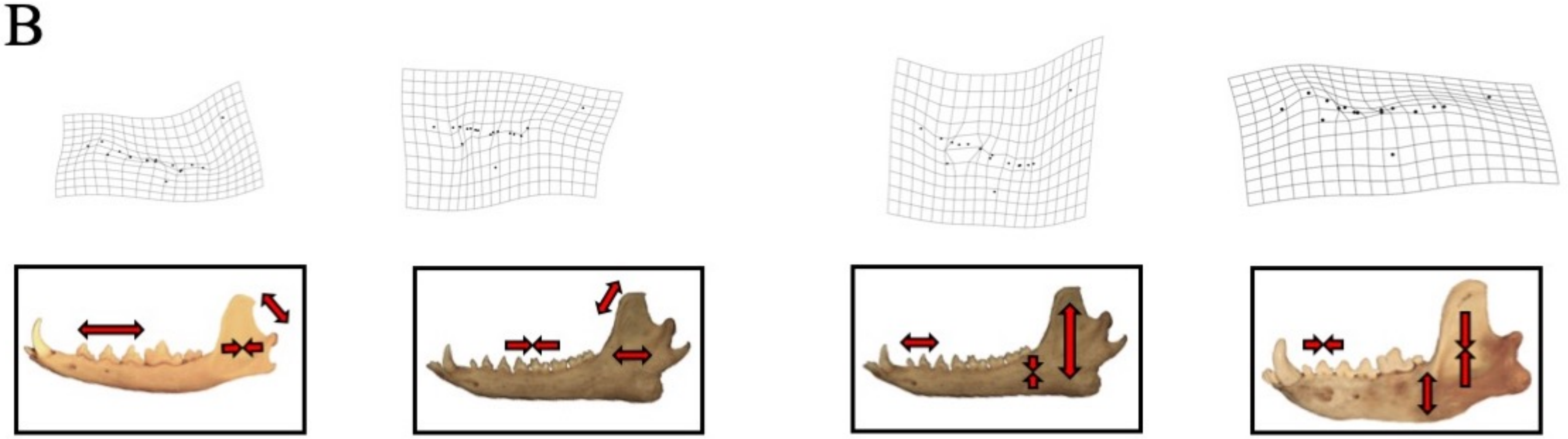


Figure III-3: Morphology associated with PC1 and PC2 of the A) cranial and B) mandibular views for all captive and wild specimens. The morphological minimum and maximum of each PC axis is represented by a thin-plate spline and an image of a specimen from the corresponding morphospace. Red arrows indicate the regions of morphology that differentiate the extremes of the PC.



Cranial PC1 (42.73%)

Cranial PC2 (21.45%)



Mandibular PC1 (42.02%)

Mandibular PC2 (18.75%)

Analyses revealed significant differences between wild and captive specimens of several different species. In the cranial view, PC1 varied significantly between nearly all wild and captive hypercarnivorous species (all except *Speothos venaticus,* Table III-2; Figure III-4A). Very little change was apparent among meso- or hypocarnivorous species along this axis, although a significant difference was detected for the hypocarnivorous *Vulpes vulpes* (t=-2.11; *P*<0.05). Analyses of PC2 of the cranial view suggested changes in the cranial morphology of several captive hypocarnivores (*Chrysocyon brachyurus,* w=159; *P*<0.05; *Otocyon megalotis,* t=3.79; *P*<0.01; *Urocyon cinereoargenteus,* t=3.36; *P*<0.05; *V. vulpes,* t=4.69; *P*<0.001; Table IV-3; Figure III-4B). Morphological differences between captive and wild populations were only detected among a single hypercarnivore (*Lycaon pictus*, t=2.62; *P*<0.05) and mesocarnivore (*V. lagopus*; t=2.46; *P*<0.05) at PC2 of the cranial view (Table III-2; Figure III-4B).

Differences were detected among several hyper-, meso-, and hypocarnivorous species at both PC1 and PC2 of the mandibular view. At PC1, two hypercarnivorous species (*Canis lupus*, t=2.35; *P*<0.05; *C. rufus*, t=4.42; *P*<0.001) and two hypocarnivorous species (*V. velox*, t=-3.03; *P*<0.05; *V. vulpes*, t=-2.76; *P*<0.05) displayed a significant difference between captive and wild populations (Table III-2; Figure III-4C). Significance was also detected among *C. lupus* (t=8.17; *P*<0.0001), *C. latrans* (t=4.28; *P*<0.001), *V. lagopus* (t=4.44; *P*<0.0001), and *V. vulpes* (t=7.56; *P*<0.0001) at the second PC of the mandibular view (Table III-2; Figure III-4D). *Vulpes*

Table III-2: Analyses of captive and wild cranial shapes for each species by carnivory type for PC1 and PC2 of the cranial and mandibular views. Carnivory types include hyper-, meso-, and hypocarnivorous species. Results are reported for Welch's Two Sample t-test (t) or Wilcoxon Rank Sum Test (w). P-values represent Benjamini & Hochberg FDR correction.

Type	Species	Cranial PC1		Cranial PC2		Mandibular PC1		Mandibular PC2	
		t/w	P-value	t/w	P-value	t/w	P-value	t/w	P-value
	Canis lupus	t=6.69	1.39e-09***	t=-1.10	0.27	t=2.35	0.03*	t=8.17	2.42e-11***
Hyper	*Canis rufus*	t=7.79	1.82e-11***	t=-1.59	0.15	t=4.42	5.16e-05***	t=-0.56	0.58
	Lycaon pictus	t=4.49	1.67e-04 ***	t=2.62	0.03*	t=-1.36	0.19	t=2.20	0.05
	Speothos venaticus	t=2.35	0.17	t=1.87	0.18	t=1.24	0.34	t=0.80	0.45
Meso	*Atelocynus microtis*	w=29	0.39	t=1.81	0.39	w=9	0.69	w=18	0.39
	Canis latrans	t=-1.08	0.28	t=1.54	0.17	t=2.23	0.06	t=4.28	0.00***
	Vulpes macrotis	t=-1.25	0.31	t=1.19	0.31	t=-2.90	0.15	t=2.32	0.15
	Vulpes lagopus	t=-0.26	0.80	t=2.46	0.04*	t=-1.50	0.19	t=4.44	0.00***
Hypo	*Chrysocyon brachyurus*	w=85	0.58	w=159	0.02*	t=0.56	0.58	t=3.01	0.021*
	Nyctereutes procyonoides	t=0.63	0.71	w=220	0.10	t=-0.22	0.83	t=2.04	0.10
	Otocyon megalotis	w=254	0.74	t=3.79	0.003**	t=-0.31	0.76	t=2.05	0.12
	Urocyon cinereoargenteus	t=1.73	0.14	t=3.36	0.013*	t=-1.82	0.14	t=1.15	0.26

Table III-2 Continued

Type	**Species**	**Cranial PC1**		**Cranial PC2**		**Mandibular PC1**		**Mandibular PC2**	
		t/w	**P-value**	**t/w**	**P-value**	**t/w**	**P-value**	**t/w**	**P-value**
	Vulpes velox	t=1.61	0.12	t=2.08	0.06	t=-3.03	0.02*	w=267	0.06
	Vulpes vulpes	t=-2.11	0.05*	t=4.69	2.15e-04***	t=-2.76	0.02*	t=7.56	7.32e-07***
	Vulpes zerda	w=251	0.26	t=1.35	0.25	t=2.3	0.06	t=2.25	0.063

P-value: 0.01-0.05*, 0.001-0.01**, 0-0.001***

Figure III-4: Mean PC scores of captive (navy) and wild (mauve) specimens of each species. Phylogenies of the species are indicated to the left of each PC figure and carnivory type is indicated by the symbols (hypercarnivores= squares, mesocarnivores=triangles, hypocarnivores=diamonds). Morphology associated with the extremes at each end of the PC axes are indicated by red arrows on the crania and mandible images. A. PC1 of the cranial view, B. PC2 of the cranial view, C. PC1 of the mandibular view, D. PC2 of the mandibular view. Species abbreviations are defined in Table III-1.

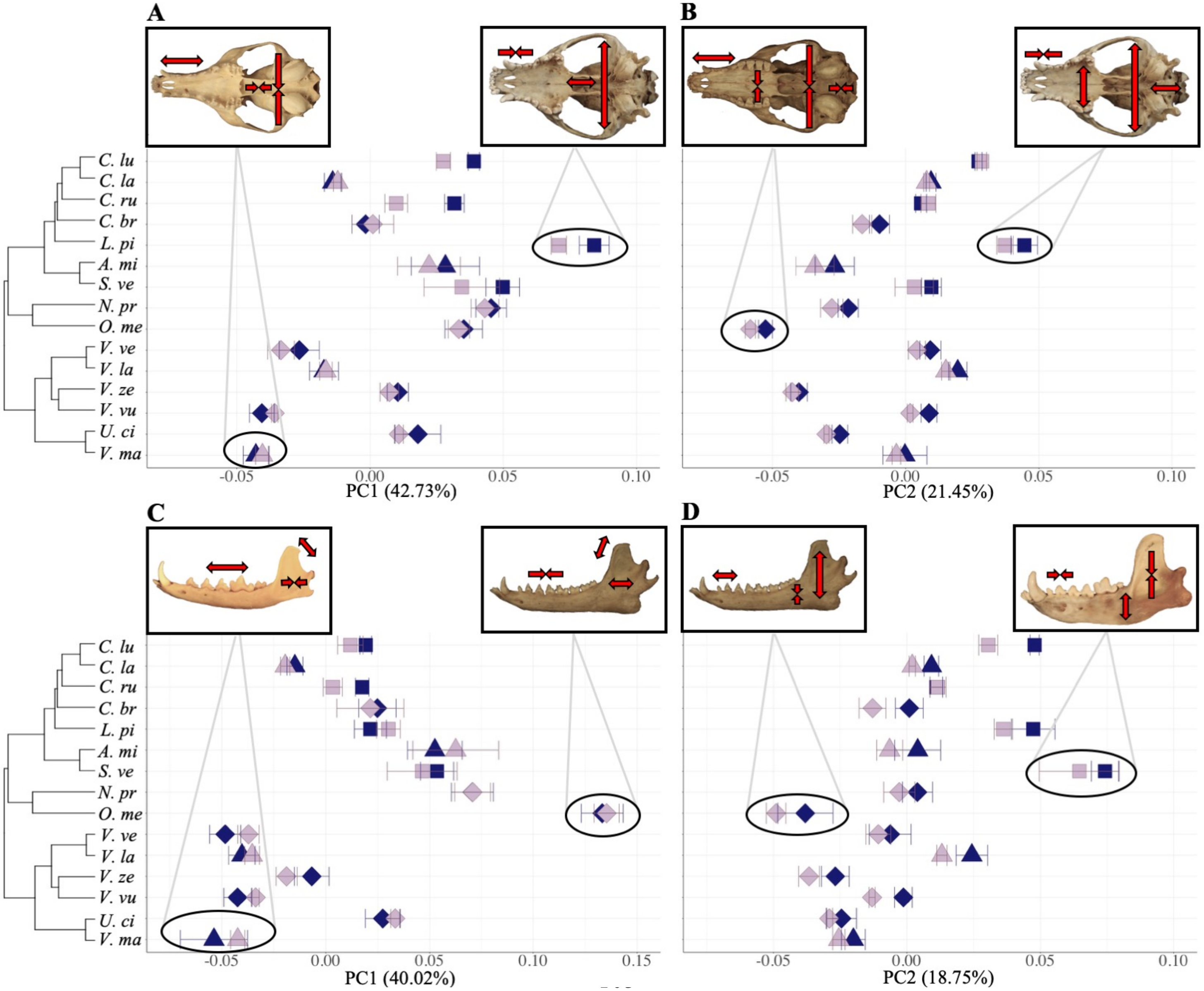

A
B
C
D
C. lu
C. la
C. ru
C. br
L. pi
A. mi
S. ve
N. pr
O. me
V. ve
V. la
V. ze
V. vu
U. ci
V. ma
-0.05
0.00
0.05
0.10
0.15
PC1 (42.73%)
PC2 (21.45%)
PC1 (40.02%)
PC2 (18.75%)

Table III-3 Phylogenetic ANOVA results of the difference values of captive and wild specimens based on carnivory types (including hyper-, meso-, and hypocarnivorous categories) with cranial and mandibular shapes (PC1 and PC2) reported as the F-statistic, pairwise posthoc Holm test results reported as t-values.

Variable	Hyper-Hypo	Hyper-Meso	Hypo-Meso	F-Statistic
Cranial PC1	-4.93^{**}	-4.91^{**}	-0.61	15.46^{**}
Cranial PC2	1.58	0.82	-0.66	1.25
Mandibular PC1	-1.69	-1.90	-0.46	2.09
Mandibular PC2	0.47	0.39	-0.03	0.12

P-value: 0.01-0.05*, 0.001-0.01**, 0-0.001***

vulpes displayed the most frequent differentiation, with significance noted for all cranial and mandibular PC's.

Phylogenetic ANOVA results revealed that differences between wild and captive specimens for PC1 of the cranial view were different by carnivory type (F=15.46, $P<0.05$; Table III-3). The posthoc Holm test showed that the difference between captive and wild populations was most apparent within hypercarnivorous species, which differed significantly from both meso- and hypocarnivores. Pairwise comparisons of meso- and hypocarnivores show the differences between wild and captive specimens were not significant (Table III-3). All hypercarnivorous species displayed a similar morphological trend associated with PC1 of the cranial view, where captive individuals displayed a higher PC score then their wild counterparts, with broader crania and a reduced toothrow length (Figure III-4A). There was no relationship between carnivory type and the degree of morphological change in captivity for PC2 of the cranial view or either mandibular PC's in the dataset (Table III-3).

Generalized Linear Mixed Model results assessing the relationship between differences in cranial shape and the date of captivity indicated PC2 of the mandibular view had a beta coefficient different from zero slope (F=4.18; $P < 0.05$; Table III-4). Carnivory did not show a beta coefficient different from zero for PC1 of the cranial or mandibular view or PC2 of the cranial view (Table III-5; Figure III-5). However, the beta coefficient was significantly different from zero among hypercarnivores in PC2 of the mandibular view ($R^2=0.18$; $P<0.0001$; Table III-5; Figure III-5D), indicating a smaller difference in mandibular shape between the wild and captive populations over time (Figure III-5D).

Table III-4: Generalized linear mixed model results associated with captive shape differential scores and the dates of captivity, presenting the fixed effect (year), interaction (year and carnivory type), and random effect (species) variance. Model conducted separately for PC1 and PC2 of both the cranial and mandibular views.

