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dissertation entitled

BEHAVIORAL AND MORPHOLOGICAL DEVELOPMENT IN
A FEMALE-DOMINATED SPECIES, THE SPOTTED HYENA
(*CROCUTA CROCUTA*)

presented by

Jaime Beth Tanner

has been accepted towards fulfillment
of the requirements for the

Doctoral degree in Zoology Program in Ecology, Evolutionary Biology and Behavior

Major Professor's Signature

12/3/07

Date

BEHAVIORAL AND MORPHOLOGICAL DEVELOPMENT IN A FEMALE-DOMINATED SPECIES, THE SPOTTED HYENA, *CROCUTA CROCUTA*

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSHOPHY

Department of Zoology
Program in Ecology, Evolutionary Biology and Behavior

2007

ABSTRACT

BEHAVIORAL AND MORPHOLOGICAL DEVELOPMENT IN A FEMALE-DOMINATED SPECIES, THE SPOTTED HYENA, *CROCUTA CROCUTA*

By

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In this dissertation, I examine changes in skull morphology and feeding behavior throughout ontogeny in order to determine if there are developmental constraints operating during the life history of a unique carnivore species, the spotted hyena (*Crocuta crocuta*). Spotted hyenas are highly durophagous, capable of cracking open and consuming bones of large diameter, and their skulls are highly specialized in order to accomplish such tasks. I use a novel approach, finite-element analysis, to investigate adaptations in the skull that enable hyenas to generate and sustain large bite forces. In particular I test hypotheses about the functional significance of a uniquely elongated fronto-parietal sinus during bone-cracking. The fronto-parietal sinus, together with a vaulted forehead, comprise a unique complex in the hyena skull that aids in dissipating stress away from the facial region and back along the sagittal crest during bone-cracking bites.

Spotted hyenas, unlike most other carnivores, are highly gregarious and live in clans that can comprise up to 80 individuals. The prey and carcasses on which hyenas feed exist as ephemeral and unpredictable patches of food. Therefore, numerous individuals are likely to be feeding on a carcass simultaneously and consequently competition can be intense. In an environment

with the dual challenges of intense competition and a durophagous diet, selection should favor individuals capable of feeding quickly and utilizing all parts of a carcass. Theory predicts that selection should favor enhanced performance during life history stages in which the risk of mortality is high yet juveniles are highly disadvantaged with respect to feeding performance relative to adults despite a long period of offspring dependence. This disadvantage is likely due to their small size and inexperience.

I utilized a unique collection of known-age skulls of wild spotted hyenas to document changes in skull morphology throughout ontogeny. Skull size continued to increase until 26 months of age shortly after reproductive maturity. However, skull shape continued to change until 35 months of age, well into adulthood. Similarly, changes in feeding performance as measured by ingestion time, continued to change until the third year of life. The results from this work suggest that there are indeed developmental constraints on skull morphology and feeding. This provides initial support for a new theory explaining the evolution of female dominance suggesting that a combination of developmental constraints and intense feeding competition led to dominant females.

Lastly, in order to investigate whether a form of play often seen in carnivores, non-nutritive chewing, reflect musculo-skeletal development, I investigated ontogenetic variation in this and other forms of play. Non-nutritive chewing does not reflect morphological changes and may instead allow young hyenas to investigate novel objects in their environment.

ACKNOWLEDGEMENTS

I have been incredibly fortunate during my graduate career and, more broadly, throughout my life to have been surrounded by many supportive people. First and foremost, I need to thank my advisor, Dr. Kay Holekamp. I am truly grateful to have had her as an advisor and mentor. Kay provided me with an opportunity that I had dreamed about since I was eight years old. More importantly, she provided me with insightful professional and scientific training and always pushed me to put forward my best effort. If I become even one-eighth of the scientist that she is, I will consider myself to be very successful.

In addition to Kay, my advisory committee has provided useful comments and support that have improved this dissertation. Dr. Joe Vorro always had kind words of encouragement when I sought his input on my work. Dr. Laura Smale provided a great deal of knowledge and advice and has been very supportive throughout my graduate career. Dr. Barb Lundrigan served as a mentor, supporter, and friend during my time at MSU and her advice and support were critical to the success of the work presented here.

The College of Natural Sciences, the Graduate School, the department of Zoology and the program in Ecology, Evolutionary Biology and Behavior at Michigan State University have all provided me with funding and logistical support. In addition, I received funding from the American Society of Mammalogists and Sigma Xi. The wonderful staff in the Zoology office were always incredibly helpful and patient with me over the past six years and I am very grateful to them. My research was part of the Mara Hyena Project, which has been funded by the National Science Foundation grants IBN9309805, IBN9630667, IBN9906445, IBN0113170, IBN0343381, and IOB0618022. I would also like the thank the Office of the President of Kenya for permission to conduct this research and to the Kenya Wildlife Service, the Narok County Council, and the Senior Warden of the Masai Mara National Reserve for their cooperation.

This dissertation includes data collected over 18 years and I am indebted to a number of individuals involved in the data collection, including Nancy Berry, Chantal Beaudoin, Erin Boydston, Aimee Cokayne, Susan Cooper, Audrey DeRose-Wilson, Stephanie Dloniak, Martin Durham, Anne Engh, Josh Friedman, Paula Garrett, Isla Graham, Tyson Harty, Cathy Katona, Karen Kapheim, Kay Holekamp, Joe Kolowski, Kieth Nelson, Karen Nutt, Gabe Ording, Wiline Pangle, Laura Smale, Jenn Smith, Micaela Szykman, Kevin Theis, Russ Van Horn, Sofia Wahaj, Kim Wiebel, Kim Wooten, and Brad White. Additionally, I have had a small army of undergraduate research assistants that have assisted me on numerous components of my work, including Stephen Bowman, Suzi Casmer, Leah DeRose-Wilson, Michelle Engmark, Melissa Gallega, Danielle Harbin, Rachel Kappler, Leanne Klocke, Lindsay Manuszak, Christine Mikkola, Jami Pawlowski, Sheri Sanders, and Kim Wooten.

I would like to thank Laura Abrazcinskas and Paula Hildebrand at the museum for their help in organizing and preparing the specimens used in this study. I have been very fortunate to collaborate with faculty, both here at MSU and at other institutions, and these women have provided great training and have served as role models to me. Dr. Sharleen Sakai in the Psychology Department

at MSU provided me with the opportunity to develop skills in analyzing CT scans and this work has led to a number of exciting projects. Dr. Miriam Zeldtich at the University of Michigan has been very generous with her time and advice on using geometric morphometrics and on statistical analyses. Lastly, Dr. Betsy Dumont at UMASS opened her lab to me for several weeks and has trained me in an exciting new technique for analyzing functional morphology. I look forward to future work with her.

I am so lucky to have lived and studied in an amazing country for several years. I can't begin to enumerate the many ways in which so many people have contributed to my success and well-being during my time in Kenya. In particular, I need to thank amazing friends from fisi camp, including John and Peris Keshe, Moses Sairowa, James Kerembe, Stephen and Lesingo Nairori. They were not only amazing people to work with, but also were incredibly loyal friends. In general, I would like to thank my many friends in Talek and Intipilukwani. Additional friends in the Mara that helped with car troubles, balloon flights, hot showers, and most importantly, laughs include Milton and Elly Kirkman, Andrew Peart, Jakob Bro-Jorgensen, Stephanie Dloniak, Howard Saunders, Bilal Butt, Nasha (Filis Tasel) and Piers Mason. My dearest friends, the Sinnarys (Abdulwahab, Amna, Mohammed, Lubaba, Mihad, Luddan),Faith Model, and Maraka Ewoi are truly like family to me and their hospitality and kindness are beyond compare.

The Holekamp lab has been a great source of friends throughout my time here. When I first started, Micaela Szykmann, Anne Engh and Russ Van Horn were great role models and friends. More recently, Katy Califf, Leslie Curren and Kate Shaw have become great friends and helped to make sure that I laughed in this past year as I struggled to finish. Sofi Wahaj has been a great friend both in East Lansing and in Kenya and we have shared many laughs and adventures together.

I am so grateful to have met Stephen Thomas and in the past two years his friendship has been incredibly valuable to me. Terri McElhinny and Pat Bills have been close friends since I started at MSU. Terri was the first person I met that was as big of a dork about skulls and bones as I was and we've spent many hours being geeky together. She has helped me in so many ways, both professionally and personally. Kevin Theis has been a huge source of support, friendship, discussions and debates, and working with him in the field was a true pleasure. I was so lucky to start my graduate career with two people who would become two of my closest friends, Joe Kolowski and Heather Watts. No matter where we are, the support we provide for one another is unparalleled. Additionally, some of my greatest support has come from friends afar, including Amanda Oxenhorn, Andrew Engell, Dan Gold and Corinne Sudberg.

I could never have achieved any success without the amazing and unconditional love and support of my family. Despite not fully understanding what would possess me to move so far away and live in a tent for years on end, my family has encouraged me in every new adventure. I can't begin to express my gratitude for such a wonderful family. Finally, I would like to thank my third grade teacher, Mrs. Weigman, for instilling in me a passion for Africa and for wildlife.