	Fixed Effects				**Interaction**		**Random Effect**
	(Year)		**(Carnivory)**		**(Year by Carnivory Type)**		**(Species)**
Variable	**Stat (F)**	***P***	**Stat (F)**	***P***	**Stat (F)**	**P**	**Variance**
Cranial PC1	1.62	0.20	1.54	0.22	1.74	0.18	4.51e-3
Cranial PC2	1.41	0.24	0.29	0.74	0.26	0.77	8.76e-6
Mandibular PC1	3.57	0.06	2.19	0.11	2.26	0.11	7.22e-3
Mandibular PC2	4.18	0.04*	1.13	0.32	1.13	0.32	5.26e-3

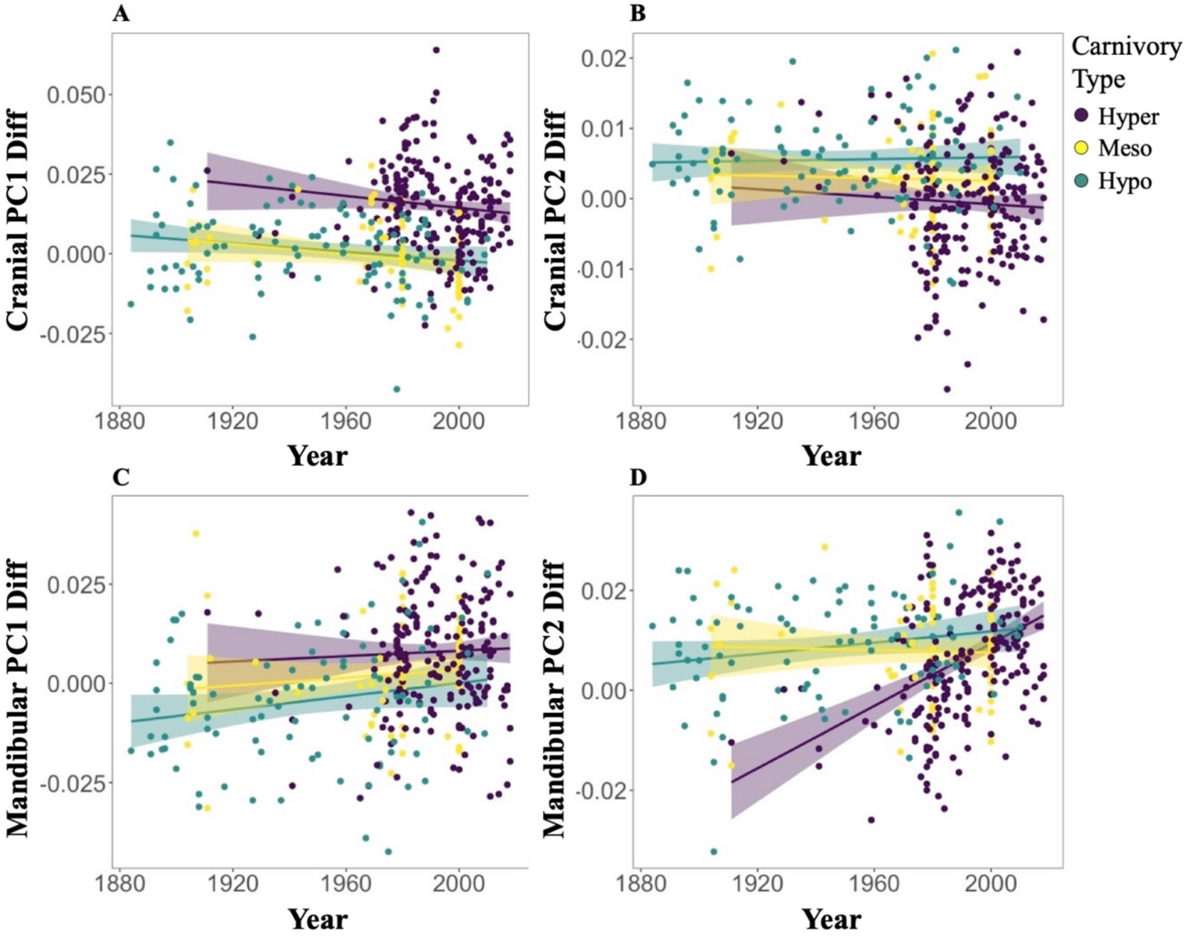
P-value significance: 0.01-0.05*, 0.001-0.01**, 0-0.001***

Table III-5: Linear regressions of cranial and mandibular PC difference scores of captive compared to wild populations and the year the animal died in captivity.

Variable	**Hyper**		**Meso**		**Hypo**	
	R^2	*P*	R^2	*P*	R^2	*P*
Cranial PC1	0.01	0.10	0.03	0.08	0.03	0.06
Cranial PC2	1.7e-3	0.42	-0.01	0.75	-0.01	0.74
Mandibular PC1	3.4e-3	0.58	0.02	0.08	0.02	0.08
Mandibular PC2	0.18	1.36e-10***	-0.01	0.75	0.02	0.09

P-value significance: 0.01-0.05*, 0.001-0.01**, 0-0.001***

Figure III-5: **Linear regressions of cranial and mandibular PC difference scores of captive compared to wild populations (where values greater or less than zero indicate a captive specimen that is further from the mean trait value of the wild population for that species) and the year the animal died in captivity. Purple represents hypercarnivores (hyper), yellow represents mesocarnivores (meso), and green represent hypocarnivores (hypo). A. PC1 of the cranial view, B. PC2 of the cranial view, C. PC1 of the mandibular view, D. PC2 of the mandibular view.**



Discussion

Changes in skull morphology can occur among canid populations in captivity compared to their wild counterparts, a result that is most acutely experienced among hypercarnivores along certain axes of morphological variation. Of the 60 morphological comparisons, I found there were 19 comparisons that document differences between captive and wild populations. In particular, hypercarnivores were prone to morphological changes associated with rostral length and zygomatic width (PC1 of the cranial view; Figure III-4A). Morphologically, captive hypercarnivores in this dataset were shifted toward a more truncated toothrow and a wider zygomatic breadth compared to their wild counterparts (Figure III-4A). This follows results noted among captive lions and tigers, which also display increased zygomatic widths (Hartstone-Rose et al., 2014; Hollister, 1917; O'Regan, 2001; O'Regan & Turner, 2004). In fact, Hartstone-Rose et al. (2014) suggested that these differences could imply enhanced cranial musculature within the captive population. Similarly, following findings that captive American alligators (*Alligator mississippiensis*) display comparatively wider skulls, Erickson et al. (2004) documented enhanced bite-performance among captive individuals. Importantly, cranial changes associated with zygomatic width (PC1 of the cranial view) will not necessarily impact the animal's functionality. Bite force is not dictated by zygomatic width alone (Damasceno et al., 2013; Elbroch, 2006; Law et al., 2018) and changing cranial elements in new and differing proportions may or may not enhance bite force (Ellis et al., 2009; Hartstone-Rose et al., 2014; Law et al., 2018). In fact, while a brachycephalic skull may increase bite force among many domestic dog breeds, abnormal cranial proportions associated with small breeds (such as chihuahuas) minimizes the space available for the masseter muscle and reduce bite force (Ellis et al., 2009). An interesting next step in this research will be to estimate the relative bite force associated with captive changes in cranial morphology to explore any functional changes among captive populations of canids.

Beyond the first principal component of the cranial view, differences in other morphological axes of variation were not different based on carnivory type. Although several hypocarnivorous species showed differences in cranial width and post-dental cranial length (PC2 of the cranial view). The extremes of this PC were represented by the hypocarnivorous *Otocyon megalotis* and the hypercarnivorous *Lycaon pictus* (Figure III-4B). All species that displayed a significant change in their distribution of PC2 values, moved in a common morphological direction, with a shorter, broader rostrum, wider zygomatic breadth, and elongated post-dental region as compared to their wild counterparts (Figure III-3B and III-4B). This included one hypercarnivore (*L. pictus*), one mesocarnivore (*Vulpes lagopus*), and several hypocarnivores (*Chrysocyon brachyurus, O. megalotis, Urocyon cinereoargenteus*, and *V. vulpes*).

Mandibular length and depth (PC1 and PC2 of the mandibular view) did not differ by carnivory type. Mandibular toothrow length and ramus width (PC1 of the mandibular view) were different for only four species, two hypercarnivores and two hypocarnivores (Figure III-4C; Table III-2). Although species across carnivory types displayed differences associated with the length and depth of the mandible (PC2 of the mandibular view) between captive and wild populations, all species moved in a common direction in morphospace (with the exception of *C. rufus* which showed almost no morphological differentiation along this PC; Figure III-4D). Nearly every species displayed a deeper mandible in captivity, with a relatively shortened mandibular ramus, and a more compact mandibular toothrow. As with the cranium, mandibular morphology is strongly associated with musculature and functionality (Bassard et al., 2020; Grossnickle, 2020), where the length of the ramus and the overall depth of the mandible is indicative of bite force (Pertoldi et al., 2006; Slater et al., 2009). Again, this may imply that captive populations have more enhanced mandibular musculature, but further analysis would be required to confirm functional differences associated with changes in these traits.

Although captive husbandry has improved over the past century (Dierenfeld, 1997; Oftedal & Allen, 1997), there was not a strong trend associated with morphological changes and date an animal died in captivity. If morphological changes were strongly dictated by a response to the relative toughness of a captive diet, we might expect the oldest captive specimens to be more differentiated from the wild population than the most recent captive specimens, but this was not the case (Figure III-5). The persistent morphological differentiation between captive and wild populations in PC1 and PC2 of the cranial view and PC1 of the mandibular view may suggest that either husbandry has not yet improved enough to avoid these effects or that captive diets are not the root of these morphological changes.

Several studies (including chapter IV of this dissertation) have implicated the effects of genetic drift, selection, or inbreeding as causal mechanisms of the morphological changes we observe in captivity (Bardo, 2012; Hartstone-Rose et al., 2014; Lynch & Hayden, 1995; McPhee, 2004). However, the captive histories (e.g., how many founding members; how many generations in captivity) may vary considerably among species; therefore, the specific mechanisms driving changes between captive and wild populations may differ. The fact that captive populations from different taxonomic backgrounds develop similar changes in captivity may suggest a common environmental problem (Figuerirido et al., 2013; Losos et al., 2011). An interesting next step in this research would be to examine these trends in other carnivorous clades, such as marsupials from Family Dasyuridae. The cranial morphology of this group displays convergent evolution with Order Carnivora (Goswami et al., 2011; Jones, 2003; Wroe & Milne, 2007). However, the ways in which captivity impacts the cranial morphology of carnivorous marsupials is largely unknown (although, Guay et al., 2012 explored changes in cranial volume among captive *Sminthopsis macroura*). Future investigations in this marsupial group may help inform the mechanisms driving morphological shifts in captivity.

The results of this study suggest that several different canid species display differences in cranial morphology in captive populations compared to wild populations, these changes were most strongly noted among hypercarnivorous species at PC1 of the cranial view and appear to be present within the captive population regardless of the year the animals were maintained in captivity. These findings are especially relevant to inform the work of zoos and other conservation-based captive facilities given that many of the species that displayed morphological changes are important for conservation initiatives (Moehrenschlager & Somers, 2004; Sergio et al., 2006). Some captive populations have been maintained with the hope that they may one day be reintroduced to the wild (Conde et al., 2011; Connolly & Cree, 2008; Willoughby et al., 2015; Witzenberger & Hochkirch, 2011) and it has been important to understand the consequences of even small changes to morphology that may influence function and impair reintroduction success and survival in the wild (La Croix et al., 2011; McPhee, 2004; Wisely et al., 2005). Reintroductions have been attempted for four canid species (*C. lupus, C. rufus*, *L. pictus*, and *V. velox*; Moehrenschlager & Somers, 2004), among which three are hypercarnivores and therefore prone to morphological changes in captivity. Developing a better understanding of why these cranial changes occur in captivity could therefore be of paramount concern to their long-term conservation success.

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CHAPTER IV ONE OF THESE WOLVES IS NOT LIKE THE OTHER: MORPHOLOGICAL EFFECTS AND CONSERVATION IMPLICATIONS OF CAPTIVITY IN MEXICAN WOLVES (*CANIS LUPUS BAILEYI*)

Introduction

Modern zoos are important conduits for conservation, helping to preserve threatened and imperiled species (Conde et al., 2011; Hutchins et al., 2003; Witzenberger & Hochkirch, 2011). In some cases, this includes preserving endangered species until they can be reintroduced to the wild (Conde et al., 2011; Frankham, 2008; Willoughby et al., 2015). However, the environment, available food, and stressors that captive populations experience are largely different from those of wild populations and may influence animal behavior, physiology, and morphology (Hartstone-Rose et al., 2014; Kapoor et al., 2016; McPhee & Carlstead, 2010; O'Regan & Kitchener, 2005), which may handicap success in the wild, where optimal functionality is imperative (Courtney Jones et al., 2018; Frankham, 2008; He et al., 2014; Wisely et al., 2005). Behavioral changes occurring in captivity (e.g., increased boldness, failure to avoid danger, poor foraging behavior; Bremner-Harrison et al., 2004; Conde et al., 2011; Grueber et al., 2017; Lei et al., 2015; Stoinski et al., 2003) have been linked to reintroduction failures. However, reintroduction failures are rarely examined in terms of morphological changes even though such changes can impede the conservation efforts of modern zoos (Lynch & O'Hely, 2001; Snyder et al., 1996; Woodworth et al., 2002).

Cranial morphology can be especially pivotal to an animal's survival and fitness in the wild (Law et al., 2018; Piras et al., 2013; Zurano et al., 2017). Certain cranial regions may be more prone to morphological change, where highly conserved regions are often intricately linked to primary functions such as vision and primary structures such as the brain (Figueirido et al., 2013; Linde-Medina et al., 2016). Morphological changes among captive carnivorans are most frequently associated with changes in sexual size and shape dimorphism, as well as rostral length, zygomatic width, and tooth row (Curtis et al., 2018; Hartstone-Rose et al., 2014; Lynch & Hayden, 1995; Zuccarelli, 2004), regions which largely dictate the size, shape, and texture of food an animal is capable of capturing and consuming (Law et al., 2018; MacNulty et al., 2009; Zurano et al., 2017). Changes to these regions should not impair an animal's survival in captivity (where animals need not capture prey). However, such changes may impact survival among reintroduced individuals who must hunt to survive.

Morphological changes occurring in captivity may be due to selection pressures, genetic drift, inbreeding, or phenotypic plasticity (Frankham, 2008; Lacy, 1987; Pelletier et al., 2009; Schulte-Hostedde & Mastromonaco, 2015; Woodworth et al., 2002). Captivity may apply unique selection pressures and neutralize pressures from the wild, potentially leading to morphological changes associated with directional or relaxed selection (Frankham et al., 1986; McPhee & McPhee, 2012; Schulte-Hostedde & Mastromonaco, 2015). Likewise, given a small population size, genetic drift or inbreeding may be probable mechanisms of morphological change in captivity (Ballou et al., 2012; McPhee, 2004; Lacy, 1987). Phenotypic plasticity (non-genetic, environmentally-induced changes to the phenotype; DeWitt & Scheiner, 2004; Hall & Witten, 2019; Whitman & Agrawal, 2009) is a frequently cited proximate cause of morphological changes occurring in captivity (Courtney Jones et al., 2018; Erickson et al., 2004; Kohn & Lubbach, 2019; O'Regan & Kitchener, 2005). Among carnivorans, plasticity associated with cranial morphology could be facilitated by the absence of appropriate masticatory mechanical stress given the relatively 'softer' texture of certain captive diets (Curtis et al., 2018; Hartstone-Rose et al., 2014; Hollister, 1917; Kapoor et al., 2016). These changes could be related to Wolff's Law (Wolff, 1892), in which the shape of the crania and other osteological elements are partially formed, and in some cases, remodeled, by mechanical stress (e.g., musculature) applied to the bone (Curtis et al., 2018; Hollister, 1917; Owen et al., 2014; Ehrlich & Lanyon, 2002; Hall & Witten, 2019; Jones et al., 1977; Pead et al., 1988). Morphological changes associated with phenotypic plasticity may be quickly lost after an animal is reintroduced (Wisely et al., 2002; Wisely et al., 2005); however, changes due to the effects of natural selection, genetic drift, or inbreeding may arise within relatively few captive generations, may be long-lasting, and may be retained many generations after reintroduction (Frankham, 2008; Lacy, 2013; McPhee, 2004; Willoughby et al., 2015; Woodworth et al., 2002).

In an attempt to understand the nature of morphological shifts occurring in captivity, their long-term impacts on reintroduced populations, and the biological mechanisms driving shifts, this study examines cranial-mandibular size and shape variation across captive, reintroduced, and historic wild populations of Mexican wolves (*Canis lupus baileyi*) using geometric morphometric techniques. If the effects of Wolff's law are occurring among captive Mexican wolves, the morphology of reintroduced individuals is expected to align closely with the morphology of historic, wild populations within a single post-captive generation. Given that the captive population of Mexican wolves was founded with seven individual animals, morphological changes due to the effects of genetic drift or inbreeding may be present (Frankham, 2008; Wisely et al., 2005; Woodworth et al., 2002). Alternatively, the drastic difference between the captive and wild environment could translate into a change in selection pressures; whether relaxed or directional, these pressures could cause rapid phenotypic differentiation (Kostow, 2004; McPhee, 2004; McPhee & McPhee, 2012; Pergams & Lacy, 2008). Ultimately, developing a better understanding of the nature of morphological changes in captivity and the biological mechanisms underlying those trends can help inform husbandry practices to avoid undesirable effects in future captive populations and promote long-term conservation success.