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GENERAL INTRODUCTION

Understanding the relationship between morphology and behavior has been one of the fundamental questions in the fields of ecology and evolutionary biology (Irschick, 2002; Lauder, 1981). Broadly, morphological traits permit some behaviors to occur while they prevent others from occurring. More specifically, variation in morphology determines the performance abilities of organisms. Wainwright (1994) defined performance as an organism's ability to accomplish "ecologically relevant tasks"; such abilities have profound effects on survival and reproduction. Thus, by understanding the constraints imposed by morphology, we may gain insight into the mediation and evolution of behavior.

In this dissertation I focus on relationships between skull morphology and feeding performance in a carnivore species that faces extreme challenges in its feeding environment, the spotted hyena (*Crocuta crocuta*). Members of the Order Carnivora are particularly interesting for the study of relationships between morphology and performance as they face major challenges in capturing and processing food. Indeed there are pronounced specializations in the skulls of predatory carnivores that facilitate prey capture such as large jaw muscles and sharp, robust canines (Van Valkenburgh, 2007). Moreover, the diversity among carnivores in their adaptations for processing prey reflects the variation in diet among members of this family. At one extreme, there are members of the Order Carnivora such as pandas (*Ailuropoda melanoleuca*) that feed exclusively on vegetation and thus have broad, rounded teeth to aid in grinding tough material.

At the other extreme are felids that specialize in eating meat and thus have a reduced and blade-like dentition.

An unusual suite of adaptations for coping with a challenging diet is found in the skulls of carnivores that crack and consume bone. Bone-cracking forms have appeared only a few times in the evolutionary history of carnivorans: among hyaenids, percrocutids, and borophagine canids. The skulls of animals capable of cracking open bone need to generate and sustain bite forces of great magnitude. Morphological traits likely to aid in these abilities and common to the skulls of bone-cracking forms include large body size, a simplified but robust dentition, a complex multidimensional structure in the tooth enamel, and a vaulted forehead (Van Valkenburgh, 2007). One additional characteristic common only to the bone-cracking members of the Family Hyaenidae, is a caudally elongated fronto-parietal sinus, which has been hypothesized to aid in stress distribution during bone-cracking (Joeckel, 1998).

The relationship between morphology and performance changes as the performance challenges facing individuals shift throughout ontogeny (Carrier, 1996; Herrel & Gibb, 2006). Although adult morphology often represents an optimal form for maximizing performance capabilities, selection also acts upon individuals during earlier life history stages. In fact, selection is expected to reduce the length of life history stages in which mortality risk is greatest and/or favor enhanced performance during these risky life history stages (Williams, 1966). Since juvenile mortality is high in most species, it would therefore be expected that selection should act to favor improved performance during this early phase of the life span (Carrier, 1996). However, juveniles are limited in their feeding capabilities due to small size, inexperience and lack of mechanical advantage of the jaws (Herrel & Gibb, 2006; Binder & Van Valkenburgh, 2000). Such limitations suggest that there are constraints acting upon individuals at early life stages. The extent to which morphology limits performance and determines the youngest ages at which adult feeding maxima can be achieved, is unknown for most mammals (Herrel & O'Reilly, 2006; Thompson et al., 2003; Erickson et al., 2003).

Spotted hyenas are large, long-lived carnivores that are capable of capturing large antelope as well as consuming almost every part of a carcass. Spotted hyenas live in social groups called clans that can contain up to 80 individuals (Kruuk, 1972). Despite a rigid linear dominance hierarchy that determines priority of access to food, *Crocuta* face challenges during feeding that are imposed by intense competition at carcasses. Thus the ability to feed quickly should be favored by natural selection. However, we know from studies of captive spotted hyenas that juveniles are at a disadvantage during competition with adults for food due to their weaker bite forces and slower feeding speed (Binder & Van Valkenburgh, 2000). In such situations it would be expected that provisioning of young might continue until individuals are capable of competing with adults. However, although spotted hyenas wean their offspring at a late age relative to those observed in other carnivores (Watts, 2007), at 14 months, there is little to no provisioning of young in this species (Holekamp & Smale, 1990). If young *Crocuta*, at the time of weaning, are not capable of competing with older conspecifics, then individual feeding success after weaning is likely to be directly limited by morphology. Here I investigate ontogenetic changes in morphology and feeding performance in spotted hyenas.

In order to investigate relationships between morphology and feeding behavior in free-living spotted hyenas I used a combination of morphological and performance data. All of the data presented here come from a study population of *Crocuta* that has been monitored continuously since 1988 in the Masai Mara National Reserve in southwestern Kenya. This rich source of information has provided a magnificent dataset with which to investigate the ontogeny of a long-lived species. Behavioral data presented here come from archived behavioral notes collected for over 15 years. Both traditional and geometric morphometrics were used to quantify changes in morphology from a collection of known-age hyena skulls housed at the MSU museum. Additionally I used finite-element (FE) analysis to investigate the relationship between form and function in the skull of an adult hyena. Finally, two sets of performance data were used in this study. The first dataset is comprised of feeding performance tests conducted by Dr. Kay Holekamp in which she presented known-age hyenas with 30g dog biscuits and measured the time it took for individual hyenas to consume the biscuit. I later spent two years in the field conducting similar performance tests, but this time presenting de-fleshed goat femurs to individuals and attempting to measure bite force from wild hyenas. This novel combination of morphological and performance data provide a comprehensive account of ontogeny in a wild carnivore.

OVERVIEW OF CHAPTERS

In Chapter One, I investigate the functional significance of a unique characteristic in the skulls of both extinct and extant bone-cracking hyenas, a caudally elongated frontal sinus (Joeckel, 1998), which extends into the parietal bone throughout the entire sagittal crest above the braincase. For this study a cutting edge technique borrowed from the field of mechanical engineering, finite-element (FE) analysis, was used to investigate patterns of stress distribution in the skull of an adult spotted hyena during unilateral biting. During bone-cracking, the hyena's skull must simultaneously be able to generate enormous bite-forces, while withstanding enormous stresses. The FE method allowed me to experimentally manipulate morphology, and to visualize the effects of these manipulations on mechanical performance. In addition, I tested alternate hypotheses about the functional role of the fronto-parietal sinus in stress dissipation. I constructed and compared three FE models: (1) a "normal" model of an adult *Crocuta* skull; (2) a model in which the fronto-parietal sinus was filled in with bone; and (3) a model in which we flattened the sagittal crest so that it resembled the plate-like crests of other mammals. Using this approach I found that during biting, an arc of stress extends from the bite point up through the vaulted forehead and along the sagittal crest. These results suggest that pneumatization of the hyena's skull does not only enhance the ability to resist bending, but that the fronto-parietal sinus, together with the vaulted forehead, play a critical role in evenly dissipating stress away from the facial region. This chapter was recently accepted with revisions for publication in the *Biological Journal of the Linnean Society*.

Although the adult *Crocuta* skull is highly modified for dealing with the stresses incurred during bone-cracking behavior, it appears to take the skulls of juveniles a long period of time to reach adult size and shape. In Chapter Two I used both traditional methods and geometric morphometrics to document the ontogenetic trajectories of skull morphology, mechanical advantage, and muscle mass in order to predict changes in feeding performance during development that are described in Chapter Three. I utilized a unique collection of 65 skulls collected from free-living spotted hyenas that were of known age when they died. The specimens in this ontogenetic series ranged in age from two months to 18 years. Linear and piecewise regressions were used to visualize the changes in shape occurring throughout ontogeny in three different views of the skull: the ventral and lateral views of the cranium and the lateral view of the mandible. A principle component analysis revealed that there were two distinct phases of ontogeny. The first phase occurred between three months and 14 months; the latter is the average age of weaning. The second phase of ontogeny occurred between weaning and 18 years. Additionally, I used nonlinear growth models to calculate the age at maturity for each skull trait, calculated as 95% of the asymptotic value. Skull size continued to increase until around or shortly after the time of sexual maturity, between 24 and 29 months of age. Skull shape reached maturity at 18 months in the lateral view and 23 months in the mandible but continued to change until 35 months in the ventral view. Mechanical advantage reached maturity at 22 months of age but muscle mass (as indicated by zygomatic arch breadth) continued to increase until 33 months of age. Overall, these findings suggest that skull morphology continues to change into adulthood despite the late age of weaning in this species. The age at maturity of a traditional estimate of performance, mechanical advantage, would suggest that feeding performance should reach maturity at 22 months of age, but actual measures of performance are presented in Chapter three where I investigate ontogenetic changes in feeding.

I used both direct and indirect measures of performance in Chapter Three to accomplish three main objectives. First, I documented ontogenetic changes in feeding performance as measured by ingestion time. Second, I examined which of the several morphological variables described in Chapter Two (dentition, skull size, skull shape, zygomatic arch breadth, or mechanical advantage of the temporalis) might act as the main limiting factors with respect to feeding performance as hyenas mature. Third, I tested the hypothesis that social rank influences feeding performance.

Although my attempts at measuring bite force in the field were largely unsuccessful, I was able to collect enough data on changes in feeding speed to document ontogenetic changes in performance. Feeding performance in wild hyenas continued to improve until late in the third year of life, between 33 and 36 months of age. Because the eruption of dentition, mechanical advantage of the temporalis and skull size reach maturity at ages much younger than feeding performance reached its asymptote, it is unlikely that these are the main factors limiting performance throughout ontogeny. However, it appears that skull shape and adductor muscle mass (as measured by zygomatic arch breadth) may be the main factors limiting performance, as these measures reach maturity at around the same age as feeding performance reaches asymptotic levels.

In addition to morphology, social factors influence feeding performance throughout hyena development. Social rank was a significant predictor of ingestion time among adults with low-ranking individuals feeding faster than high-ranking hyenas. Detailed investigation of rank-related variation in skull morphology revealed that lower-ranking females had significantly larger skulls than did higher-ranking females, which might explain the rank-related variation observed in adult feeding performance. Among juvenile hyenas, however, the influence of social rank was in the opposite direction from that observed in adults, with higher-ranking cubs feeding faster than their lower-ranking peers. Previous research (Wahaj & Holekamp, 2006; Hofer & East, 1996) suggests that high-ranking cubs grow at faster rates than do low-ranking cubs, and the fact that higher-ranking youngsters are large might explain their surperior feeding performance.

Lastly, in Chapter Four, I examined the possible functions of a behavior often observed in young carnivores, a form of object play referred to as non-nutritive chewing. I investigated whether ontogenetic variation in this behavior might be related to morphological changes during development in skull size and shape. Alternatively, non-nutritive chewing might merely reflect “teething” or functions similar to those served by other forms of play. Therefore, I investigated ontogenetic rates of non-nutritive chewing, and compared these to rates of other forms of play. Consequently, in this chapter I first documented the ontogenetic patterns of the various forms of play in order to draw inferences about potential functions of each type of play. Additionally, given the sex-role reversal in this species I investigated sexual dimorphism in specific play behaviors. Finally, I tested hypotheses suggesting social and energetic influences on play. With this comprehensive work I was able to suggest potential functions of each type of play.

Temporal peaks in non-nutritive chewing did not coincide with either periods of tooth replacement or significant changes in skull morphology. Instead, rates of this activity peaked quite early in development, between two and four months of age, and then steadily declined after that. This suggests that non-nutritive chewing does not reflect musculoskeletal development, but rather that it serves as a way for young hyenas to explore and gain information about their environments via sensory receptors in and around the mouth. This chapter is currently in press to be published in the *Journal of Developmental Processes*.

Collectively, this work provides a great deal of insight into developmental relationships between morphology and behavior in *Crocuta*. My results suggest that development is a slow and prolonged process in spotted hyenas, consistent with the hypothesis that there are developmental constraints in juvenile spotted hyenas that affect their performance capabilities and thus their need for assistance from their mothers. In order to determine whether development is delayed or prolonged in this species relative to that in other carnivores, and whether this might be one of the selective forces leading to the evolution of female dominance, comparable studies on other carnivores are needed.

This dissertation is the result of a great deal of effort and insight from a number of collaborators, and each chapter was prepared as a manuscript for publication with some of those collaborators. Therefore, the term “we” is used throughout.

CHAPTER ONE

OF ARCS AND VAULTS: THE BIOMECHANICS OF BONE-CRACKING IN SPOTTED HYENAS (*CROCUTA CROCUTA*)

INTRODUCTION

Organisms that cope effectively with extreme challenges in their environment provide excellent opportunities for exploring the relationship between form and function (Wainwright & Reilly, 1994). The skulls of carnivores are especially interesting in this context because they allow us to examine the interplay between forces shaping the highly developed neural and sensory organs critical for locating and capturing prey, and those shaping a feeding apparatus designed for both the capture of uncooperative prey and the processing of their carcasses. The skulls of bone-cracking animals represent extreme examples. Bone-cracking forms have appeared only a few times in the evolutionary history of carnivorans: among hyaenids, percrocutids, and borophagine canids, with only hyaenids surviving to the present. Comparative analysis reveals some striking morphological similarities among the various bone-cracking forms, including large body size, a simplified but robust dentition, a complex multidimensional structure in the tooth enamel, and a vaulted forehead (Van Valkenburgh, 2007).

Although extant carnivores provide useful models for the study of skull form and function, *in vivo* studies of the forces and strains generated during biting are often logistically and practically impossible to accomplish. Only scant data are available documenting bite forces generated by carnivores, and almost all of these are from captive animals (Binder & Van Valkenburgh, 2000; Dessem, 1989). Captive studies yield bite force maxima that almost certainly underestimate the true feeding capabilities of these animals in the wild (Stefen, 1997). Such limitations force us to look elsewhere for methods to more accurately assess the relationship between form and function in carnivore skulls. Here we use finite element (FE) analysis to evaluate this relationship in the skulls of spotted hyenas (*Crocuta crocuta*).

Spotted hyenas are highly durophagous animals that are capable of breaking open and consuming bones of large diameter. These gregarious carnivores are recently descended from carrion-feeding ancestors (Lewis & Werdelin, 2000). Spotted hyenas share with other bone-cracking hyaenids, and with percrocutids and borophagine canids, a suite of cranio-dental characteristics that maximize their ability to feed on virtually all components of a carcass. Bone-cracking carnivorans use their premolar teeth (p3 in hyaenids and percrocutids, and p4 in borophagine canids) for opening large bones (Werdelin, 1989; Van Valkenburgh, 2007). As a result, the main bone-cracking premolar is often robust and pyramidal in shape (Van Valkenburgh, 2007; Werdelin, 1989; Biknevicius & Van Valkenburgh, 1996). Likewise in hyaenids, percrocutids, and at least one borophagine (*Borophagus secundus*), there is a highly derived enamel microstructure that aids in preventing fracture (Rensberger & Wang, 2005; Rensberger, 1999; Stefen, 1997). Furthermore, the height of the mandibular corpus and the thickness of the cortical bone in the dentary in bone-cracking carnivores is greater than that found in carnivores not specialized for bone-cracking; presumably these mandibular characteristics enhance resistance to dorsoventral bending (Biknevicius & Ruff, 1992; Biknevicius & Van Valkenburgh, 1996; Therrien, 2005).

Bone-cracking mammals typically have larger jaw muscles than their non-durophagous relatives (Werdelin, 1989). Associated with this enhanced jaw musculature is usually a prominent sagittal crest that provides a relatively large surface area for attachment of the temporalis muscle, as well as a relatively wide bi-zygomatic breadth that increases attachment area for the masseter, and allows more space in which to accommodate the enlarged jaw adductors. Additionally, bone-cracking hyaenids, percrocutids and borophagine canids have uniquely vaulted foreheads created by an enlarged frontal sinus. Based on the work of Buckland-Wright (1978; 1971), which suggested that compressive forces travel in an arc from the bite point dorsally through the region anterior to the eye orbit, Werdelin (1989) hypothesized that this vaulting creates an arc of bone that transfers stresses incurred during biting from the facial region caudally along the sagittal crest.

Although this suite of adaptations for durophagy occurs in all bone-cracking carnivores, a rare trait shared by extinct and extant bone-cracking members of the Family Hyaenidae is a caudally-elongated frontal sinus that invades the parietal bones (from now on referred to as the fronto-parietal sinus) and completely overlies the braincase (Negus, 1958; Buckland-Wright, 1969;

Paulli, 1900; Joeckel, 1998). Thus, the skull is not only pneumatized anteriorly between the postorbital processes (as in borophagines), but also all along the sagittal crest (Figure 1.1A). There are other species in which the frontal sinus invades the parietal bone (ie. Brown bears, pigs, elephants, etc.), however, the sinus does not lie underneath the sagittal crest as it does in hyenas. Thus, the presence of the elongated frontal sinus within the sagittal crest of bone-cracking hyenas results in the unique triangular cross section (Figure 1.1B) that is dramatically different from the laterally compressed, plate-like sagittal crest of most other mammals (Figure 1.1 C).

Joeckel (1998) was able to rule out hypotheses suggesting that the elongated fronto-parietal sinus functions to enhance olfaction, dissipate shock to the brain, cool the brain, or maximize muscle attachment area. Instead, he likened the space created by the caudal portion of the elongated fronto-parietal sinus to an architectural shell or arch, and suggested that the sinus increases structural support by enhancing resistance to muscular loads applied to the sagittal crest during bone cracking. Based on principles derived from structural engineering, he predicted that the uniquely pneumatized sagittal crest in hyenas should be more resistant to vertical loading by the temporalis muscles than the plate-like sagittal crests of other mammals. Joeckel concluded that the hollow, arch-like sagittal crest makes the crest more resistant to bending than a vertical flat crest, and simultaneously lightens the skull. Although these are interesting ideas, the relative importance of the elongated fronto-parietal sinus in strengthening and lightening the skull remains unclear. For example, it is

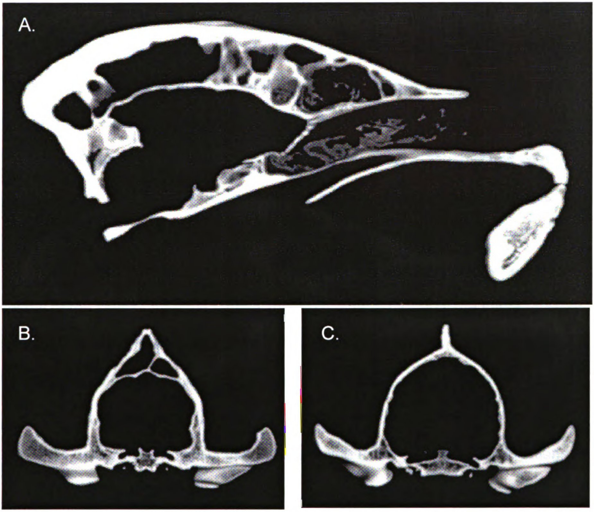


Figure 1.1. CT slices of sagittal (A) and coronal (B) views of a *Crocuta* skull showing the pneumatized sagittal crest, and (C) a coronal view of a wolf (*Canis lupis*) showing the more typical plate-like sagittal crest found in non-hyaenid mammals.

possible that it is the expanded base of the crest, rather than the fact that the crest is hollow that makes it more resistant to large muscle forces during biting. Here we used the FE method to predict patterns of stress distribution in an adult *Crocuta* skull during a bone-cracking bite and test Joeckel's hypothesis about the role of the fronto-parietal sinus in bending resistance of the sagittal crest. We also evaluate more broadly the function of the sinus in the context of stresses generated by both muscle loading and bite forces.