Methods

Study System

Mexican wolves are an exemplary species of conservation concern that have been reared in zoos in an effort to reintroduce them into the wild (Greely, 2018; U.S. Fish & Wildlife Service, 2017). Mexican wolves are native to southwestern portions of the United States into Central Mexico and were nearly extinct by 1976 (Hedrick &

Fredrickson, 2010; Kalinowski et al., 1999). Similar to many large carnivores, these wolves experienced major population losses due to poaching and eradication initiatives (Lynch, 2005). However, remarkable efforts were made to breed seven wild-caught individuals to found a captive population whose progeny were eventually reintroduced to limited regions of their former geographic range (Greely, 2018; Hedrick & Fredrickson, 2010; Kalinowski et al., 1999). Mexican wolves were first reintroduced to the Animas Mountains of eastern Arizona in 1998 (Kalinowski et al. 1999) and in 2011, they were reintroduced to Mexico in the Sierra San Luis complex of Northern Sonora (Greely, 2018; U.S. Fish & Wildlife Service, 2017). As of 2018, there were 390 Mexican wolves spread across 49 captive facilities (Greely 2018; Hedrick & Frederickson, 2010) and an estimated 193 animals in reintroduced populations (163 in the United States and roughly 30 individuals in Mexico; U.S. Fish & Wildlife Service, 2020). Although their reintroduced population has grown by about 15% each year throughout the past decade (U.S. Fish & Wildlife Service, 2020), future outlooks for the subspecies are far from certain as it suffers from dangerously limited genetic diversity owing to its small founding population (Mechak et al., 2016).

As a highly charismatic group, disproportionate efforts and funds have been devoted to canid conservation (Keulartz, 2015; Moehrenschlager & Somers, 2004; Sergio et al., 2006). For Mexican wolves, this interest has translated into meticulously curated captive studbooks, radio-collared reintroduced individuals, and the long-term preservation of cranial and skeletal materials in museum collections (Greely, 2018). As a result, a great deal of data are available regarding captive animal pedigrees and the number of generations an individual animal has been removed from the wild, or alternatively, the number of generations a reintroduced animal has been removed from captivity.

Specimen Collection and Definition

A total of 251 Mexican wolf crania and mandibles were photographed from five museum collections, predominately from the Museum of Southwestern Biology (Albuquerque, NM) and the Smithsonian National Museum of Natural History (Washington, D.C.; Supplementary File 1, Table V-7). Specimens were grouped into one of three categories: wild, captive, or reintroduced based on museum specimen data and the Mexican wolf studbook (Greely, 2018). Captive and reintroduced individuals were often associated with a studbook number. Studbooks provide a full account of each animal in the recorded captive history, including information on sex, parentage, date of birth and death, and a full list of localities inhabited during the lifetime of each individual. Captive and reintroduced individuals were differentiated based on where they spent the majority of their early development (at least 60% of their first year). Animals with a captive lineage and an associated studbook number that were born in the wild or spent the majority of their first year in the wild were considered reintroduced. Given that wolf cranial morphology primarily forms during early development (Frederickson & Hedrick, 2002; Wayne, 1986), animals that spent the majority of their first year in captivity but were later reintroduced to the wild were still defined as 'captive'. Any specimen labeled ‘captive’ without a studbook number was removed from the study if it was either collected after reintroductions began (in 1998) or if it had no associated date available. Wild specimens included only those animals collected from the historic wild populations. To prevent confusion with reintroduced individuals, all specimens in the ‘wild’ group were collected between 1894 and 1958, prior to the start of the species reintroduction. As of 2018, there were two populations of reintroduced Mexican wolves, the Blue Free population of the Animas Mountains in Arizona and the population in the Sierra San Luis complex of Northern Sonora (Greely 2018; U.S. Fish & Wildlife Service, 2017). Due to specimen availability, only the Blue Free population was included in this study. In total, this dataset consisted of 77 wild, 138 captive, and 35 reintroduced Mexican wolves, representing up to 13 captive generations and 5 reintroduced generations (Supplementary File 1).

Generation Calculations

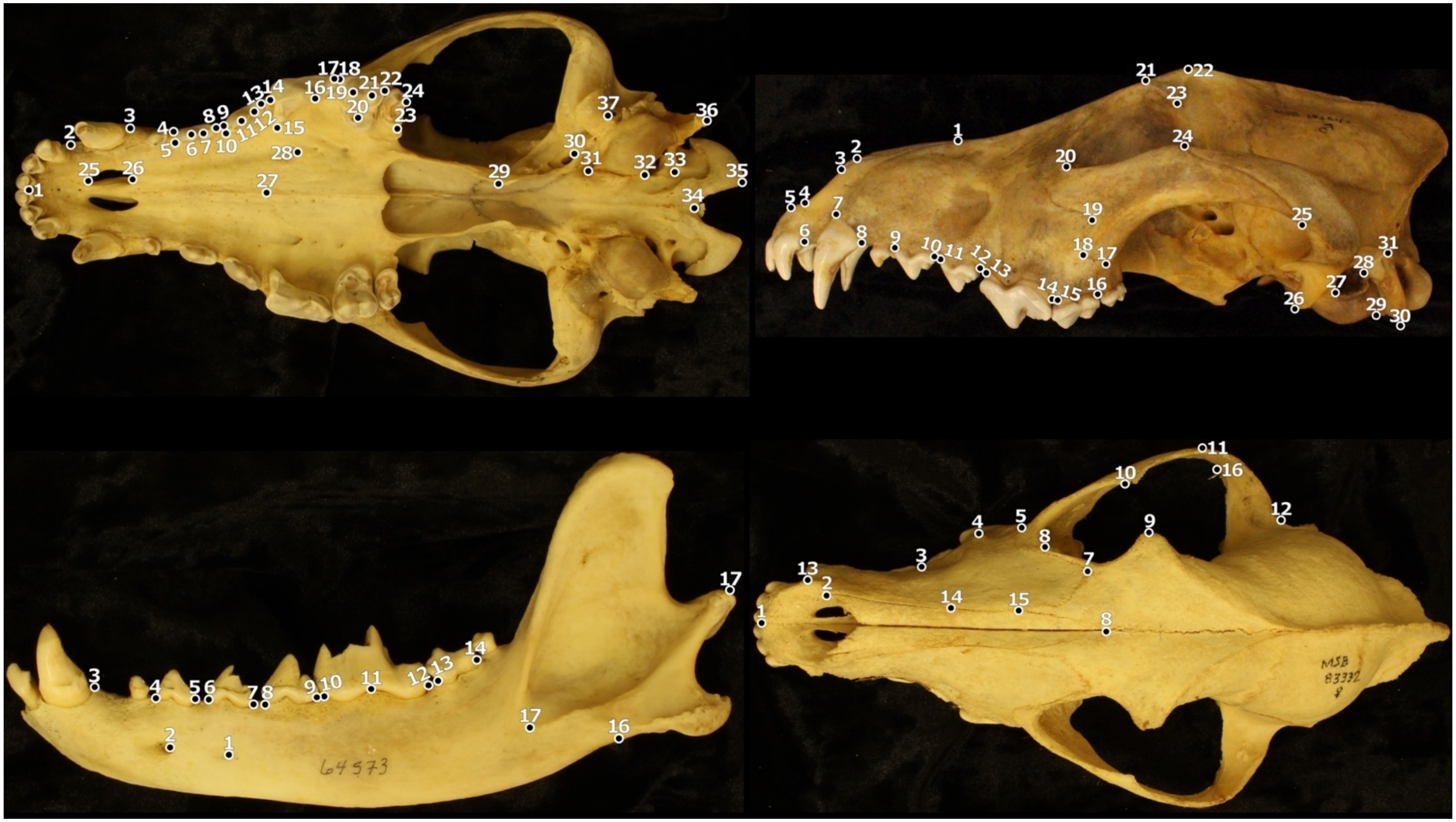
Generations in captivity and generations removed from captivity were calculated using the studbook (Greely, 2018). These calculations were only applicable to non-wild individuals and only those specimens designated as ‘reintroduced’ were assigned values for both generations in captivity and generations reintroduced (e.g., generations removed from captivity). Each generation was determined based on the animal’s pedigree, where one captive generation represented an animal who was born and raised in captivity, but whose sire and dam were both wild caught. Generations were assigned by taking the maximum number of generations experienced by either parent and adding one additional generation (Coster, 2013; Sinnwell & Therneau, 2019). In the few cases where the lineage was not fully known, generation calculations were based on the known ancestors. Given that none of the specimens in the dataset were founding members of the captive population, the oldest captive specimens represented at least two captive generations. Captive individuals without a recorded studbook number were not included in any analyses using generations. Reintroduced generations for each individual were calculated similarly, where the number of generations removed from captivity were tabulated and assigned (Supplementary File 1). All analyses were conducted in R 3.6.1 (R Core Team 2017), generations were calculated using *pedigree* (Coster, 2013) and *kinship2* packages (Sinnwell & Therneau, 2019).

Image Digitization

Crania and mandibles were photographed for use in geometric morphometric analyses with a Canon Rebel EOS T5i camera. Depending on the condition and availability of specimen materials, each cranium was photographed from dorsal, ventral, and lateral views and each mandible was photographed in a lateral orientation. In certain circumstances, not all views were available for each specimen due to missing or damaged materials. Only adult specimens were included in subsequent analyses (age approximations were determined by the presence of deciduous teeth, cranial sutures, and when possible, verified by studbook data).

Landmarks were applied to each of the views (right-side only landmarks were used for dorsal and ventral views) using tpsDig232 (Rohlf, 2001) (Figure IV-1; Table V-8). Landmark locations were selected to assess regions related to cranial and mandibular musculature (associated with jaw strength and bite force) as well as relative skull length and width, regions which are hypothesized to shift over captive generations (Curtis et al., 2018; Hollister, 1917). In many cases, the caudal region of the crania (braincase) had been removed during specimen preparation. Given that these cranial regions are less prone to morphological differentiation (Figueirido et al., 2013; Linde-Medina et al., 2016) and in an effort to preserve the sample size of this dataset, landmarks were not applied to the braincases. Since *Canis lupus* subspecies are known to display sexual dimorphism (Morris & Brandt, 2014; Fredrickson & Hedrick, 2002), separate analyses were conducted of each sex for each view. To eliminate the variation associated with rotation, translation, and scale, a Procrustes superimposition was applied to each analysis (Lawing & Polly, 2010; Rohlf, 1990). Geometric morphometric analyses were conducted using the *geomorph* package in R (Adams et al., 2020).

Figure IV-1: Landmark schemes used for each skull orientation. A. Ventral, B. Lateral, C. Mandibular, D. Dorsal. See Table V-5 for additional information on each landmark.



Size and Shape Analyses

Centroid size was calculated for each specimen and used as a proxy for size independent of shape (Mitteroecker & Gunz, 2009; Webster & Sheets, 2010). Centroid size is the square root of the sum of squared differences between all landmarks and the geometric center of the landmark scheme (Zelditch et al., 2004). Size differences among wild, captive, and reintroduced populations were assessed using ANOVA's and post-hoc Tukey's Honest Significant Difference (HSD). The standard index of sexual size dimorphism (ISSD) was calculated with the ratio of mean centroid sizes, where the average centroid size of the larger sex is divided by the smaller sex (Ljubisavljević et al. 2010; Porobic et al. 2016). Male and female centroid sizes were compared using Welch's two-sample t-tests.

For each skull view (dorsal, ventral, lateral, and mandibular), landmarks were ordinated using a Principal Components Analysis (PCA). The derived PC scores describe independent axes of shape variation and were used for further analyses of shape differences (Klingenberg, 2013; Lawing & Polly, 2010; Webster & Sheets, 2010). The first 20 principal components explained roughly 90% of the overall variation in shape for all skull views (ventral 87.8%, lateral 90.1%, dorsal 97.9%, and mandibular 98.8%, respectively), therefore these were the only axes retained for further analysis. Sexual shape dimorphism (SShD) was assessed for each skull view using multivariate analyses of variance (MANOVA) in conjunction with PC scores and sex. Analyses of Variance (ANOVA) tests were used to detect differences among sexes for individual PC's and

MANOVA’s were used to detect differences associated with the population type (captive, wild, or reintroduced; Cooke & Terhune, 2015). The degree of shape variance, that is, the amount of morphospace occupied by each group, was calculated using the distribution of PC scores. ANOVA’s and Tukey’s HSD tests were applied to these distributions to detect disparities associated with population type. Linear discriminant function analyses (LDFA) were conducted to detect whether reintroduced specimens aligned more closely with captive or wild population types. The significance of LDFA assignments was further assessed using Welch two sample t-tests.

Generational Change

For captive and reintroduced populations, number of generations in captivity and number of generations reintroduced were compared to size and shape. MANOVA’s were applied to PC scores to examine the relationship between cranial-mandibular shape, generations captive or reintroduced, and sex. To examine shifts in sexual size dimorphism, analyses of covariance (ANCOVA) were conducted to examine the relationship between centroid size and sex over increasing generations. Similarly, ANCOVA’s were performed to examine whether LDFA assignments (i.e., the likelihood that reintroduced individuals aligned with the captive or wild populations) were related to sex or number of captive or reintroduced generations (Collyer & Adams, 2013; Cooke & Terhune, 2015).

Inbreeding and Heritability

To assess the relative impact of inbreeding on size and shape, inbreeding coefficients (F) were calculated and assigned to each individual with a recorded studbook number using the R *pedigree* package (Coster, 2013). Inbreeding coefficients were used as independent variables in linear regressions to investigate their explanatory power on size (centroid size) and shape (PC's 1-3) variables. Narrow-sense heritability (h^2) of size and shape variables was calculated using ASReml 4.1 (Gilmour et al., 2015), as the ratio of the additive genetic variance to the phenotypic variance (Falconer & MacKay, 1996). Mixed linear models were used to calculate narrow-sense heritability and included sex as a fixed variable and the fixed linear regression of traits (size or shape) on age of the specimen (in days as calculated from the studbook). These fixed effects were excluded if probability values of the F statistic of the effects were greater than 0.15. Individual specimen was included as a random effect in all analyses; that is, covariances among specimens were modeled by the incorporation of a matrix of estimated relatedness among pairs of wolves based on the studbook.

Results

Sexual Dimorphism

Regardless of the population type or cranial-mandibular view, males consistently displayed larger crania and mandibles and occupied a different position in shape space from females (Table V-9), with comparatively narrow, elongated rostra and bowed zygomatic arches. Sex was a consistently significant factor in analyses of shape and size dimorphism, generations in captivity was occasionally significant, but the interaction between sex and captive generations was never significant (Table V-10). Analyses across reintroduced generations showed sex as a consistently significant variable and generations as an occasionally significant variable, the interaction between the variables was significant only from the dorsal view (Table V-11). These differences also appear to have been retained within all five reintroduced generations available in this study (Table V-11).

Size and Shape

Specimens from the historic wild populations were consistently and significantly larger than reintroduced specimens across all cranial-mandibular view (Table IV-1; Figure IV-2). Tukey's HSD results revealed that the greatest pairwise difference between the groups existed between the wild and reintroduced populations, where the reintroduced population was smaller. Males displayed more significant size differences between captive, wild, and reintroduced individuals compared to females.

Shape analyses revealed a significant difference between captive, wild, and reintroduced populations, a trend which was apparent regardless of the cranial-mandibular view (Table IV-2; Figure IV-3). Principal components analyses revealed a trend in which captive and wild specimens inhabited differing regions of morphospace (with overlap) and reintroduced populations largely represented an intermediate morphology between the two (Figure IV-3). Shape differences between the groups were associated with the spacing of the tooth row, skull width, and skull length (Figure IV-3; Figure IV-4). Regardless of the view, captive and wild populations inhabited regions of morphospace that were unexploited by the other group.

In the ventral view, significant PC's were most strongly associated with skull elongation; PC1 (28.7% of the variation) largely reflected the relative distance between the rostral and caudal areas of the skull, whereas PC2 (11.1% of the variation) was driven predominately by tooth spacing (Figure IV-3A; Figure IV-4A). Relative toothrow elongation largely represented the primary axis of variation in the lateral view (PC1 = 20.4% of the variation), where PC2 was predominately driven by rostral elongation (18.1% of the variation; Figure IV-3B; Figure IV-4B). In the mandibular orientation, variation was strongly associated with the arrangement of the toothrow, where PC1 generally represented a convex arrangement of the teeth relative to the mandible (34.7% of the variation) and PC2 represented a more concave arrangement (14.9% of the variation; Figure IV-3C; Figure IV-4C). The dorsal view was characterized by relative rostral (PC2, 15% of the variation) and zygomatic breadth (PC3, 11.1% of the variation) (Figure IV-3D; Figure IV-4D).