We built and compared three different FE models for this study: a detailed model of the skull of an adult female spotted hyena, a model of the same specimen in which the parietal portion of the pneumatized fronto-parietal sinus was filled in with bone, and a third model in which we modified and flattened the sagittal crest so that it resembled the more plate-like crests of other mammals. If Joeckel's hypothesis is correct that the elongated fronto-parietal sinus functions to resist bending of the sagittal crest, then the model with the normal sagittal crest should be less stressed during biting than the model with the flattened crest. If the function of the sinus is purely to lighten the skull, then normal and "filled" models should undergo similar stress regimes. Because the FE method allows us to experimentally manipulate morphology and visualize the effects of these manipulations on mechanical performance, we can use it to evaluate the influence of competing demands for bite force, bone strength, and skull mass in the evolution of skull form among these durophagous carnivores.

METHODS

The three FE models used in this study were all based on the skull of one 55 month old adult female *Crocuta crocuta* (Michigan State University Museum – MSU 36567). This specimen originated from a longitudinal study population of free-living spotted hyenas in the Masai Mara National Reserve in southwestern Kenya (Frank et al., 1995). The specimen was scanned on a General Electric Discovery ST 16 slice scanner at the Department of Radiology at Michigan State University, with a slice thickness of 0.625mm. Methods for generating a 3D FE model from CT scans follow Dumont et al. (2005). First, we produced a 3D surface model from the scans using VGstudioMax software (Volume Graphics Inc., Heidelberg, Germany). This model was then imported to Geomagic Studio (Raindrop Geomagic Inc., Research Triangle Park, North Carolina) where we repaired scanning defects (holes, beam hardening, etc) and simplified regions that, in our experience, can be difficult to mesh with finite elements. These included very small foramina and thin structures such as the turbinates, which are unlikely to be load-bearing. Cranial sutures of adult hyenas are completely fused (Schweikher, 1930) and therefore were not included in the model.

We then used Geomagic to alter the surface model based on the scans (the "normal" model, Figure 1.2 A) to create the two modified morphologies. For the "filled" model (Figure 1.2 B), the sinus cavity was truncated at the post-orbital processes; the anterior portion of the fronto-parietal sinus and the external morphology of the sagittal crest were not altered in any way. For the "flattened" model (Figure 1.2 C) we again removed the sinus cavity caudal to the post-orbital

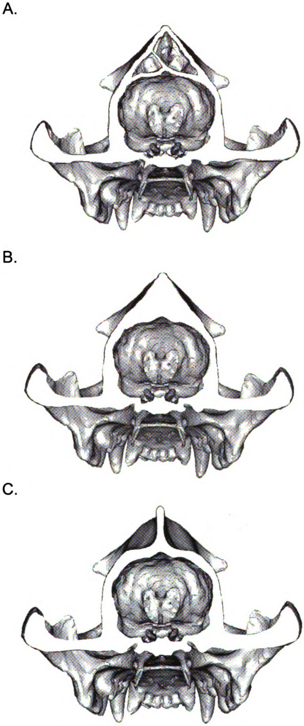


Figure 1.2. Comparison of STL surface representations with a posterior coronal cross-section illustrating the different morphologies of the (A) normal, (B) filled, and (C) flattened models.

processes and also flattened the crest to form a thin blade-like crest that was similar in shape to those found in most other crest-bearing mammals (Figure 1.1C). All three surface models ("normal", "filled" and "flattened") were saved from Geomagic as STL files and then imported to the FE analysis tool Strand7 (G+D Computing, Sydney, Australia). We used the solid mesh generation algorithm in Strand7 to create a volumetric mesh composed of 4-noded tetrahedrals from each surface model. Despite their morphological differences, the resulting FE models were similar in size ("normal" model = 1,319,307 elements, "filled" model = 1,332,181 elements, "flattened" model = 1,255,253 elements).

Unfortunately, there are no data available that summarize Young's modulus or Poisson's ratio values for bone in hyena skulls. Therefore, we assigned our models values for the material properties of cortical bone used by Verrue et al. (2001) for FE models of dog skulls ($E = 1.37 \times 10^4$ MPa; $v = 0.3$). We modeled bone as homogeneous and isotropic, even though this is not likely to be the case (Currey, 2002). Therefore the absolute values of stress predicted by our model may not reflect actual values, and should be interpreted with caution. Past studies have shown that, although varying elastic properties may cause slight variations in the magnitude of stress distribution, overall gross patterns of stress distribution are quite robust (Strait et al.2005). Because we applied the same material properties to each of our models, we can confidently compare stress distributions among the three models, and attribute any differences observed to modifications in the morphology of the sagittal crest.

We applied loads to our models that mimic the forces produced by the three main jaw-adductor muscles: temporalis, masseter, and pterygoid. Data summarizing the average mass of unilateral temporalis and masseter muscles ($\bar{x}$ = 247 ± 18.2 g and $\bar{x}$ = 136 ± 9.3 g, respectively) were drawn from four necropsies of *Crocuta* conducted in the field (JBT). Based on the relative size of the pterygoids in hyenas, and on data from felids (Gorniak & Gans, 1980), we estimated that the pterygoids contribute approximately 18% to the total mass of the jaw adductors. Therefore, we modeled the relative contribution of temporalis:masseter:pterygoid to adductor muscle force production as 50:32:18. The areas of attachment for each of the three muscle groups were based on descriptions of *Crocuta* and *Hyaena* myological studies by Buckland-Wright (1969) and also from our own field necropsies. To define the vectors of muscle force, we identified a node on the mandible within the region of attachment of each muscle. As in previous studies (Strait et al., 2005) we constrained the models at one node on each articular surface of the temporomandibular joint. These constraints defined an axis around which the models could rotate in response to muscle forces. A node on the occlusal surface of the third premolar was also constrained in order to generate a reaction force that models bite force. Hyenas, like other carnivores, use unilateral biting during feeding (Biknevicius & Van Valkenburgh, 1996) and therefore we loaded our model to reflect forces incurred by one side of the jaw.

After defining muscle attachment sites and constraints, we used the Visual Basic program BoneLoad to apply muscle forces to FE models (Grosse et al., In

Press). This loading algorithm applied muscle forces based on both the traction muscles generate and the normal forces that accumulate as muscle fibers wrap around curved bone surfaces. For each FE model an initial analysis was run using an arbitrary total muscle force. Based on the resulting bite reaction force (measured perpendicular to the palate at the constrained premolar), we then adjusted the muscle loads in order to obtain a biologically relevant bite force. In captivity, measures of bite forces up to 3500 N have been obtained from spotted hyenas (Binder & Van Valkenburgh, 2000). However, Stefen (1997) and others (Meers, 2002; Therrien, 2005; Erickson et al.1996) estimate that 7000-9000 N are required to crack open the long bones of antelope, such as wildebeest (*Connochaetes taurinus*), on which wild hyenas commonly feed. Therefore we applied muscle loads to each FE model here to generate a conservative bite force of 5500 N.

The resulting FE analyses were compared in two ways. First, we visually inspected the results to compare and contrast the magnitude and distribution of von Mises stress, a predictor of failure for ductile materials due to distortion or shear. Stresses were quantified in units of megapascals (MPa). These will be referred to as our "qualitative" results. Second, we constructed histograms to compare the distribution of stress within two separate regions of the skull: the face and the cranial vault. The face included the entire skull anterior to the post-orbital processes, and the vault included the lateral and posterior walls of the braincase and the sagittal crest. Third, we compared the absolute muscle forces required to generate 5500 N of bite force in each model. The histograms and muscle forces will be referred to as our "quantitative" results.

RESULTS

The patterns of stress distribution in the normal adult *Crocuta* model during unilateral right-sided biting are illustrated in Figure 1.3 A. When sufficient muscle loads were applied to generate 5500 N of bite force, high areas of stress appeared on the working side maxilla anterior to the orbit and dorsal to the infraorbital foramen. Additionally, there was a hotspot of stress just anterior to the jugal-squamosal suture in the zygomatic arch. As predicted by Buckland-Wright (1971; 1978) and Werdelin (1989), an arc of stress extended from the bite point through the region anterior to the orbit, and along the vaulted forehead. This arc of stress then gradually dissipated posteriorly along the sagittal crest. The anterior portion of the braincase experienced somewhat higher concentrations of stress than did the rest of the braincase. Stress along the crest was distributed around the external surface of the fronto-parietal sinus and along the trabeculae within it (Figure 1.4 A).

Both the filled and flattened models deviated from the patterns seen in the normal model. Overall the filled model experienced less stress than that observed in the normal model (Figure 1.3 B). As in the normal model, an arc of stress extended from the bite point to the vaulted forehead, however the stress pathway terminated just posterior to the postorbital processes. Therefore, there was less stress along the sagittal crest and braincase than that observed in the

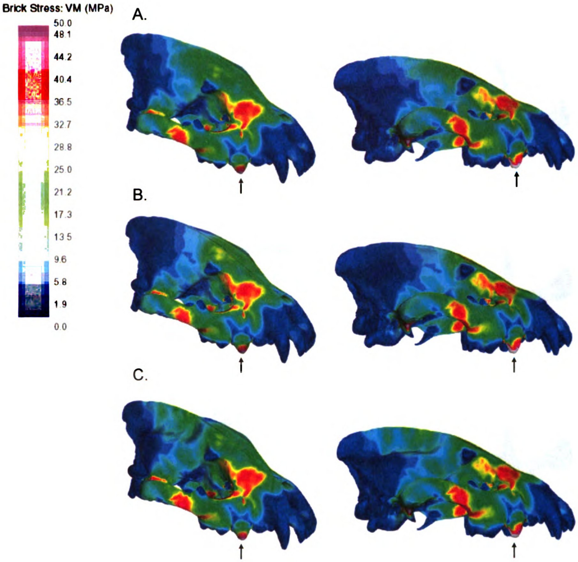


Figure 1.3. Von Mises stress during unilateral right-side biting in the three-quarter lateral view (left) and the lateral view (right) for the (A) normal, (B) filled, and (C) flattened models. Stress is measured in megapascals (MPa) with warmer colors representing higher stress. Arrows indicate the bite point on the third premolar in each model.

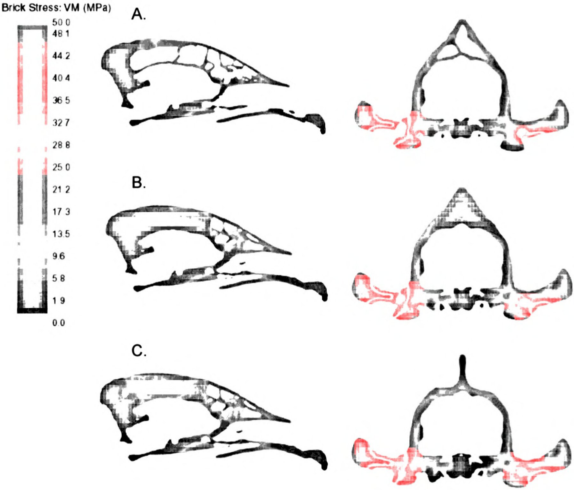


Figure 1.4. Sagittal (left) and coronal (right) slices showing Von Mises stress distribution (in MPa) in the sagittal crests of the (A) normal, (B) filled, and (C) flattened models. Note the distribution of stress to the trabeculae within the frontal sinus as well as along the other bones. The warm colors and white areas on the left temporomandibular joint illustrate the large stresses on the contralateral jaw joint during unilateral right-side biting.

normal model (Figure 1.4 B). Although the filled model was generally less stressed, there were higher concentrations of localized stress on the working side of the vaulted forehead (as illustrated by the yellow area of stress adjacent to the postorbital process on the working side), and on the anterior edge of the orbit, than in the other two models.

The flattened model exhibited by far the greatest stress values along the sagittal crest, as well as slightly elevated stress in the anterior portion of the braincase (Figure 1.3 C). There were high values of stress throughout the crest but the highest stress values were in the dorsal region as indicated in a coronal cross-section (Figure 1.4 C). Additionally, the stresses on the working side maxilla were higher than in the other two models.

We observed only slight differences among the three models with respect to the amount of localized stress in the facial region, with the flattened model showing the highest stress values in this area. The most obvious differences among the models were seen in the area between left and right post-orbital processes, and along the sagittal crest. The flow of stress from the face to the sagittal crest evident in the normal and flattened models was interrupted in the filled model. The normal model, although more heavily stressed than the filled model, illustrated an intermediate pattern of stress distribution relative to the other two models. The stresses incurred during unilateral biting appeared to be more evenly distributed in the normal model than in either of the other models, with lower stresses on the edge of the orbit and in the forehead than in the filled model, as well as lower stresses along the sagittal crest and zygomatic arch than in the flattened model.

Quantitative differences among the three models in the percent of total skull volume stressed in the face and cranial vault mirrored the results of the qualitative comparisons (Figure 1.5). For the cranial vault, the filled model was clearly the least stressed, and the flattened model the most stressed. Although variation in the proportion of the facial skeleton experiencing stress was very slight, the areas that were most highly stressed differed among the three models. The filled model had higher concentrations of stress along the edge of the orbit and on the forehead than did the other two models, whereas the flattened model had higher values in the maxilla.

In considering these results it is important to point out that the muscle forces required to produce 5500 N of bite force differed among the three models (Table 1.1). The filled model required the least amount of muscle force, whereas the flattened model required the greatest amount of force to produce the same bite force; the normal model was intermediate. From a slightly different perspective, the muscle forces required to produce a 5500 N bite force in the normal model produced a bite force of 5750 N in the filled model, and a bite force of 5350 N in the flattened model. Thus the normal model was intermediate in terms of the efficiency of transferring muscle forces to bite force, and the filled model was most efficient.

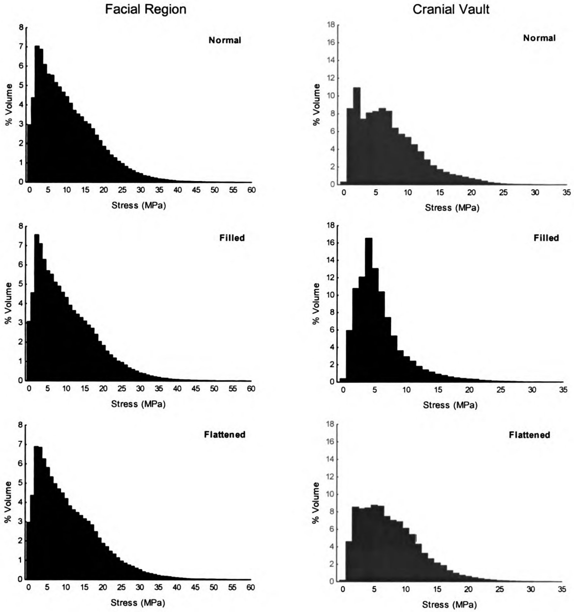


Figure 1.5. The percent of total volume of the facial region (left) and cranial vault (right) stressed (in MPa) during biting in (A) normal, (B) filled, and (C) flattened models. The area under the curve represents 98-99% of the volumes of the cranial vault and facial region, respectively.

Table 1.1. Muscle forces applied to each model in order to produce 5500N of bite force. Values (in Newtons) represent the sum of identical forces applied on the left and right.

Model	Temporalis (N)	Masseter (N)	Pterygoid (N)	Total (N)
Normal	101.6	64.6	45.2	211.4
Filled	97.2	61.8	43.2	202.2
Flattened	104.5	66.4	46.5	217.4

DISCUSSION

Here, for the first time, we examined patterns of stress distribution during biting in the skull of a bone-cracking hyaenid. The FE method allowed us to experiment with alternative morphologies and take a holistic approach to predicting patterns of force distribution during biting behavior. This kind of detailed "snapshot" of the stress state throughout the entire skull is impossible to achieve using any other technique, and highlights the utility of this method in comparative biology, especially when investigating areas of the skull, such as the elongated fronto-parietal sinus, from which it would otherwise be impossible to collect data (Ross, 2005). Although FE analysis is a modeling technique that necessarily requires certain assumptions, it greatly enhances our ability to generate theories and predictions about relationships between form and function in the vertebrate skull. Only through the use of FE analysis were we able to understand the interplay of biting forces and visualize what this might look like. Thus this study illustrates the usefulness of the FE method in hypothesis-testing, and its potential for addressing questions about form-function relationships in organisms that cope with extreme conditions.

Given the unique challenges of a highly durophagous diet and the unique morphology seen in the feeding apparatus of the hyena, this study provides compelling evidence that the form of the fronto-parietal sinus, and consequently the pneumatized sagittal crest, represent adaptations to cope effectively with the large stresses experienced and forces imposed during bone-cracking. If the sagittal crest of hyenas functioned simply to increase muscle attachment area, then we would expect to find a morphology similar to that in our flattened model. On the other hand, if the main selective pressure was simply for resisting higher muscle forces in the cranial vault, we might expect the skull of a hyaenid to more closely resemble that of our filled model. Consequently, the pneumatized sagittal crest appears to represent the most efficient structure for meeting the concurrent demands of the massive jaw musculature necessary for generating large bite forces, and skull strength sufficient for resisting large stresses, without increasing skull weight.