Regardless of skull orientation, captive individuals consistently showed the greatest variation in shape (Table IV-3; Figure IV-5). Both wild and reintroduced populations showed reduced shape variation, where the reintroduced group had the least variation. Post-hoc Tukey's HSD indicated that although significant differences existed between all pairwise comparisons, the smallest differences were typically found between wild and reintroduced populations (Table IV-3). Slightly different trends were apparent between males and females, but the overall p-values tended to be similar (Table IV-3).

LDFA results indicated that the cranial shape of reintroduced individuals most frequently aligned with captive, not wild, populations, particularly among reintroduced females (Table IV-4). This trend was most evident from the ventral view, where 93.8% of female and 89.5% of male reintroduced specimens grouped with the captive morphotype. Welch two sample t-test results confirmed a significant difference in the wild and captive assignments for all but the females in the lateral view of the cranium. ANCOVA results did not detect a significant relationship between the number of reintroduced generations and LDFA prediction scores (Table V-12).

Table IV-1: Tukey's and ANOVA HSD results for centroid size for C=captive, W=wild, and R=reintroduced specimens for ventral, lateral, mandibular, and dorsal views across all males (M) and females (F).

	Sex	N	Tukey's R-C	Tukey's W-C	Tukey's W-R	F-Statistic
Ventral	M	129	0.12	2.55e-3**	1.46e-4***	10.02***
	F	87	0.07	4.4e-6***	2.0e-7***	20.13***
Lateral	M	98	0.02*	0.52	6.02e-3**	5.38**
	F	74	0.07	1.1e-2*	3.13e-4***	8.45***
Mandibular	M	136	0.12	0.50	0.03*	3.39*
	F	104	0.85	0.09	0.11	2.89
Dorsal	M	131	0.10	0.02*	6.53e-4***	7.69***
	F	86	0.07	0.22	4.64e-3**	5.30**

P-value significance: 0.01-0.05*, 0.001-0.01**, 0-0.001***

Figure IV-2: Relative centroid sizes of captive (purple), reintroduced (red), and wild (yellow) specimens for A) ventral, B) lateral, C) mandibular, and D) dorsal views. Figure is representing males only; female trends are similar.

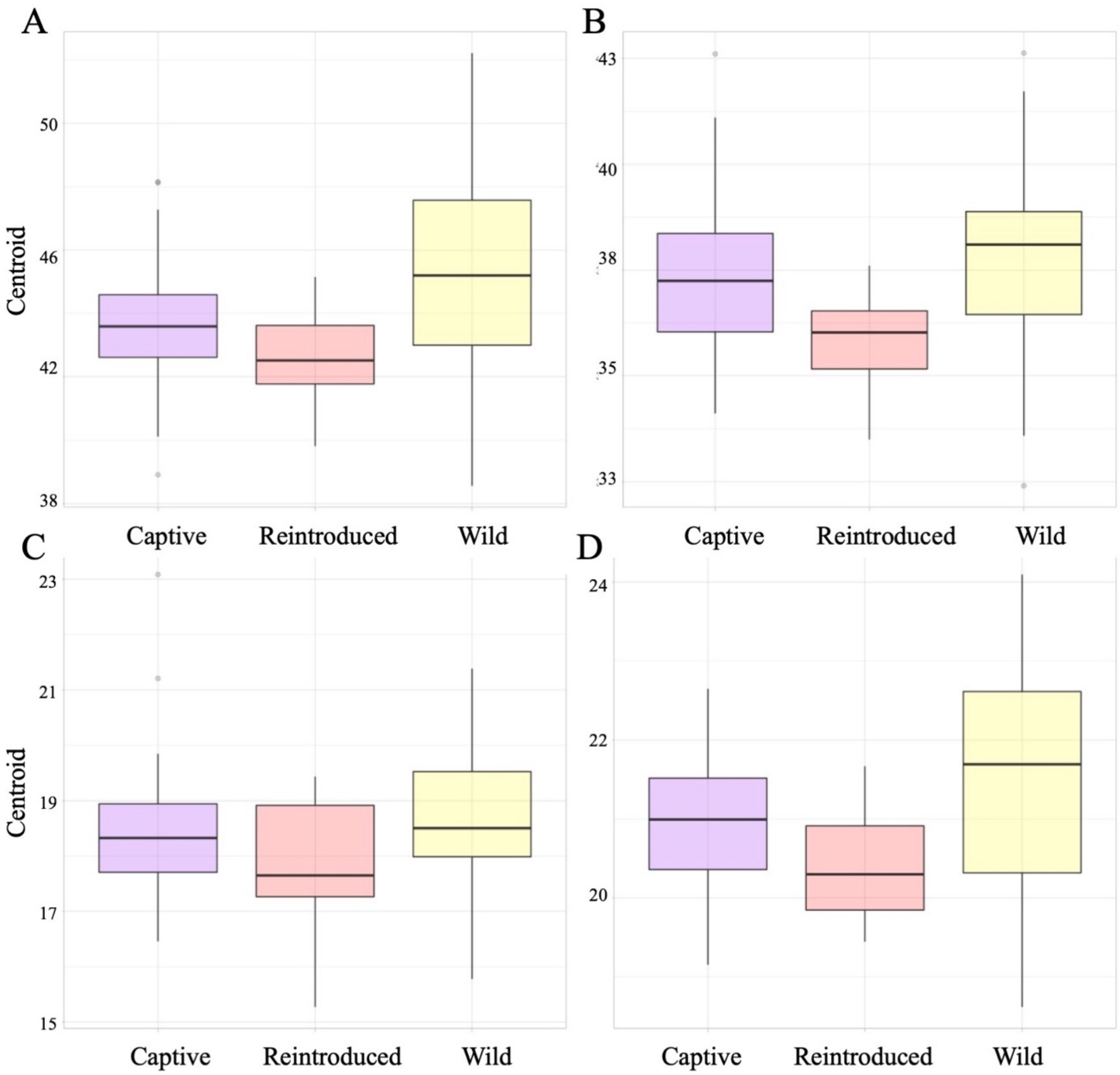


Table IV-2: MANOVA results for shape associated with population type (captive, wild, or reintroduced) and sex arranged for ventral, lateral, mandibular, and dorsal cranial views across all males (M), females (F), and the pooled dataset of both males and females (P). Sex and the interaction between sex and type was assessed only for the pooled samples.

	Sex	N	Type: Wilks	Sex: Wilks	Type * Sex: Wilks
Ventral	M	129	0.18^{***}		
	F	87	0.11^{***}		
	P	227	0.18^{***}	0.60^{***}	0.77
Lateral	M	98	0.14^{***}		
	F	74	0.28^{***}		
	P	182	0.26^{***}	0.62^{***}	0.67
Mandibular	M	136	0.23^{***}		
	F	104	0.11^{***}		
	P	249	0.21^{***}	0.64^{***}	0.72
Dorsal	M	131	0.26^{***}		
	F	86	0.17^{***}		
	P	228	0.28^{***}	0.69^{***}	0.74

P-value significance: 0.01-0.05*, 0.001-0.01**, 0-0.001***

Figure IV-3: Principal components analyses of all skull orientations: A) ventral, B) lateral, C) mandibular, and D) dorsal. Specimen types are represented by colors with 95% confidence intervals, where yellow is wild, red is reintroduced, and purple is captive. Figure is representing males only; female trends are similar.

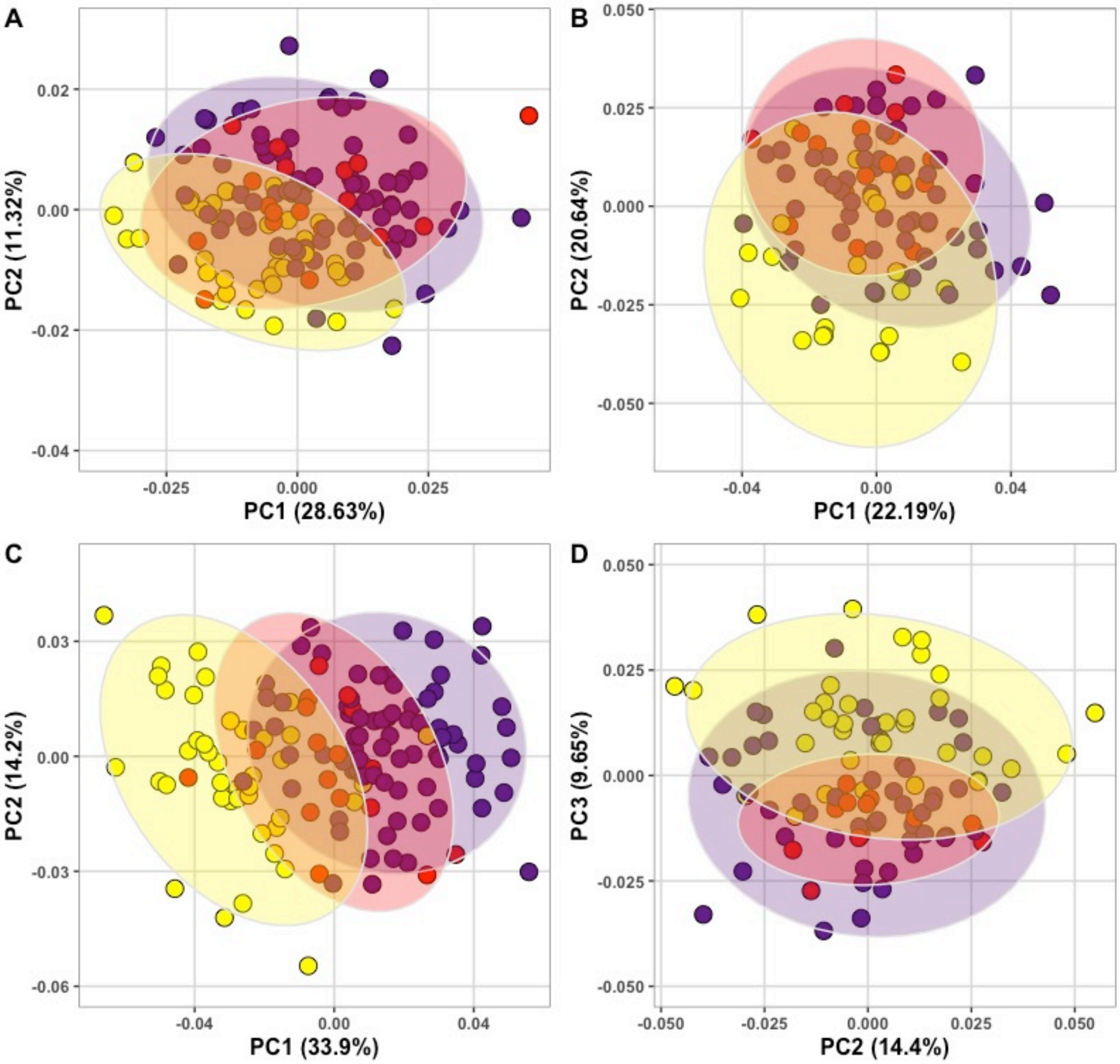
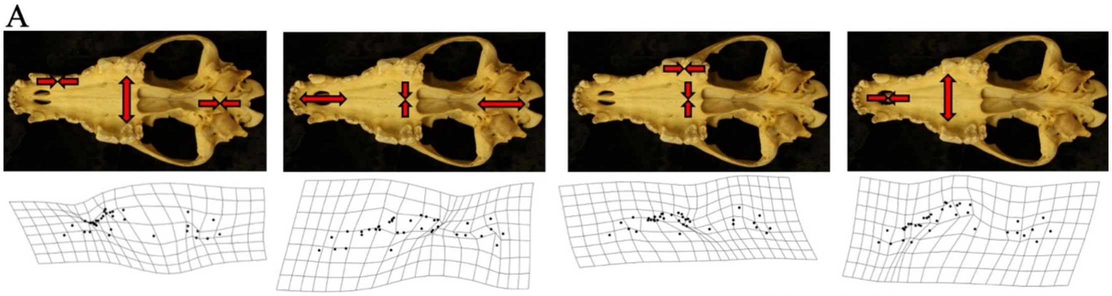
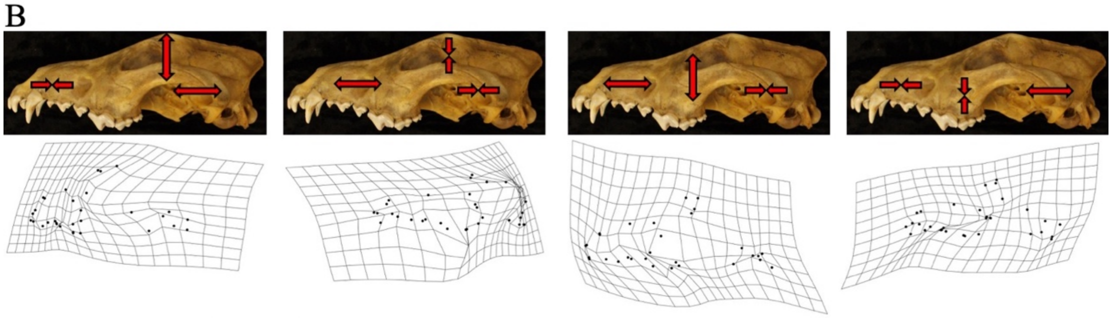


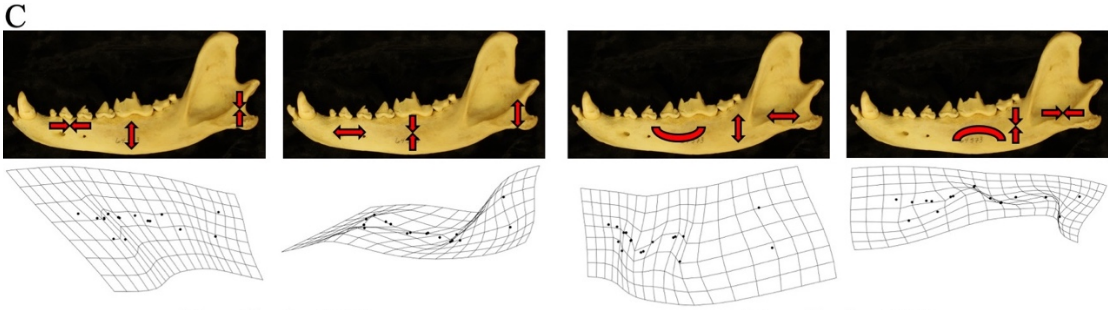
Figure IV-4: Morphology associated with PC1 and PC2 of the A) ventral, B) lateral, C) mandibular, and D) dorsal views for all captive and wild specimens. The morphological extremes of each PC axis are represented by a thin-plate spline. Red arrows indicate the regions of morphology that differentiate the extremes of the PC.