The general pattern of stress distribution observed in our normal model conforms to predictions made by Buckland-Wright (1978; 1971) and Werdelin (1989), who concluded that forces generated in hyenas during premolar biting must pass through the face anterior to the orbit, and then continue along the vaulted forehead to the sagittal crest. The greater stress in the sagittal crest of the flattened model than in the normal model also supports Joeckel's (1998) hypothesis that the elongated fronto-parietal sinus helps to resist loads by dissipating stress along its curvature (Figures 1.3 & 1.4). Specifically, the stresses are dissipating laterally along the edges of the sinus (Figure 1.4 A) in addition to the pathway of stress dissipating caudally (Figure 1.3 A). However, although Joeckel predicted that the highest areas of stress in a plate-like crest would be found at the base, our flattened model revealed higher stress along the top of the crest (Figure 1.4 C). This may be due to the tensile stresses caused by the simultaneous pull of the muscles on either side of the plate-like crest in our FE model. If temporalis activity were asymmetrical, it is likely that there would indeed be bending stress at the base of the crest, but stresses were applied symmetrically in our models. In any case, Joeckel was correct in his interpretation that the vaulted sagittal crest provides more structural support during biting than does a flattened crest. Certainly, the greatest variation in stress among our three models occurred in the cranial vault. It is noteworthy that, despite the limited technology available to these earlier researchers, their insights into these unique adaptations have proven to be remarkably accurate.

Including the filled model in our analysis allowed us to extend Joeckel's work by teasing apart the relative importance of the fronto-parietal sinus in resisting muscle loads versus lightening the skull. The volume of the fronto-parietal sinus in the specimen used here was 89.7 cm^3 (Sakai, Tanner, Lundrigan & Holekamp, In Prep). Therefore, assuming the density of bone is similar to that found in primate and canid skulls (around 1.62 g/cm^3) (Novecosky & Popkin, 2005; Wang et al., 2006), our filled model would result in an addition of 145g of bone. It is difficult to estimate the extent to which this small additional mass might affect the animal's ability to hunt and capture prey. We can, however, predict the effects that a solid sagittal crest would have on patterns of stress in the skull during biting.

Our quantitative results showed that, overall, the presence of a sinus actually increased the amount of stress in the skull during biting. That is, in comparison to the filled model, the normal model exhibited significantly higher stresses in the cranial vault (Figure 1.5). The presence of the sinus also decreased the efficiency of transferring muscle force into bite force. The filled model was more efficient, requiring less muscle force to produce a bite force of 5500 N. This suggests that a filled arch-shaped crest would allow for the generation of higher bite forces than a pneumatized crest, which might be advantageous for an animal that makes its living cracking open bones. However, we have observed extant spotted hyenas cracking open giraffe long bones up to 7 cm in diameter, so their pneumatized skulls allow them to cope effectively even with bones much larger than those of their usual prey. Furthermore, the filled model exhibited slightly higher stress concentrations in the facial region. The sinus, therefore, appears to reflect a trade-off between maximizing efficiency in force production and minimizing regions of stress concentration.

Although the normal model experienced more stress than the filled model, the qualitative comparisons indicate that the skull is more evenly stressed in the normal model. This is particularly striking in light of the fact that the normal model required higher muscle forces to achieve the same bite force than the filled model. Therefore, even under larger loads, the normal model has fewer areas with very high concentrations of stress. For instance, the areas of highly concentrated stress in the orbit and forehead of the filled model predict that the bone in these areas would reach the point of failure under lower muscle loads than the same areas in the normal model. This highlights the significant role played by the fronto-parietal sinus in moving forces away from the face, through the forehead to the cranial vault. Thus, there appears to be a unique complex comprised of the elongated fronto-parietal sinus and the vaulted forehead that is critical for stress dissipation in the skulls of these bone-cracking hyenas. When the sinus in the sagittal crest is absent, as in our filled model, the force transmission pathway from the face to the crest is broken. A critical next step in understanding the biomechanical adaptations for durophagy would be to compare the arc of stress dissipation we have demonstrated in hyaenids with that in percrocutids or borophagine canids, which both have similar vaulting in the forehead but lack an elongated fronto-parietal sinus beneath the sagittal crest. We would expect to find a pattern similar to that in our filled model, lacking the advantages of an elongated fronto-parietal sinus to evenly dissipate stress away from the facial region.

CHAPTER TWO

ONTOGENETIC CHANGE IN SKULL MORPHOLOGY AND MECHANICAL ADVANTAGE IN THE SPOTTED HYENA (*CROCUTA CROCUTA*)

INTRODUCTION

The ability to obtain and process food is essential for the survival of all mammals after weaning, yet an individual's functional abilities to perform such tasks may still be quite immature at the time of nutritional independence (Monteiro et al., 1999; Wainwright & Reilly, 1994; Herrel & Gibb, 2006; Herrel & O'Reilly, 2006). The functional challenges associated with weaning may be especially demanding for carnivorous mammals, as they must feed on uncooperative and unpredictable prey, and they must also often cope with fierce competitors. Morphological changes during development are rarely isometric, so juvenile carnivores are not miniature replicas of their adult counterparts (Figure 2.1). Instead their adult form is the result of changes in both size and shape, and these changes have functional significance with respect to feeding performance. Wainright (1994) defines performance as an organism's ability to carry out ecologically relevant tasks. Both morphology and behavior delimit the boundaries of performance, which in turn constrain the environmental resources that an organism can utilize, and thereby affect fitness (Wainwright, 1994).

One approach adopted by ecomorphologists interested in constraints is to study the functional capacities of organisms that live in extreme environments (Wainwright, 1994). Here, we investigate developmental changes in skull

Figure 2.1. Photographs of *Crocuta* skulls illustrating dramatic changes in size and shape throughout development a) in frontal view (from left to right) at 3 months, 20 months, and 11 years of age, and b) in lateral view at 3 months (left) and 11 years.

morphology of a carnivore, the spotted hyena (*Crocuta crocuta*), that must simultaneously cope effectively with two types of extreme conditions. First, the intense feeding competition characteristic of spotted hyenas represents an extreme environment in which selection has apparently favored traits, including female 'masculinization,' that set these animals apart from other gregarious mammals (Frank, 1997). Second, spotted hyenas have a highly durophagous diet that demands cracking and consumption of bones of large diameter (Kruuk, 1972; Werdelin, 1989). Enhanced feeding performance in such an extreme environment may have profound effects on an individual's fitness. Mortality is high among juvenile spotted hyenas, with less than half of all cubs surviving to adulthood (Frank, 1997; Holekamp et al., 1996), so selection should favor rapid improvement in feeding performance among juveniles after weaning. However, past studies indicate that juvenile spotted hyenas may in fact be at a disadvantage for rapid feeding long after weaning, possibly due to their relatively small size and poor mechanical advantage of the feeding apparatus (Biknevicius, 1996; Binder, 1998; Binder & Van Valkenburgh, 2000).

Adult spotted hyenas can consume 1.3 kg of meat and bone per minute (Kruuk, 1972), so they are capable of very rapid feeding. Furthermore, the skull of an adult spotted hyena is highly modified to deal with the performance demands of bone-cracking. Like other extant and extinct bone-cracking members of the Family Hyaenidae, as well as extinct percrocutids and borophagine canids, adult spotted hyenas possess a number of morphological characteristics that enhance their ability to generate and sustain bite forces of great magnitude.

These adaptations for durophagy include a robust and simplified dentition, large jaw adductors, a pronounced sagittal crest, a vaulted forehead, wide bi-zygomatic arch breadth, increased cortical thickness of the dentary bone and large body size (Van Valkenburgh, 2007). Furthermore, all extant bone-cracking hyaenids have an unusually long lactation period relative to other carnivores, lasting over one year and extending to 24 months in some cases (Kruuk, 1972; Holekamp et al., 1996; Watts, 2007), suggesting that development of the specialized morphology of bone-cracking forms might require a great deal of time.

In contrast to most other carnivores, including the other extant bone-cracking hyaenids, spotted hyenas are highly gregarious. The size of their social groups, called clans, can reach 80 individuals (Kruuk, 1972). Each clan is structured by a rigid linear dominance hierarchy, and social rank determines priority of access to food. The challenges posed by weaning in spotted hyenas may thus be exacerbated both by slow development of the specialized traits needed to cope with a durophagous diet, and also by intensive feeding competition with conspecifics. It is noteworthy that there is little or no provisioning of young by adult spotted hyenas (Holekamp & Smale, 1990). Therefore, aside from potential maternal assistance during feeding competition, individual feeding success after weaning in this species is likely to be directly limited by morphology.

One method traditionally used to inquire about changes in feeding ability during mammalian development has been to model the jaw as a lever in order to draw inferences about mechanical advantage of the feeding apparatus (Radinsky, 1981b; Greaves, 1985; Greaves, 1983; Thomason, 1991; Smith, 1993). In lever-arm models, relative bite force is inferred by estimating size and mechanical advantage of the primary masticatory muscles, the temporalis and masseter (Binder, 1998; Hildebrand, 1984; Radinsky, 1981a). The traditional indicator of adductor muscle mass on Mammalian skulls is maximal width across the zygomatic arches, in-lever arm lengths are indicated by distances separating muscle insertion points, and out-lever (moment arm of resistance) length by the distance from the jaw joint to the bite point on the mandible (Binder, 1998; Gittleman & Van Valkenburgh, 1997; Radinsky, 1981b). As the length of the temporalis and masseteric moment arms increase relative to the length of the resistance arm, mechanical advantage increases (Hurov et al., 1988; Gittleman & Van Valkenburgh, 1997). These linear measurements have been used in myriad studies of functional jaw morphology.