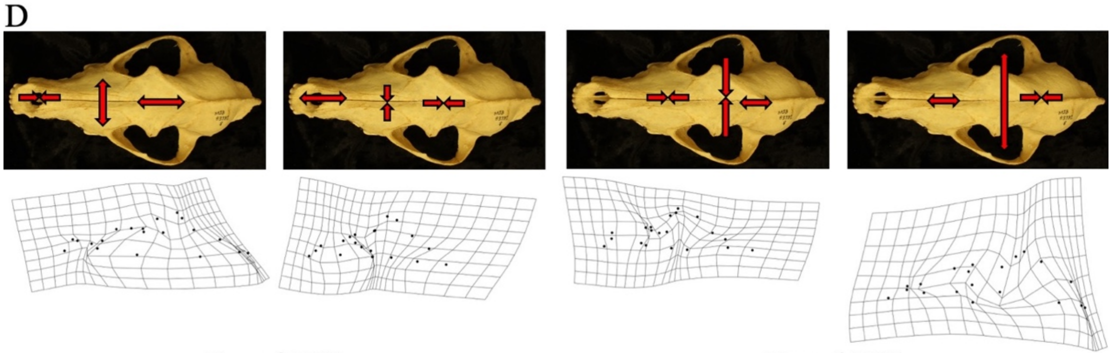
Ventral PC1 Ventral PC2



Lateral PC1 Lateral PC2



Mandibular PC1 Mandibular PC2



Dorsal PC2 Dorsal PC3

Table IV-3: **Tukey's HSD and ANOVA results for shape variation for C=captive, W=wild, and R=reintroduced specimens for ventral, lateral, mandibular, and dorsal cranial views across all males (M) and females (F).**

	Sex	N	Tukey's R-C	Tukey's W-C	Tukey's W-R	F-Statistic
Ventral	M	129	0^{***}	0^{***}	0.67	48.85^{***}
	F	87	$3.62e\text{-}5^{***}$	$6.58e\text{-}3^{**}$	0.32	13.10^{***}
Lateral	M	98	0.27	0.70	0.18	1.62
	F	74	0.06	0.85	0.15	2.79
Mandibular	M	136	0.95	$3.30e\text{-}6^{***}$	0.05	12.23^{***}
	F	104	0.99	0^{***}	$2.73e\text{-}5^{***}$	34.46^{***}
Dorsal	M	131	0^{***}	0.05^{*}	0^{***}	62.15^{***}
	F	86	0^{***}	0^{***}	0.78	61.70^{***}

P-value significance: 0.01-0.05*, 0.001-0.01**, 0-0.001***

Figure IV-5: Degree of cranial and mandibular shape variation, where purple represents captive, yellow is wild, and red is reintroduced for A) ventral, B) lateral, C) mandibular, and D) dorsal cranial views. Figure is representing males only; female trends are similar.

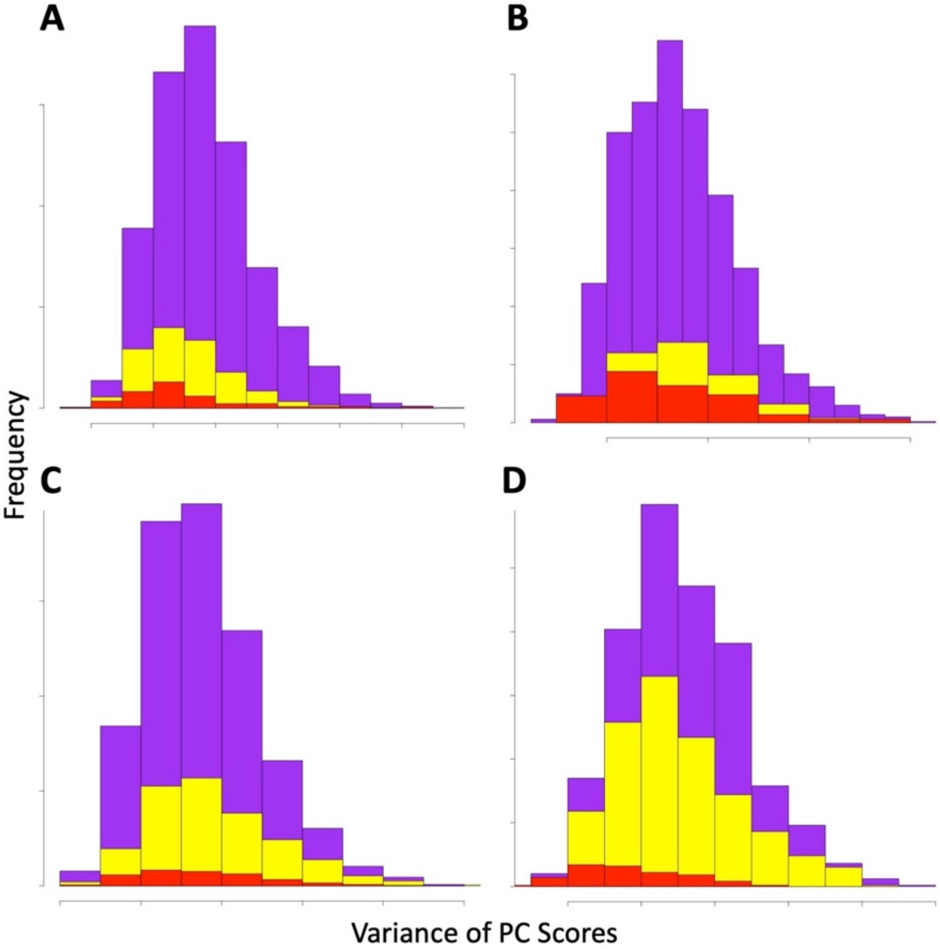


Table IV-4: Linear discriminant function analysis (LDFA) results predicting whether the morphology of reintroduced specimens aligns more closely with captive or wild specimens for ventral, lateral, mandibular, and dorsal views across all males (M) and females (F). N represents the total number of reintroduced specimens in each analysis, where % scores indicate the percentage of reintroduced specimens that align more closely with the morphology of wild or captive specimens, at a threshold of 65%. Asterisks represent the results of a Welch Two Sample T-Test.

	Sex	**N**	**% Wild**	**% Captive**
Ventral	M	19	10.5%	89.5%***
	F	16	0	93.8%***
Lateral	M	16	18.8%	43.8%*
	F	13	30.8%	53.8%
Mandibular	M	17	17.6%	47.1%*
	F	16	25%	68.8%**
Dorsal	M	18	16.7%	61.1%***
	F	14	0	85.7%***

P-value significance: 0.01-0.05*, 0.001-0.01**, 0-0.001***

Inbreeding and Heritability

The average inbreeding coefficient in the captive population ranged from 0 (an indication of no inbreeding) to 0.61 (an indication of high inbreeding), with an average coefficient of 0.17. Regardless, no significant relationships were detected when inbreeding coefficients were regressed against centroid size. Several shape variables (PC 1-3) were significant when regressed against the inbreeding coefficients; however, with the exception of PC3 of the lateral and mandibular views, these were generally weak correlations (Figure V-7). Estimates of narrow-sense heritability (h^2) ranged from 0.13 to 0.77 across size and shape traits (Table V-13). However, each of these values was associated with sizeable standard errors and should therefore be interpreted cautiously. Heritability associated with centroid size ranged from 0.20 in the lateral orientation to 0.36 in the dorsal orientation. The highest heritability values associated with PC1 were found in the lateral view ($h^2 = 0.66$) and the lowest heritability values were associated with the dorsal view ($h^2 = 0.26$). Heritability estimates were generally highest among the shape variables, although PC2 from the mandibular view did recover the lowest value ($h^2 = 0.13$) (Table V-13).

Discussion

Index of sexual size dimorphism (ISSD) results suggest that sexual dimorphism was not lost in this captive populations of Mexican wolves. *Canis lupus* subspecies are known to display sexual dimorphism (Morris & Brandt, 2014); however, *C. l. baileyi* had not been examined in previous studies. Regardless of population type, Mexican wolves show a relatively sizable degree of sexual size and shape dimorphism. Given that captive populations are generally not afforded mate choice, the loss of sexual selection can cause a corresponding loss of sexual dimorphism in captivity, particularly in populations whose breeding behavior is highly regulated (Courtney Jones et al., 2018; O'Regan & Kitchener, 2005; Schulte-Hostedde & Mastromonaco, 2015; Wisely et al., 2005). However, sexual size and shape dimorphism appears to have been retained among both captive and reintroduced populations of Mexican wolves.

The crania and mandibles of the historic wild specimens were consistently larger than captive or reintroduced individuals. This supports Frederickson & Hedrick (2002) who found a decrease in body size among captive Mexican wolves. Previous studies have shown that captive mammals frequently display a change in body size, either becoming larger due to the heightened availability of high-quality foods (Araújo et al., 2000; Crossley & Del mar Miguelez, 2001; Terranova & Coffman, 1997) or smaller, potentially due to the effects of an inbreeding depression or genetic drift (Fredrickson & Hedrick, 2002; Laikre & Ryman, 1991). Captive populations of Mexican wolves are known to have experienced fairly extensive inbreeding (Asa et al., 2007), although inbreeding coefficients were not correlated with centroid size (Figure V-7). However, inbreeding coefficients calculated from captive studbooks may represent an underestimation given that these values assume no inbreeding occurred prior to the founding captive population (Rudnick & Lacy, 2008; Witzenberger & Hochkirch, 2011). Regardless, inbreeding coefficients provide useful estimates for individual animals which may display the most extensive and recent inbreeding. The retention of the smaller body size among captive and reintroduced populations and the heritability of this trait may imply that it has been driven by genetic drift and may be expected to be retained in future generations (Ballou et al., 2012; Lynch & O'Hely, 2001; Wisely et al., 2002).

Black-footed ferrets (*Mustela nigripes*) are also a species that has benefited from extensive conservation efforts. Not unlike Mexican wolves, black-footed ferrets were reintroduced from captively-bred populations after a massive bottleneck in their wild population (Wisely et al., 2002; Wisely et al., 2005). Unlike the reintroduced ferrets (Wisely et al., 2005), reintroduced Mexican wolves have not recovered their historic wild body size. In fact, reintroduced wolves consistently displayed the smallest size overall compared to wild and captive animals. While Wisely et al. (2005) interpreted the rapid size increase among reintroduced ferrets as an indication of phenotypic plasticity, the retention of the smaller size among reintroduced Mexican wolves implies an evolutionary mechanism. While a reduction in size may be irrelevant to captive animals, whose diets are fully provisioned, it may be a substantial obstacle for reintroduced individuals. Size decreases can have significant fitness consequences for reintroduced animals that may rely on their body size to capture prey (Brzeski et al., 2014). MacNulty et al. (2009) demonstrated that the prey items captured by wild wolves in Yellowstone National Park are highly correlated to their body size; smaller wolves may capture more agile prey, but only large wolves captured large prey (MacNulty et al., 2009).

Although there was overlap, captive and wild Mexican wolves occupied different areas of morphospace. Similar to the findings of previous studies, captive skulls were primarily differentiated by their relative elongation, breadth, and arrangement of the tooth row (Curtis et al., 2018; Hartstone-Rose et al., 2014; Zuccarelli, 2004). Plastic morphological changes in captive mammal crania are frequently linked to differences in the texture of captive and wild diets (Curtis et al., 2018; Hartstone-Rose et al., 2014; Wisely et al., 2005). The diet and habitat of reintroduced populations is presumably quite similar to the environment inhabited by the historic wild population (or at least more closely aligned than what is available in captivity). The fact that reintroduced Mexican wolves have not returned to the historic wild morphospace suggests that phenotypic plasticity is not the sole mechanism of morphological change. Instead, these changes may have partially resulted from the effects of inbreeding or genetic drift, especially since the captive population was founded with very few individuals (n=7) (Greely, 2018; Hedrick & Fredrickson, 2010). Given that shape was generally heritable (Table V-13) and was not strongly correlated with inbreeding coefficients (Figure V-7), genetic drift appears to be the likely mechanism behind the observed morphological change.

Morphological differences between reintroduced and wild populations are particularly important to identify for a species of such heightened conservation interest as these cranial regions largely reflect jaw strength and the relative ability to secure and capture prey in the wild (Law et al., 2018; Wroe et al., 2005). Given that these cranial shape changes are associated with functionality and may impact fitness, these differences may represent an obstacle to reintroduction success (Blanchet et al., 2008; McPhee, 2004; Wisely et al., 2005). However, a direct link between the observed shape changes and functionality remains speculative. An interesting next step in this line of research will be to measure functionality and diet, perhaps through bite force and stable isotope analyses between reintroduced and wild Mexican wolves, to assess whether there has been any detectable shifts.

Captive individuals consistently displayed the greatest variation in morphological space (Figure IV-5; Table IV-3). Without the selective pressures of living in the wild, captive individuals display a variety of phenotypes without fitness consequences (Bryant & Reed, 1999; Lynch & O’Hely, 2001; McPhee & McPhee, 2012). The broader morphological space inhabited by the captive wolves suggests a degree of relaxed selection (Bryant & Reed, 1999; Lynch & O’Hely, 2001; McPhee & McPhee, 2012). Interestingly, reintroduced individuals displayed the least variation in morphospace. This may partially be the result of the population bottleneck the species experienced and may also suggest that the reapplication of wild pressures is forcing a return to the wild morphospace.

Neither the number of captive nor reintroduced generations had an impact on morphology (Table V-10). In fact, morphological differences seem to have occurred quickly within the captive population and do not appear to differ substantially from what is displayed by specimens that represent up to 12 captive generations (although trends vary among different size and shape variables, see Figure V-8). The speed with which these differences appeared in the population may reflect phenotypic plasticity (Courtney Jones et al., 2018; Mason et al., 2013; Renaud et al., 2015). However, the population bottleneck experienced by the species may have made it prone to the effects of genetic drift (Frankham, 2008; Lacy, 1989; Wisely et al., 2005; Willoughby et al., 2015). Given the 16-year gap between the most recent wild caught individual (ca. 1958) and the oldest captive individual (ca. 1974) in the dataset, the effects of genetic drift may have arisen in the wild (at some point after 1958) and simply continued in the captive population. The retention of these heritable traits may suggest a genetic change in the population, where drift may be likely. While differing morphology due to phenotypically plastic changes can be apparent within a single generation, the retention of traits within the reintroduced population suggests that plasticity is not the sole causal mechanism.

Reintroduced animals tended to display an intermediate morphotype between captive and wild populations, regardless of the number of post-captive generations. The relative explosion in morphospace inhabited by the captive population followed by the precipitous loss in morphospace among the reintroduced population may suggest that once wild pressures are reapplied and there are fitness consequences associated with certain areas of occupied morphospace, what remains in the reintroduced population represents all of the practical morphological variation (that can sustain life in the wild) left after the bottleneck. The fact that the reintroduced population still aligns with the captive population in many regards may be the product of a founder effect, in which a population lacks genetic diversity given the small number of originating founders (Kraaijeveld-Smith et al., 2006; Montgomery et al., 1997; Schulte-Hostedde & Mastromonaco, 2015).

Overall, the findings of this study suggest that the skulls of captive, wild, and reintroduced Mexican wolves are differentiated in size and shape. Sexual dimorphism was retained within all of the captive and reintroduced populations. The number of generations in captivity or reintroduced to the wild appears to be irrelevant to the resulting morphotype. Given the wide breadth of morphological trait values displayed in captivity, this may imply relaxed selection within the captive population. The retention of similar size and shape values between captive and reintroduced populations, along with the heritability of these traits, suggests that these morphological changes are also driven by genetic drift. Given the population bottleneck experienced by the species and the small number of founding individuals used to establish the captive population, genetic drift is likely to have occurred (Kraaijeveld-Smit et al., 2006; Lacy, 1987; Willoughby et al., 2015). Although inbreeding was not strongly correlated to the changes in these populations, certain members of the captive population have extremely high inbreeding coefficients (up to 0.61), so continued genetic monitoring and careful breeding recommendations are imperative for the continued success of the captive population. The effects of inbreeding and drift are frequently deleterious and may have substantial fitness consequences for reintroduced populations (Araki et al., 2007; Araki et al., 2009; Lacy, 1989; Lynch & O'Hely, 2001). However, reintroduced populations of Mexican wolves have seen modest, but continued population growth (Greely, 2018), suggesting that these effects have not yet overwhelmed the viability of the species.

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CHAPTER V

CONCLUSIONS

General Framework

Captivity often represents the last refuge for threatened and endangered species (Alroy, 2015; Balmford et al., 2011; Conde et al., 2011). Zoos can provide a safe environment for species that may otherwise exist in perilous conditions (Alroy, 2015; Balmford et al., 2011; Conde et al., 2011). To fulfill their potential, zoos must preserve species in a way that captive animals remain appropriate proxies for their wild conspecifics so that they could successfully be reintroduced if wild habitats become hospitable again (McPhee, 2004; Rudnick & Lacy, 2008; Shepherdson, 1994; Willoughby et al., 2015; Wisely et al., 2005). However, previous studies suggest that captive mammals frequently display differentiation in cranial morphology compared to their wild counterparts (e.g., Antonelli, 2015; Curtis et al., 2018; Groves, 1966; Groves, 1982; Hartstone-Rose et al., 2014; Hollister, 1917; McPhee, 2004; O'Regan, 2001; O'Regan & Kitchener, 2005; Selvey, 2018; Van Velzen, 1967; Wisely et al., 2002; Wolfgramm, 1894); although the commonality, directionality, and frequency of these morphological changes has largely remained unclear. In this dissertation, I examined morphological changes in captivity by quantitatively assessing the existing literature, examining cranial morphology in the Family Canidae, and conducting a case study of reintroduced captively-bred wolves. In doing so, this dissertation addressed some of the long-standing hypotheses in the field of comparative morphology and provided husbandry suggestions to avoid morphological differentiation in future captive generations.