One shortcoming of this traditional approach is that it is difficult to integrate the results into an accurate visualization of overall shape change in the feeding apparatus as it matures. A more effective method for describing such change is geometric morphometric methods (Bookstein, 1991; Rohlf & Marcus, 1993; Zelditch et al., 2004), which combines traditional multivariate statistics with the graphical tradition introduced by D'Arcy Thompson (1917), and allows us to investigate changes in skull size and shape independently. Here we use both traditional methods and geometric morphometrics to document ontogenetic variation in skull size, skull shape, and mechanical advantage in spotted hyenas in order to predict changes in feeding performance during development.

MATERIALS AND METHODS

Samples

In this study we utilized an ontogenetic series of 68 *Crocuta* skulls, representing individuals ranging in age from two months to 18 years (Figure 2.1). All specimens are catalogued into the Michigan State University Museum (Table A.1). Most originated from a study population of spotted hyenas that has been monitored since 1979 (Frank, 1986) in the Masai Mara Game Reserve in southwestern Kenya, but the youngest specimen, a two month old, was obtained from a captive colony of spotted hyenas at UC Berkeley. For 37 specimens, including all subadults, exact age at death was known because these individuals were born into the study population, and their birth dates were estimated (to ± 7 days) based on the appearance of cubs when first observed above ground (Holekamp et al., 1996). For all other adult individuals we estimated age (± 6 months) based on canine height and occlusal wear on the surface of the third lower premolar as described in Van Horn et al. (2003).

Geometric Morphometrics

To quantify changes in skull size and shape we used 2D geometric morphometrics. Homologous landmarks were chosen from photographs of all specimens in the ventral (27 landmarks) and lateral (19 landmarks) views of thecranium. Whenever available, mandibles (n=62) were also photographed in lateral view (39 landmarks). Landmarks at suture points and homologous structures were digitized using tpsDig (tpsDig; SUNY, Stony Brook, NY). Sutures on the cranium are completely fused in adult hyenas (Schweikher, 1930), and there are few suitable landmarks on the mandible. Therefore, semi-landmarks, points evenly spaced along curves, were used in lateral views of the cranium and mandible to capture overall shape. The landmarks and semi-landmarks in each view are shown in Figure 2.2 and described in Table A.2. Each specimen was photographed and digitized three times on separate days by the same observer (JBT) in order to estimate measurement error. The data for each individual were then averaged so that each specimen had one mean value for every landmark in each view.

For the ventral view specimens were arranged so that the palate was parallel to the photographic plane. Landmarks were digitized on both sides, but to avoid inflating degrees of freedom the coordinates for bilaterally homologous landmarks were subsequently reflected and averaged to yield 16 landmarks. In the lateral views of the cranium and mandible, the camera was oriented perpendicular to the sagittal plane of the specimen. A 1cm scale was used in all photographs for all views.

A Generalized Least-Squares Procustes superimposition algorithim was used to align the landmark and semi-landmark data. This removes information not related to shape, i.e. scale, position and orientation (Zelditch et al., 2004; Rohlf & Slice, 1990). Skull size was calculated using centroid size, which is the

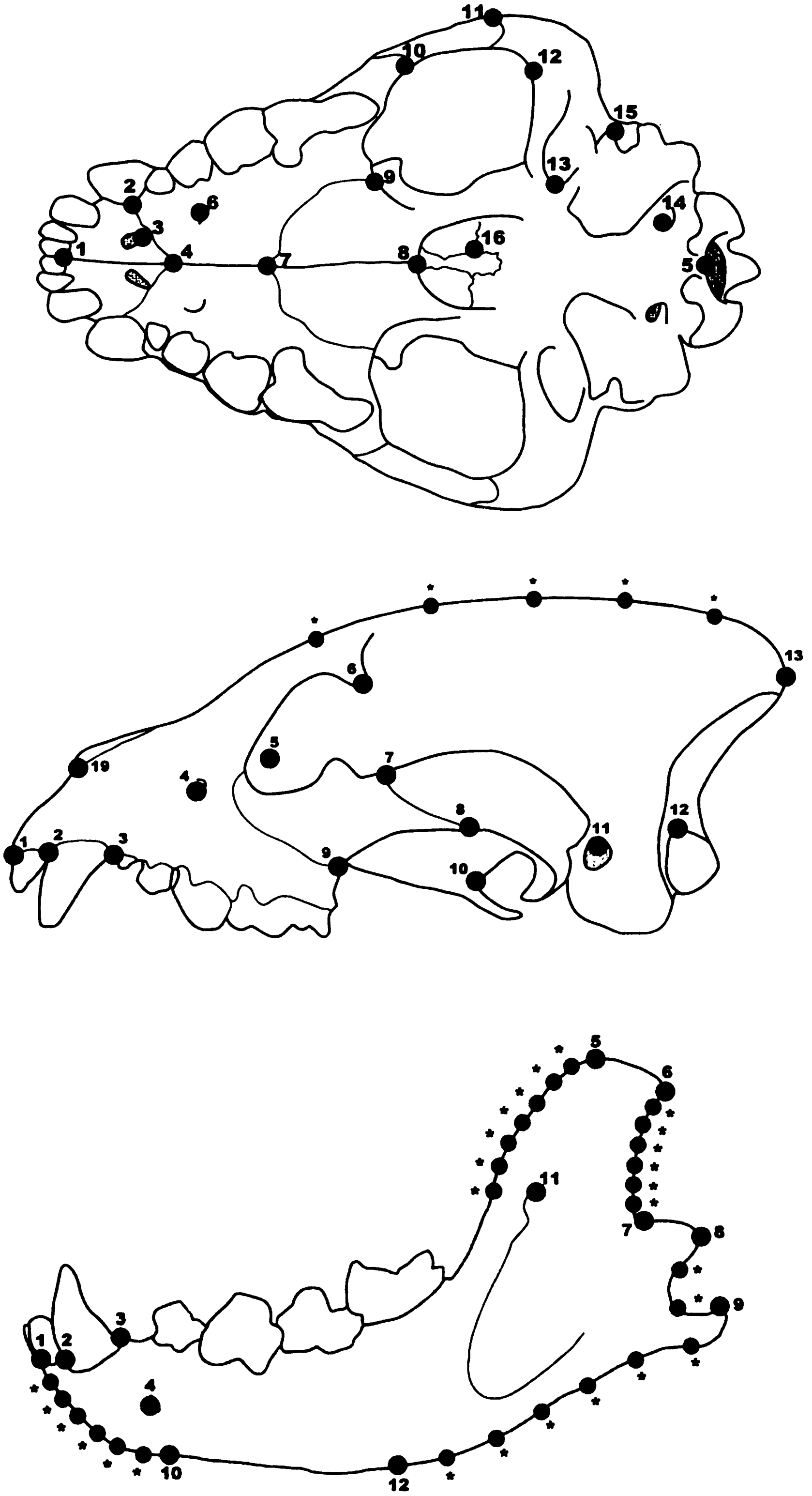


Figure 2.2. Landmarks digitized in each view used in the study, (*) indicate semi-landmarks in the lateral cranium and mandible views. See appendix for the descriptions of each landmark point.

square root of the summed squared distances from each landmark to the geometric center of the object. Procrustes distance was used to quantify the magnitude of differences in shape, and the thin-plate spline function was used to draw the pictures (Bookstein, 1996; Zelditch et al., 2004). Superimposition was done using CoordGen for the ventral and lateral views of the cranium and SemiLand for the lateral view of the mandible (Sheets, Canisius College, Buffalo, NY).

For each view of the skull we performed a linear regression of shape against centroid size to illustrate the dominant linear changes in shape throughout ontogeny and generate deformation grids. In order to determine whether a linear model was appropriate for our ontogenetic data, we performed an exploratory analysis using principal component analysis (PCA).

Mechanical Advantage and muscle mass

To estimate mechanical advantage of the jaw adductors we used traditional linear measurements. Following Radinsky (1981b), mechanical advantage was calculated as the in-lever divided by the out-lever, with the in-lever (or moment arm) of the temporalis measured as the distance from the dorsal tip of the coronoid process to the mandibular condyle, and the in-lever for the masseter measured as the distance from the mandibular condyle to the middle of the ventral masseteric depression. The out-levers for both the temporalis and the masseter were measured as the distance from the mandibular condyle to the bite point. The third lower premolar was used as the bite point in this study because that is the main tooth used for bone-cracking in this species (Werdelin, 1989; Van Valkenburgh, 1988). Zygomatic arch breadth at the widest point on the skull was used as an estimate of jaw adductor mass.

Age at Maturity

To determine the age at which skull size and shape reached adult values, we used a nonlinear growth model to calculate asymptotic values. A number of different growth models were fitted to the data using an IMP program, GrowChoice (Sheets, Canisius College, Buffalo, NY), and Aikaike's Information Criterion (AIC) weights were calculated for each model. For each measure (size, shape, mechanical advantage and zygomatic arch breadth) the monomolecular model (following Gaillard et al., 1997) had the highest AIC weight; we therefore used this model to estimate asymptotic values. The monomolecular model is appropriate when initial growth is rapid, but then levels off (Karkach, 2006). The formula used was

$$x(t)= A\{1\text{-}\exp^{K(t_0-t)}\}$$

where x(t) is the measurement of interest at time t, A is the asymptotic adult value, K is the rate of approach to adult value, and t_0 is the age at which growth begins (Gaillard et al., 1997; Zelditch et al., 2003). We report age at maturity as 95% of the asymptotic value. After 95% of the asymptote was determined, this age (in months) was used as a breakpoint and a regression was performed on the subset of data after this age to ensure that age was no longer a significant predictor of the measurement of interest.