Objectives and Conclusions

Commonality and Magnitude of Morphological Variation

One of the primary objectives of this dissertation was to assess whether captive populations display predictably distinct cranial morphology compared to their wild counterparts. For over a century, morphology studies have documented significant differences between populations of captive and wild mammals (e.g., Hollister, 1917; Wolfgramm, 1894). While commonalities appeared to exist within these sources, it has remained unclear whether disparate taxa display similar changes or changes of the same magnitude. To assess this, I examined the published literature regarding cranial morphology in captive mammals using a phylogenetic meta-analysis to detect the nature of morphological changes in captivity.

Wild and captive mammals often differ in cranial morphology and the nature and magnitude of their cranial differences varied considerably across taxa (Chapter II). In general, captive carnivorans displayed a widening of the crania, whereas other groups, including many primate and ungulate species, displayed the opposite trend. This implies that the nature of cranial changes in captivity may be specific to particular taxonomic groups. However, it may be possible to establish trends across smaller taxonomic units (e.g., mammalian orders or families), or even disparate groups that utilize their cranial morphology in a similar way. Trends associated with the carnivorous marsupial *Sminthopsis macroura*, for example, largely reflected what was observed among the eutherian order Carnivora. The magnitude of the trends also varied widely between individual species. While the majority of species displayed significant differentiation in captivity compared to wild populations in at least one cranial measure, the degree of changes varied. Certain species, most notably black-footed ferrets (*Mustela nigripes*), displayed such a major shift (often in the opposite direction of other carnivorans) that they were clear outliers in most analyses. While other species, such as chimpanzees (*Pan troglodytes*), displayed little to no differentiation in captivity compared to the wild. The extreme morphological change among black-footed ferrets may be related to the population bottleneck experienced by the species prior to captivity, as well as the small number of founders that originated the captive population. Likewise, the lack of change among chimpanzees may be related to their generalist diet or their taxonomic history, given that many primates in this dataset showed minimal morphological differences in captivity compared to the wild. This suggests that morphological changes in captivity cannot be generalized across all mammals and that individual species history and taxonomic groupings are an important consideration.

Similar results were detected within Family Canidae (Chapter III). Many canids displayed a common trajectory of changes in captive populations, including a widening of the zygomatic arch and palate, as well as a deepening of the mandibular body. Among the 15-canid species included in this analysis, several shifted considerably in morphological space from their wild counterparts, although some species showed no changes in captivity. Certain species, such as red foxes (*Vulpes vulpes*) showed heightened differentiation at every axis of variation that was assessed. However, other species, such as short-eared dogs (*Atelocynus microtis*) and raccoon dogs (*Nyctereutes procyonoides*) did not display differentiation at any of the morphological axes. These changes may suggest a corresponding shift in functionality, perhaps including a change in maximal bite force.

Predicting Morphological Variation in Captive Populations with Species Ecology

Captivity does not induce a universal shift in any particular morphological feature across captive mammalian populations, so it is useful to understand key predictors of observed shifts. Correlations have been suggested between the morphological variation of captive populations and certain traits, such as species diet or home range size (Curtis et al., 2018; Duckler, 1998; Hartstone-Rose et al., 2014; Hollister, 1917; O'Regan & Kitchener, 2005). The results of the meta-analysis suggest that carnivorous species with limited dietary breadth display the most predictable changes, particularly associated with skull widening (Chapter II). Species with a more generalized diet displayed much less morphological change in captivity. Home range size was less predictive than diet, although species with the smallest home ranges were associated with a greater magnitude of change (Chapter II). This suggests that diet is more predictive of morphological changes occurring in captivity than home range size and that carnivory specialists may display the most distinct cranial changes compared to populations in the wild.

Analyses conducted exclusively on canid species revealed similar trends where hypercarnivorous species generally displayed the greatest morphological changes in captivity compared to the wild (Chapter III). These morphological changes were associated with a widening of the skull and shortening of the toothrow, potentially indicative of an increased bite force among the captive population (Erickson et al., 2004; Hartstone-Rose et al., 2014). In general, mesocarnivorous species, including coyotes (*Canis latrans*) and short-eared dogs (*A. microtis*), showed the least morphological changes in captivity. However, regardless of carnivory type, many captive canid species displayed an increasingly wide skull and shortened toothrow. This means that although hypercarnivores display the greatest morphological changes in captivity, all species display a morphological shift in a similar direction, perhaps suggesting a common mechanism of change across canid species.

Morphological Differentiation of Captive and Reintroduced Mexican Wolves

Mexican wolves (*Canis lupus baileyi*) have received substantial restoration efforts in the form of intensive captive maintenance, breeding, and reintroduction initiatives (Greely, 2018; Hedrick & Fredrickson, 2010; Kalinowski et al., 1999; Lynch, 2005). Given the massive resources devoted to their conservation, it is useful to note whether their morphology has remained static after generations of captivity. A significant difference was detected in the skull sizes of these animals, where wild specimens were the largest and reintroduced specimens were the smallest (Chapter IV). Captive specimens also displayed significantly differentiated cranial shapes in terms of their zygomatic width and tooth row length, which could imply a change in their cranial functionality. In general, reintroduced specimens displayed an intermediate morphology between the captive and historic wild populations. Captive individuals displayed the greatest overall variation in shape, positioned in regions of morphospace unused by the wild or reintroduced populations. Although captive mammals have been known to display a change in sexual size and shape dimorphism association with cranial and mandibular traits (Kamaluddin et al., 2019; Lynch & Hayden, 1995; Wisely et al., 2005), sexual dimorphism did not differ between captive and wild Mexican wolves.

Mechanisms of Changes

To understand the mechanisms that drive morphological changes in captivity, it is useful to know whether the changes are retained among reintroduced populations, the number of founders used to establish the captive population, and the ways in which the traits differ over captive and reintroduced generations. Among Mexican wolves, the morphological changes that arose within the captive population were retained among all reintroduced generations (Chapter IV)**.** Given that phenotypically plastic effects associated with captivity should be absent in the reintroduced population as individuals quickly revert to the wild morphology (Wisely et al., 2005), the retention of these traits suggests that there are other mechanisms driving morphological changes in captivity. The small size of the founding population along with the increased variation in cranial morphology among captive Mexican wolves suggests that morphological changes in captivity may be related to genetic drift and relaxed selection. Although small population sizes can also lead to the effects of inbreeding (Rudnick & Lacy, 2008; Wisely et al., 2002; Witzenberger & Hochkirch, 2011), inbreeding coefficients were not correlated with the changes in morphology that I observed. The reintroduced populations largely represented intermediate forms between the captive and wild individuals and displayed a much narrower trait distribution in cranial morphology. This may imply that the morphology displayed among reintroduced Mexican wolves may be a product of genetic drift and the reapplication of wild natural selection pressures.

The cranial morphology of hypercarnivorous species is expected to display the largest changes in captivity given the absence of tough materials in their captive diet as compared to what they may consume in the wild (e.g., large-bodied animals; Curtis et al., 2018; Hartstone-Rose et al., 2014; Hollister, 1917; Wolff, 1892). In this study, hypercarnivorous captive canid species did display significant changes in their cranial morphology associated with their skull width, toothrow (Chapter III) confirming the idea that hypercarnivores display the largest morphological changes in captivity, at least among canid species. However, regardless of carnivory type, many of the canid species displayed a similar change in morphology.

Although animals have been maintained in captivity for centuries, the quality of their care and the diets they are provided has improved steeply over the past several decades (Dierenfeld, 1997; Oftedal & Allen, 1997). Among other factors, these improvements have translated into enhanced nutrition and efforts to provide the appropriate dietary texture to captive populations (Bond & Lindburg, 1990; Kapoor et al., 2016; Lindburg, 1988; Vosburgh et al., 1982; Young, 1997). If diets are the proximate cause of cranial shape changes in captive populations, and if efforts to improve diets have been successful among captive canid species, we might expect a corresponding decrease in cranial differentiation, where individuals that were from a captive population more recently might display less cranial differentiation than older specimens. However, results associated with these trends were generally not significant, suggesting that either 1) past diets did not drastically affect cranial morphology, 2) recent diets may still have an inappropriate diet texture (either too soft or too tough) for captive canids, 3) diet texture does not drive cranial changes in captive animals (Chapter III).

Recommendations

Captive hypercarnivorous canids display heightened cranial differentiation, a trend which does not appear to have decreased over time (Chapter III). Whether or not diet has impacted morphology, I recommend that zoos continue to provide additional opportunities for hypercarnivorous species to use their substantial cranial musculature.

Providing a tougher diet may not only reduce the amount of morphological change occurring in captivity (Fitch & Fagan, 1982; Kapoor et al., 2016; Young, 1997) and benefit oral health (Bond & Lindburgh, 1990; Fitch & Fagan, 1982; Glatt et al., 2008; Kapoor et al., 2016;), it can also provide environmental enrichment (e.g., provisioning of species-appropriate stimuli intended to engage natural behaviors), which may reduce stress levels and deter stereotypic behavior (Bond & Lindburgh, 1990; Glatt et al., 2008; Lindburg, 1988; McPhee 2002; Young, 1997). Captive facilities are not always comfortable providing naturalistic diets, such as animal carcasses, given the risk of broken teeth, the relative difficulty associated with obtaining such items, the potential for disease transmission, as well as the amount of cleaning and preparation required to provide such a diet (Glatt et al., 2008; Young, 1997). However, similar effects may be achieved with tough enrichment items including Kongs, tires, bark (Cloutier & Packard, 2014; Glatt et al., 2008), or other objects that motivate the animal to bite, gnaw, or chew.

Although some morphological changes occurring in captivity may be associated with diet texture (see suggestions above), some of the differences may be related to selection, genetic drift, or inbreeding. Given that morphological changes occurring in captivity are unlikely to benefit a reintroduced animal (McPhee, 2004; Pelletier et al., 2009; Wisely et al., 2005), it is important to avoid them when possible. In the case of Mexican wolves, a small captive population size was unavoidable, which made the population prone to drift (McPhee, 2004; Kraaijeveld-Smit et al., 2006; Witzenberger & Hochkirch, 2011), an unfortunate, but necessary measure to preserve the species from extinction. Breeding remnant populations of endangered species often represents the only hope for their ongoing survival. Although many zoo animals are not presently endangered or threatened (Alroy, 2015; Balmford et al., 2011; Fa et al., 2014; Frynta et al., 2013), the current rate of anthropogenic destruction may imperil many additional species (Baronsky et al., 2011; Ceballos et al., 2017). Therefore, all captive animals should be maintained with a similar goal of preservation, regardless of their present endangerment status. In this effort, enhancing the effective population size by maintaining gene flow among captive populations is essential to avoid the deleterious effects of drift or inbreeding. Captivity can represent an essential reservoir for animal populations, protecting and preserving these species even when wild conditions become inhospitable. However, this can only be achieved if captive animals remain suitable approximations of wild populations.

Captive specimens are frequently avoided in morphological research given that they are suspected to display abnormalities which may not be representative of the morphology observed among wild populations (Drumheller et al., 2016; Hartstone-Rose et al., 2014). Certain mammalian groups may display greater morphological differentiation in captivity than others; those species that are especially adept at gnawing, chewing, or consuming particularly tough materials appear to be most prone to morphological changes in captivity. However, the changes occurring among ungulate and primate species appear to be less apparent and much more variable, possibly related to their less specialized cranial musculature and diets (Chapter II). This suggests that the crania of captive hypercarnivores and gnawing rodents should continue to be avoided in morphology studies; however, captive species with less specialized cranial musculature may be useful in future morphology studies on a case-by-case basis, with preliminary investigation.

Recommendations for Future Work

Future studies examining specific captive facilities and how they relate to morphological differentiation could be beneficial to our broader understanding of the morphological changes that occur in captivity. In particular, the ways in which the diets, enrichment, and enclosure designs of different zoos relate to the degree of morphological differentiation could help inform future captive management trends. Likewise, not all captive facilities share the same imperatives as AZA-accredited zoos. It would be useful to explore the cranial morphology of species housed in non-accredited captive facilities that display animals for entertainment (e.g., road-side zoos, circuses, or other show animals) or material goods (e.g., fur farms). Such exploration would help determine whether enhanced husbandry practices mandated by the AZA help to preserve wild morphotypes as well as the unintended impacts of artificial selection on the cranial morphology of exotic species. Additional temporal data could also be useful in determining whether any notable changes in cranial morphology have been associated with specific regulatory improvements at individual facilities or throughout the AZA at large.

The cranial changes observed in this dissertation were generally related to zygomatic width or toothrow, traits which are often associated with an animal's functionality including their bite force and dietary niche (Meloro et al., 2017; Van Valkenburgh, 1989; Wroe et al., 2007). An interesting next step in this research will be to examine specific relationships that exists between variation in cranial morphology and functionality. In particular, additional analyses could detect whether the change in cranial morphology has translated into an enhanced bite force as expected based on the increased zygomatic breadth (Erickson et al., 2004; Hartstone-Rose et al., 2014) displayed by many captive specimens. Likewise, given that the cranial morphology of reintroduced Mexican wolves mirrors the captive population, a stable isotope analysis of fur samples could help indicate whether these changes have translated into dietary differences among these animals.

Exploring the morphology of captive taxa with even greater dietary diversity could also be quite instructive. For example, the carnivoran family Ursidae includes the hypercarnivorous polar bear (*Ursus maritimus*) and the herbivorous giant panda (*Ailuropoda melanoleuca*), which predominantly consumes bamboo (Sacco & Van Valkenburgh, 2004). Although these species have widely different dietary preferences, we might expect them to display similar morphological change in captivity given the amount of musculature required to process both diets (Curtis et al., 2018; Law et al., 2018). Likewise, these trends may also be investigated in the context of convergence by exploring the morphology of non-carnivoran carnivores, such as marsupial species belonging to the family Dasyuridae. If captive husbandry is the primary factor influencing morphological changes in captivity, we would expect hypercarnivorous marsupials to display changes similar to their eutherian counterparts. Examining these trends would help explore the degree to which cranial functionality explains the nature of the morphological changes we observe in captivity.

Summary

Although morphological changes occurring in captivity have been repeatedly demonstrated, especially in particular mammal groups, the reliability and predictability of these changes is not well understood. By examining the existing data using a quantitative approach, this dissertation helps to establish the directionality of these changes, their magnitude, and the species that may be most at risk of morphological differentiation. This dissertation shows that certain species groups may be more prone to morphological change, particularly hypercarnivores. Additionally, although Mexican wolves represent a group of particular conservation interest, the cranial and mandibular morphology of the captive and reintroduced populations were previously undescribed until this study. While my research shows that captive individuals did differentiate from their historic wild population, the reintroduced population did not return to the wild morphotype, suggesting that the morphological differences noted among the captive population may not have resulted exclusively due to phenotypic plasticity.

For most of their history, the primary goal of captive facilities has been entertainment and recreation; however, in the past few decades that focus has shifted dramatically (Fa et al., 2011; Hosey et al., 2009; Zimmerman et al., 2007). Given their housing of rare and endangered species, zoos are uniquely instrumental in conservation efforts. To successfully fulfill that role, captive animals should remain relatively morphologically and genetically unchanged so that they may be appropriate proxies for their wild conspecifics. Although previous studies have suggested that animals differentiate in captivity (O'Regan & Kitchener, 2005), these trends have remained largely untested. This is of particular concern given that morphological changes occurring in captivity could have substantial impacts on the long-term success of reintroduced populations (Bryant & Reed, 1999; Edwards, 2014; Frankham et al., 1986; Håkansson & Jensen 2008; McPhee & Silverman, 2004; McPhee, 2004).

Canids in particular are frequent targets of conservation initiatives, including the reintroduction of captively-bred Mexican wolves (*Canis lupus baileyi*), red wolves (*Canis rufus*), African painted dogs (*Lycaon pictus*), and swift foxes (*Vulpes velox*) (Moehrenschlager & Somers, 2004). Each of these initiatives required extensive coordination, man-hours, and monetary resources (Beck et al., 1994; Hutchins et al., 2003). Reintroductions often fail more than they succeed, representing massive financial losses and often the death of individuals belonging to imperiled species (Beck et al., 1994; Frankham, 2008). Understanding how species change in captivity, if at all, helps point to the challenges facing reintroduction efforts and the ways captive management should be altered to help mitigate those effects. If captive animals show dramatic morphological changes, their reintroduction success may be limited (McPhee, 2004; Pelletier et al., 2009; Wisely et al., 2005) and they may no longer accurately represent wild populations, making them less useful for conservation purposes. By documenting and understanding the nature of morphological changes occurring in captivity, we can avoid those effects in the future and promote the long-term conservation goals of captive facilities and contribute to the grand challenge of conserving biodiversity.

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APPENDIX A

CHAPTER II APPENDIX AND SUPPLEMENT

Table V-1: Ecological covariate categories applied to species in this dataset as found in the PanTHERIA (Jones et al., 2009). Trophic levels include carnivores (carn), omnivores (omn), and herbivores (herb). Dietary breadth refers to the number of dietary items consumed by the species, either 1, 2-3, or 4+ items. Home range sizes ranged from small (S), to medium (M), to large (L). See Table I.1 for taxonomic information each species.

Species	Trophic Level	Dietary Breadth	Home Range Size
Canis lupus	Carn	1	L
Canis latrans	Carn	1	M
Chlorocebus aethiops	Omn	4+	S
Dicerorhinus sumatrensis	Herb	2-3	M
Equus asinus	Herb	1	L
Equus hemionus	Herb	1	M
Gorilla gorilla	Omn	2-3	S
Hydrochoerus hydrochaeris	Herb	4+	S
Lemur catta	Omn	4+	S
Mustela nigripes	Carn	1	S
Pan troglodytes	Omn	4+	M
Panthera leo	Carn	1	L
Panthera tigris	Carn	1	L
Peromyscus polionotus	Herb	2-3	S

Table V-1 Continued

Species	**Trophic Level**	**Dietary Breadth**	**Home Range Size**
Pongo pygmaeus	Omn	4+	S
Rhinoceros unicornis	Herb	2-3	S
Sminthopsis macroura	Carn	2-3	S

Table V-2: Data extracted from studies. Type refers to geometric (geo) or traditional morphometric (trad) analyses. Source is the section of the publication from which the data were extracted. Data were reported as a mean and variance measure or a t-statistic. Variance measures included standard deviations (StDev), standard error (SE), or the prognostic method (Prog), which was used to estimate the standard deviation. See Table V-1 for taxonomic information each species.

Study	Species	Type	Source	Data Reported
Aeschbach et al., 2016	*Hydrochoerus hydrochaeris*	Geo	Supplement	Mean, StDev
Antonelli, 2015	*Mustela nigripes*	Trad	Table 3.2	Mean, StDev
Curtis et al., 2018	*Canis latrans*	Trad	Table 4	t-statistic
Groves, 1966	*Equus asinus,* *Equus hemionus*	Trad	Table 1	Mean, Prog
Groves, 1982	*Dicerorhinus sumatrensis,* *Rhinoceros unicornis*	Trad	Tables 1, 2, & 4	Mean, Prog
Guay et al., 2012	*Sminthopsis macroura*	Trad	Table 1 & from author	Mean, StDev
Hartstone-Rose et al., 2014	*Panthera leo,* *Panthera tigris*	Trad	Supplement	Mean, StDev
McPhee, 2004	*Peromyscus polionotus*	Geo	Figure 4	Mean, StDev

Table V-2 Continued

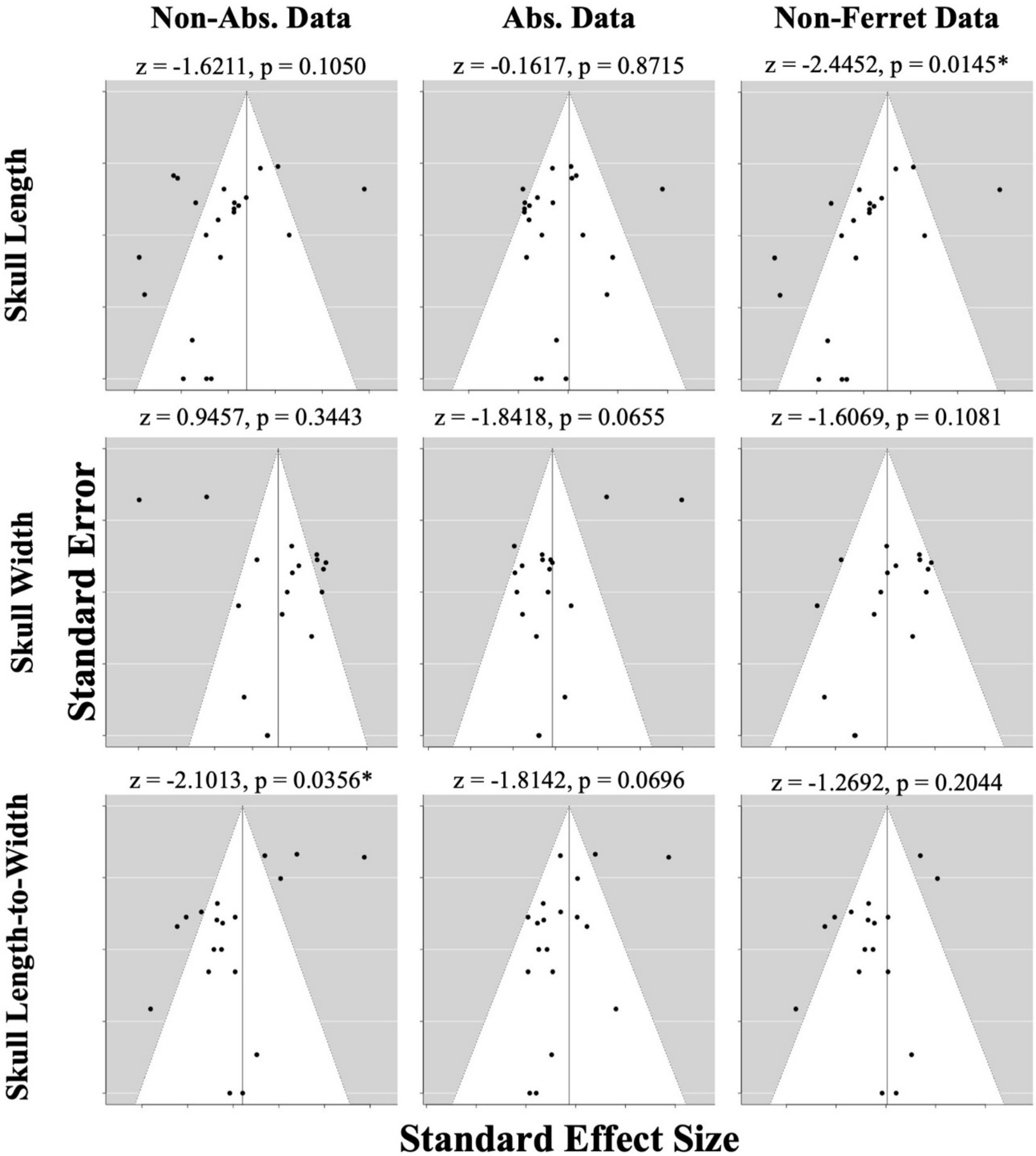
Study	**Species**	**Type**	**Source**	**Data Reported**
Selvey, 2018	*Lemur catta*	Trad	Figure 3.1	Mean, StDev
Turner et al., 2016	*Chlorocebus aethiops*	Trad	Table 2 & 3	Mean, StDev
van Velzen, 1967	*Gorilla gorilla,* *Pan troglodytes,* *Pongo pygmaeus*	Trad	Figures 5, 7, 9	Mean, StDev
Wisely et al., 2002	*Mustela nigripes*	Trad	Table 3	Mean, SE
Wolfgramm, 1894	*Canis lupus*	Trad	Table 2	Mean, StDev

Table V-3: Pagel's λ results reporting the mean lambda estimate from 2,000 randomly selected phylogenic trees and the standard deviation (StDev) around that mean for skull length, skull width, and skull length-to-width (L:W) analyses.

Feature	Analysis	λ Mean	λ StDev
Skull Length	Directional	0.92^{***}	0.009
	Directional w/o ferrets	0.87^{**}	0.011
	Magnitude	0.69^{*}	0.088
Skull Width	Directional	0.79^{*}	0.015
	Directional w/o ferrets	0.51^{*}	0.015
	Magnitude	0.70	0.011
Skull L:W	Directional	0.88^{*}	0.009
	Directional w/o ferrets	0.72^{*}	0.006
	Magnitude	0.41	0.041

P-value significance: 0.01-0.05*, 0.001-0.01**, 0-0.001***

Figure V-1: **Funnel plots exploring publication bias in the dataset for each meta-analysis conducted. Asymmetry was assessed with Egger's regression.**



APPENDIX B

CHAPTER III APPENDIX AND SUPPLEMENT

Table V-4: Definitions of appendix columns and abbreviations

Column	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**
Label	Collection	Number	Genus	Species	Subspecies	Type	Country	State	Year	Sex	Carnivory
Summary	Natural history collection where specimen is housed	Museum catalogue number				How the specimen was defined for this research			Specimen date from museum record	Sex of the specimen	Carnivory type
Types	See Table S2					**C**: Captive **W**: Wild				**F**: Female **M**: Male **U**: Sex unknown	**hyper**: hypercarnivore **hypo**: hypocarnivore **meso**: mesocarnivore

Table V-5: Museum list

Collection Code	**Institution**	**Location**
AMNH	American Museum of Natural History	New York, New York
BRTC	Texas A&M University Biodiversity Research and Teaching Collections	College Station, Texas
CAS	California Academy of Sciences	San Francisco, California
DMNS	Denver Museum of Nature & Science	Denver, Colorado
FMNS	Field Museum of Natural History	Chicago, Illinois
HSU	Humboldt State University	Arcata, California
KU	University of Kansas Biodiversity Institute	Lawrence, Kansas
LACM	Natural History Museum of Los Angeles County	Los Angeles, California
LSU	Louisiana State University Museum of Natural Science	Baton Rouge, Louisiana
MSB	Museum of Southwestern Biology	Albuquerque, New Mexico
MSU	Michigan State University Museum	Lansing, Michigan
OMNH	Sam Noble Oklahoma Museum of Natural History	Norman, Oklahoma
PSM	James R. Slater Museum of Natural History	Tacoma, Washington
ROM	Royal Ontario Museum	Toronto, Canada
UCLA	University of California, Los Angeles Museum	Los Angeles, California
UMMZ	University of Michigan Museum of Zoology	Ann Arbor, Michigan
USNM	National Museum of Natural History, Smithsonian Institute	Washington, D.C.

Table V-6: Anatomical landmark definitions. Landmark types based on Bookstein (1991) typology of landmarks, where Type 1 refers to a point at which several sutures meet, Type 2 refers to a landmark whose location is informed by the presence of another feature (e.g. maximum point of curvature), and a Type 3 landmark refer to a point defined by several other anatomic features (e.g. meeting of two curves) (Cooke & Terhune, 2015; Weber & Bookstein, 2011).

Orientation	Number	Type	Definition
Ventral	1	2	Basion: medial, posterior point of occipital
Ventral	2	1	Hypoglossal canal
Ventral	3	2	Anterior point of the tympanoocipital fissure
Ventral	4	1	Spinous foramen
Ventral	5	1	Retroarticular foramen
Ventral	6	1	Lateral suture of the temporal and zygomatic bones
Ventral	7	2	Posterior point of P4 alveoli, lateral edge of maxilla
Ventral	8	2	Medial, anterior point of P4 alveoli
Ventral	9	1	Anterior notch of P4 alveoli
Ventral	10	2	Lateral, anterior cusp of P4 tooth, lateral edge of maxilla
Ventral	11	2	Posterior point of P3 alveoli, lateral edge of maxilla
Ventral	12	1	Posterior cusp of P3
Ventral	13	2	Posterior point of P2 alveoli, lateral edge of maxilla
Ventral	14	2	Medial, Anterior point of P2 alveoli
Ventral	15	2	Posterior point of canine alveoli, lateral edge of maxilla
Ventral	16	2	Posterior point of I3 alveoli, lateral edge of premaxilla
Ventral	17	1	Prosthion, rostral tip of premaxilla, medial diastema of I1
Ventral	18	2	Incisive foramen, rostral end of palatine fissure
Ventral	19	2	Incisive foramen, caudal end of palatine fissure

Table V-6 Continued

Orientation	Number	Type	Definition
Ventral	20	1	Medial suture of the premaxilla and maxilla
Ventral	21	1	Medial suture of the maxilla and palatine
Ventral	22	1	Major palatine foramen
Mandibular	1	1	Rostral-most tip of prothion
Mandibular	2	2	Posterior point of canine alveoli
Mandibular	3	1	Rostral mental foramen
Mandibular	4	2	Anterior point of P2 alveoli
Mandibular	5	2	Posterior point of P2 alveoli
Mandibular	6	2	Anterior point of P3 alveoli
Mandibular	7	2	Posterior point of P3 alveoli
Mandibular	8	2	Anterior point of P4 alveoli
Mandibular	9	2	Posterior point of P4 alveoli
Mandibular	10	2	Anterior point of M1 alveoli
Mandibular	11	2	Point of maximum curvature of M1
Mandibular	12	3	Ventral-most extension of landmark 11
Mandibular	13	2	Posterior point of M1 alveoli
Mandibular	14	2	Anterior point of M2 alveoli
Mandibular	15	2	Posterior point of M2 alveoli
Mandibular	16	2	Caudal-most point of toothrow
Mandibular	17	2	Dorsal-most tip of the coronoid process

Bookstein F.L. 1991. Morphometric tools for landmark data: Geometry and biology. Cambridge New York: Cambridge University Press.

Cooke, S. B., & Terhune, C. E. (2015). Form, function, and geometric morphometrics. *Anatomical Record*, *298*(1), 5–28.

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APPENDIX C CHAPTER IV APPENDIX AND SUPPLEMENT

Table V-7: Definitions of Chapter IV appendix columns and abbreviations.

Column	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
Label	Collection	Number	Year	Type	Sex	Studbook	Gens_C	Gens_R
Summary	Natural history collection where specimen is housed	Museum catalogue number	Date of specimen death from museum record	How the specimen was defined for this research	Sex of the specimen	Studbook number associated with the specimen	Number of generations since the specimen was captive	Number of generations since the specimen was reintroduced
Type	**KU:** University of Kansas Biodiversity Institute;			**C**: Captive **R**: Reintroduced **W**: Wild	**0**: Female **1**: Male **U**: Sex Unknown		**0**: Wild **U**: Unknown	**0**: Captive **Na**: Wild

Table V-7 Continued

Column	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
	LSU: Louisiana State University Museum of Natural Science; **MSB**: Museum of Southwestern Biology; **MSU**: Michigan State University Museum;							

Table V-7 Continued

Column	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
	TCWC: Texas A&M University Biodiversity Research and Teaching Collections; **USNM**: National Museum of Natural History, Smithsonian Institute							

Table V-8: Anatomical landmark definitions. Landmark types based on Bookstein (1991) typology of landmarks, where Type 1 refers to a landmark whose location is most clearly indicated, such as a point where several sutures meet, Type 2 refers to a landmark whose location is informed, in part, by the presence of another feature, for instance the maximum or minimum point of a structure's curvature, while Type 3 landmarks refer to a point that is defined by several other anatomic features, for example, the meeting of two curves (Cooke & Terhune, 2015; Weber & Bookstein, 2011).

Orientation	Number	Type	Definition
Ventral	1	1	Prosthion, rostral tip of premaxilla, medial I1 diastema
Ventral	2	2	Posterior point of I3 alveoli, lateral edge of premaxilla
Ventral	3	2	Posterior point of canine alveoli, lateral edge of maxilla
Ventral	4	2	Anterior point of P2 alveoli, lateral edge of maxilla
Ventral	5	2	Medial, Anterior point of P2 alveoli
Ventral	6	1	Anterior cusp of P2
Ventral	7	1	Posterior cusp of P2
Ventral	8	2	Posterior point of P2 alveoli, lateral edge of maxilla
Ventral	9	2	Anterior cusp of P3 alveoli, lateral edge of maxilla
Ventral	10	2	Lingual, anterior cusp of P3 alveoli
Ventral	11	1	Anterior cusp of P3
Ventral	12	1	Posterior cusp of P3
Ventral	13	2	Posterior point of P3 alveoli, lateral edge of maxilla
Ventral	14	2	Anterior cusp of P4 alveoli, lateral edge of maxilla
Ventral	15	2	Medial, anterior point of P4 alveoli
Ventral	16	1	Medial P4 depression between paracone and metacone
Ventral	17	2	Posterior point of P4 alveoli, lateral edge of maxilla

Table V-8 Continued

Orientation	Number	Type	Definition
Ventral	18	2	Anterior point of M1 alveoli, lateral edge of maxilla
Ventral	19	1	Paracone cusp of M1
Ventral	20	1	Protocone cusp of M1
Ventral	21	1	Metacone cusp of M1
Ventral	22	2	Posterior point of M1 alveoli, lateral edge of maxilla
Ventral	23	2	Lingual edge of M2
Ventral	24	1	Metacone cusp of M2
Ventral	25	2	Incisive foramen, rostral end of palatine fissure
Ventral	26	2	Incisive foramen, caudal end of palatine fissure
Ventral	27	1	Medial suture of the maxilla and palatine
Ventral	28	1	Major palatine foramen
Ventral	29	2	Hamulus of pterygoid
Ventral	30	1	Spinous foramen
Ventral	31	1	Foramen lacerum
Ventral	32	2	Anterior point of the tympanooccipital fissure
Ventral	33	1	Hypoglossal canal
Ventral	34	2	Basion: medial, posterior point of occipital
Ventral	35	2	Posterior point of occipital condyle
Ventral	36	2	Posterior point of jugal process
Ventral	37	1	Retroarticular foramen
Lateral	1	1	Suture of incisive and maxilla
Lateral	2	2	Point of greatest curvature of the nasal bone

Table V-8 Continued

Orientation	Number	Type	Definition
Lateral	3	2	Rostral tip of nasal bone
Lateral	4	2	Point of least curvature in nasal aperture depression
Lateral	5	2	Point of greatest curvature of incisive
Lateral	6	2	Posterior point of I3 alveoli
Lateral	7	2	Anterior point of canine alveoli
Lateral	8	2	Posterior point of canine alveoli
Lateral	9	2	Anterior point of P2 alveoli
Lateral	10	2	Posterior point of P2 alveoli
Lateral	11	2	Anterior point of P3 alveoli
Lateral	12	2	Posterior point of P3 alveoli
Lateral	13	2	Anterior point of P4 alveoli
Lateral	14	2	Posterior point of P4 alveoli
Lateral	15	2	Anterior point of M1 alveoli
Lateral	16	2	Posterior point of M1 alveoli
Lateral	17	1	Distal-most suture of maxilla and zygomatic bones
Lateral	18	1	Rostral-most suture of maxilla and zygomatic bones
Lateral	19	1	Proximal-most suture of maxilla and zygomatic bones
Lateral	20	1	Suture of lacrimal and zygomatic bones
Lateral	21	2	Anterior edge of the frontal bone zygomatic process
Lateral	22	2	Posterior edge of zygomatic process, greatest curvature of frontal bone
Lateral	23	2	Tip of the frontal bone zygomatic process

Table V-8 Continued

Orientation	Number	Type	Definition
Lateral	24	1	Anterior-most suture of zygomatic and temporal bones, frontal process of zygomatic bone
Lateral	25	1	Posterior-most suture of zygomatic and temporal bones
Lateral	26	2	Distal-most tip of retroarticular process
Lateral	27	3	Anterior edge of external auditory meatus
Lateral	28	3	Posterior edge of external auditory meatus
Lateral	29	2	Distal-most attachment of tympanic bulla and paracondylar process
Lateral	30	2	Anterior-most distal tip of paracondylar process
Lateral	31	2	Posterior-most proximal tip of paracondylar process
Mandibular	1	1	Caudal mental foramen
Mandibular	2	1	Rostral mental foramen
Mandibular	3	2	Posterior point of canine alveoli
Mandibular	4	2	Anterior point of P2 alveoli
Mandibular	5	2	Posterior point of P2 alveoli
Mandibular	6	2	Anterior point of P3 alveoli
Mandibular	7	2	Posterior point of P3 alveoli
Mandibular	8	2	Anterior point of P4 alveoli
Mandibular	9	2	Posterior point of P4 alveoli
Mandibular	10	2	Anterior point of M1 alveoli
Mandibular	11	2	Point of maximum curvature of M1

Table V-8 Continued

Orientation	Number	Type	Definition
Mandibular	12	2	Posterior point of M1 alveoli
Mandibular	13	2	Anterior point of M2 alveoli
Mandibular	14	2	Posterior point of M2 alveoli
Mandibular	15	2	Tip of condyloid process
Mandibular	16	2	Tip of angular process
Mandibular	17	2	Distal-most point of masseteric fossa
Dorsal	1	1	Prothion, rostral tip of incisive
Dorsal	2	1	Rostral-most suture of nasal and maxilla bones
Dorsal	3	2	Rostral tip of alveolar process between P2 and P3
Dorsal	4	2	Point of greatest curvature of maxilla
Dorsal	5	1	Caudal-most suture of maxilla and zygomatic bones
Dorsal	6	3	Rostral-most point of orbital margin on lacrimal bone
Dorsal	7	1	Caudal-most suture of the maxilla and frontal bones
Dorsal	8	1	Caudal-most suture of the nasal and frontal bones
Dorsal	9	2	Tip of the frontal bone zygomatic process
Dorsal	10	1	Rostral-most suture of zygomatic and temporal bones
Dorsal	11	1	Caudal-most suture of zygomatic and temporal bones
Dorsal	12	3	Point of least curvature at base of temporal bone, caudal to the zygomatic arch
Dorsal	13	2	Anterior canine alveoli
Dorsal	14	1	Suture of the incisive, maxilla, and nasal bones
Dorsal	15	1	Caudal-most suture of the maxilla and frontal bones

Table V-8 Continued

Orientation	Number	Type	Definition
Dorsal	16	3	Point of maximum curvature of zygomatic arch on the temporal bone

Bookstein F.L. 1991. Morphometric tools for landmark data: Geometry and biology. Cambridge New York: Cambridge University Press.

Cooke, S. B., & Terhune, C. E. (2015). Form, function, and geometric morphometrics. *Anatomical Record*, *298*(1), 5–28.

Weber, G.W., & Bookstein, F.L. (2011). Virtual anthropology: a guide to a new interdisciplinary field.

Table V-9: Shape and size sexual dimorphism result for all raising styles pooled for dorsal, ventral, lateral, and mandibular cranial views. Abbreviations are as follows: CS Mean: average centroid size +/- the standard deviation; ISSD: standard index of sexual size dimorphism, which is the mean of the larger sex (males) divided by the smaller sex (females). T-statistics represent the results of the Welch's 2-sample t-test of centroid size for both sexes. F-statistics SShD represent the results of the MANOVA examining the relationship between shape and sex.

	Sex	**N**	**CS Mean**	**ISSD**	**T (SSD)**	**F (SShD)**
Ventral	M	129	43.96 +/- 2.44	1.06	7.74***	4.66***
	F	87	41.43 +/- 2.29			
Lateral	M	98	37.23 +/- 1.88	1.06	8.47***	2.95***
	F	74	35.05 +/- 1.48			
Mandibular	M	136	18.38 +/- 1.11	1.06	7.92***	4.25***
	F	104	17.36 +/- 0.88			
Dorsal	M	70	20.96 +/- 0.85	1.06	8.76***	3.48***
	F	44	19.71 +/- 0.67			

P-value significance: 0.01-0.05*, 0.001-0.01**, 0-0.001***

Table V-10: Cranial -mandibular shape and size across captive generations. F-statistic, Size represents the results of the ANCOVA examining centroid size in relationship to sex and captive generations (Gens). F-statistic, Shape represents the results of the MANOVA examining shape (PC 1-20) in relationship to sex and generations captive.

		N (M)	**N (F)**	**F, Size**	**F, Shape**
Ventral	Sex	68	45	78.47^{***}	2.98^{***}
	Gens			0.63	3.85^{***}
	Sex * Gens			2.50	1.25
Lateral	Sex	58	44	66.26^{***}	2.92^{***}
	Gens			0.36	3.75^{***}
	Sex * Gens			3.46	1.50
Mandibular	Sex	71	50	48.76^{***}	3.15^{***}
	Gens			0.32	1.96^{*}
	Sex * Gens			2.33	0.92
Dorsal	Sex	70	44	67.93^{***}	3.86^{***}
	Gens			0.07	4.36^{***}
	Sex * Gens			0.83	10.88

P-value significance: 0.01-0.05*, 0.001-0.01**, 0-0.001***

Table V-11: Cranial-mandibular shape and size across reintroduced generations. F-statistic, Size represents the results of the ANCOVA examining centroid size in relationship to sex and reintroduced generations (Gens). F-statistic, Shape represents the results of the MANOVA shape (PC 1-20) in relationship to sex and generations reintroduced to the wild.

		N (M)	**N (F)**	**F, Size**	**F, Shape**
Ventral	Sex	17	15	31.75***	2.31
	Gens			5.43*	1.22
	Sex * Gens			0.12	1.37
Lateral	Sex	17	13	20.55***	2.37
	Gens			1.90	0.79
	Sex * Gens			0.57	0.58
Mandibular	Sex	17	15	3.26	2.00
	Gens			1.10	2.60
	Sex * Gens			0.22	0.89
Dorsal	Sex	19	15	41.36***	4.64
	Gens			3.67*	1.84*
	Sex * Gens			0.41	2.76**

P-value significance: 0.01-0.05*, 0.001-0.01**, 0-0.001***

Table V-12: ANCOVA results of LDFA captive prediction scores among reintroduced specimens. F, Gens C represent the results of the ANCOVA examining the LDFA prediction score (the likelihood that the reintroduced specimen aligned more closely with the captive population) in relationship to sex and the number of captive or reintroduced generations. F, Gens R represents the results of the ANCOVA examining the LDFA prediction score in relationship to sex and the number of reintroduced generations.

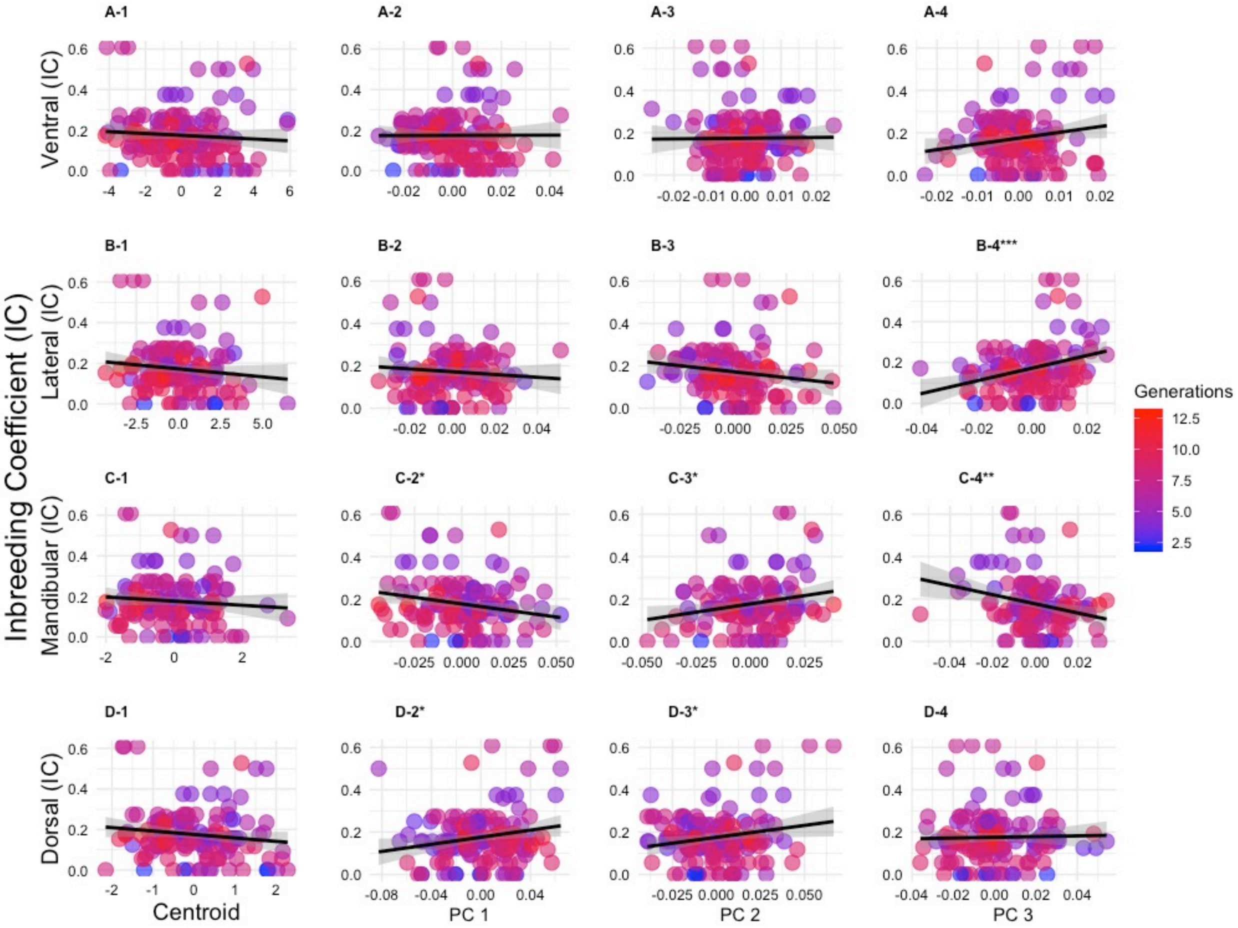
		N (M)	**N (F)**	**F, Gens C**	**F, Gens R**
Ventral	Sex	17	15	5.09*	5.03*
	Gens			0.67	0.57
	Sex X Gens			0.22	0.05
Lateral	Sex	17	13	0.19	0.20
	Gens			0.05	0.18
	Sex X Gens			1.43	3.00
Mandibular	Sex	17	15	1.11	1.01
	Gens			0.37	0.58
	Sex X Gens			5.70*	3.24
Dorsal	Sex	18	14	0.31	0.21
	Gens			3.78	0.32
	Sex X Gens			0.14	0.06

P-value significance: 0.01-0.05*, 0.001-0.01**, 0-0.001***

Table V-13: Narrow-sense heritability (h^2) of size (centroid) and shape (PC 1-3) variables and the associated standard error (SE) for ventral, lateral, mandibular, and dorsal cranial views.

		N	**h^2**	**SE**
Ventral	Centroid	85	0.29	0.19
	PC1		0.49	0.22
	PC2		0.59	0.25
	PC3		0.41	0.21
Lateral	Centroid	130	0.20	0.18
	PC1		0.66	0.19
	PC2		0.42	0.18
	PC3		0.77	0.14
Mandibular	Centroid	128	0.21	0.22
	PC1		0.57	0.21
	PC2		0.13	0.21
	PC3		0.68	0.17
Dorsal	Centroid	139	0.36	0.21
	PC1		0.26	0.16
	PC2		0.24	0.16
	PC3		0.61	0.20

Figure V-2: Linear regressions examining the relationship between inbreeding coefficients and size (centroid; column 1) and shape (PC's 1-3; columns 2-4) variables for ventral (row A), lateral (row B), mandibular (row C), and dorsal (row D) views. Colors represent the number of captive generations, where warmer colors indicate a longer captive history.



P-value significance: 0.01-0.05*, 0.001-0.01**, 0-0.001***

Figure V-3: Linear regressions of PC difference scores of captive and reintroduced compared to wild populations (where values greater or less than zero indicate a specimen that is further from the mean trait value of the wild population) and the generations captive (purpule) or reintroduced (red). Row A) ventral view, Row B) lateral View, Row C) mandibular View, and Row D) dorsal View; Column 1) centroid, Column 2) PC1 difference, Column 3) PC2 difference, Column 4) PC3 difference.