RESULTS

Ontogenetic changes in Size and Shape

The linear regression of change in shape in the ventral view from two months to 18 years of age (Figure 2.3) shows that ontogeny is characterized by a relative increase in the bi-zygomatic arch breadth and lengthening of the zygomatic arches. Additionally there is a relative narrowing of the basicranium and palate. Although skull length increases relative to breadth, this change is less than the changes in the zygomatic arches, which dominate the deformation grid in this view.

In the lateral view (Figure 2.4), we observed the typical mammalian pattern in which the braincase becomes less bulbous during ontogeny, as illustrated by the downward direction of the vectors from semi-landmarks along the mid-dorsal surface of the cranium. This flattening of the cranial profile is further accentuated by a relative increase in height at the posterior end of the skull, representing expansion of the nuchal and occipital crests. The lengthening of the zygomatic arches is also evident in this view, as is the lengthening of the rostrum compared to the rest of the skull.

Ontogenetic change in the mandible (Figure 2.5) is dominated by expansion of the masseteric fossa and a marked anterior-dorsal reorientation of the coronoid process resulting in a more vertical orientation in adults than in

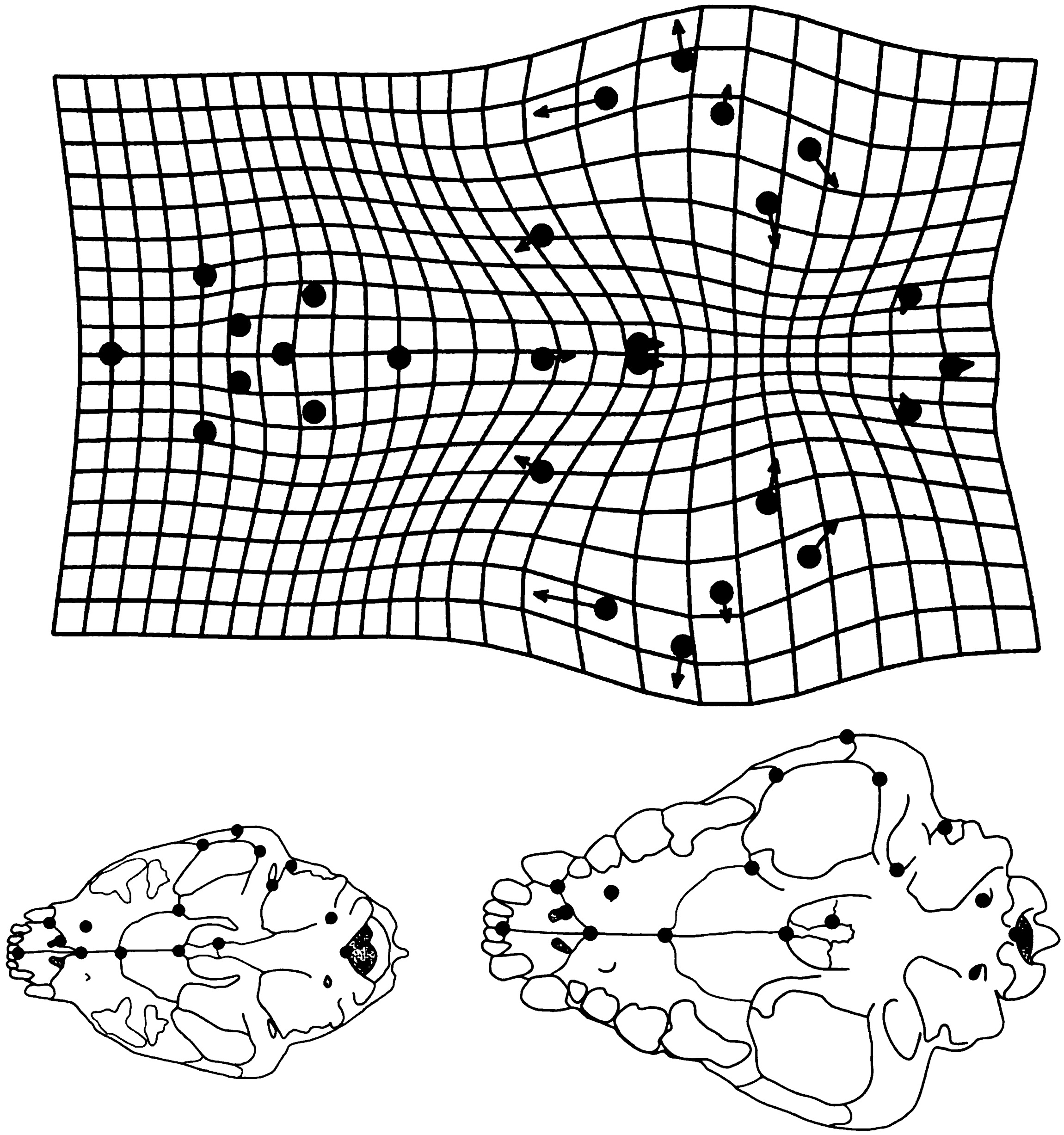


Figure 2.3. Deformation grid showing ontogenetic change in skull shape in the ventral view. The landmarks have been back-reflected for ease of interpretation. Vectors show the direction and magnitude of change from the youngest to the oldest specimens after centroid size is scaled to the same size for each specimen.

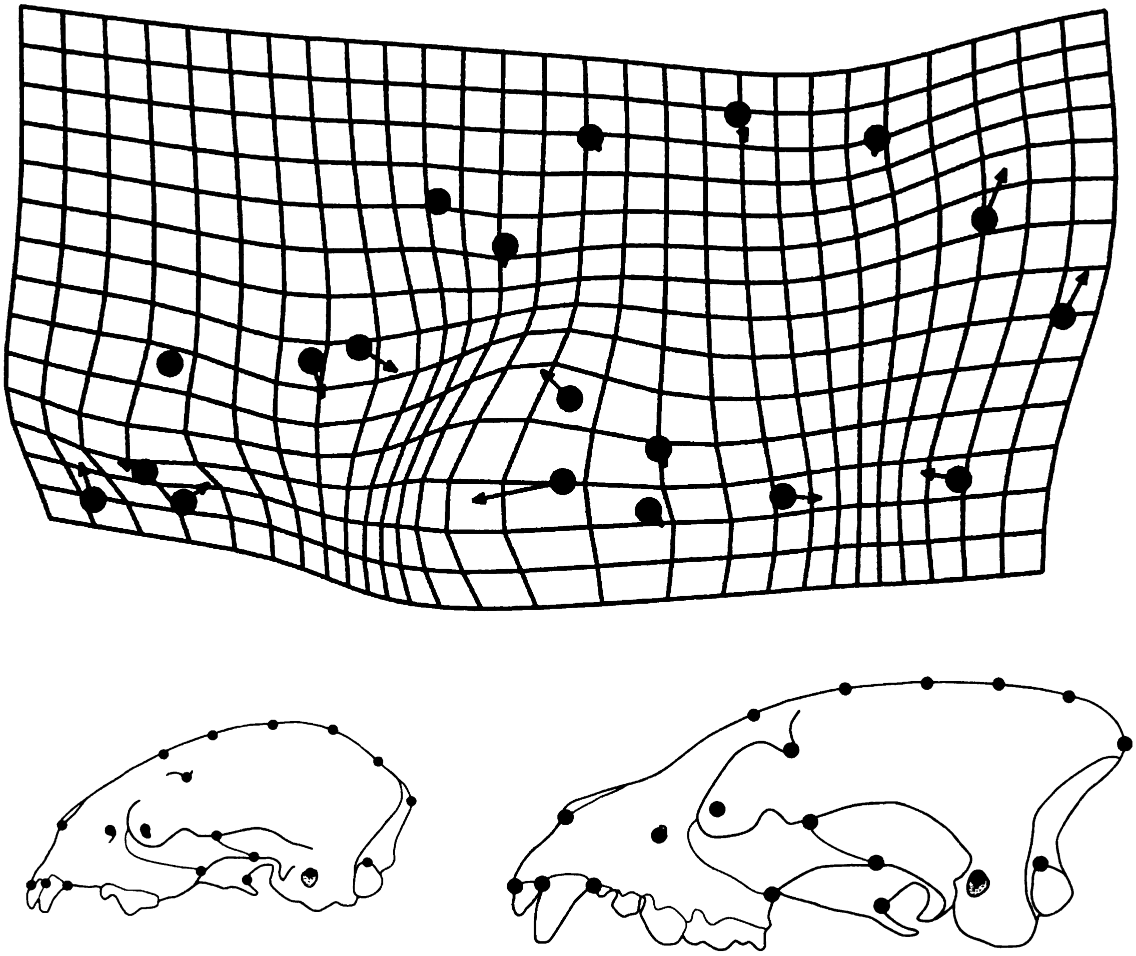


Figure 2.4. Deformation grid showing ontogenetic change in skull shape in the lateral view. Vectors show the direction and magnitude of change from the youngest to the oldest specimens after centroid size is scaled to the same size for each specimen.

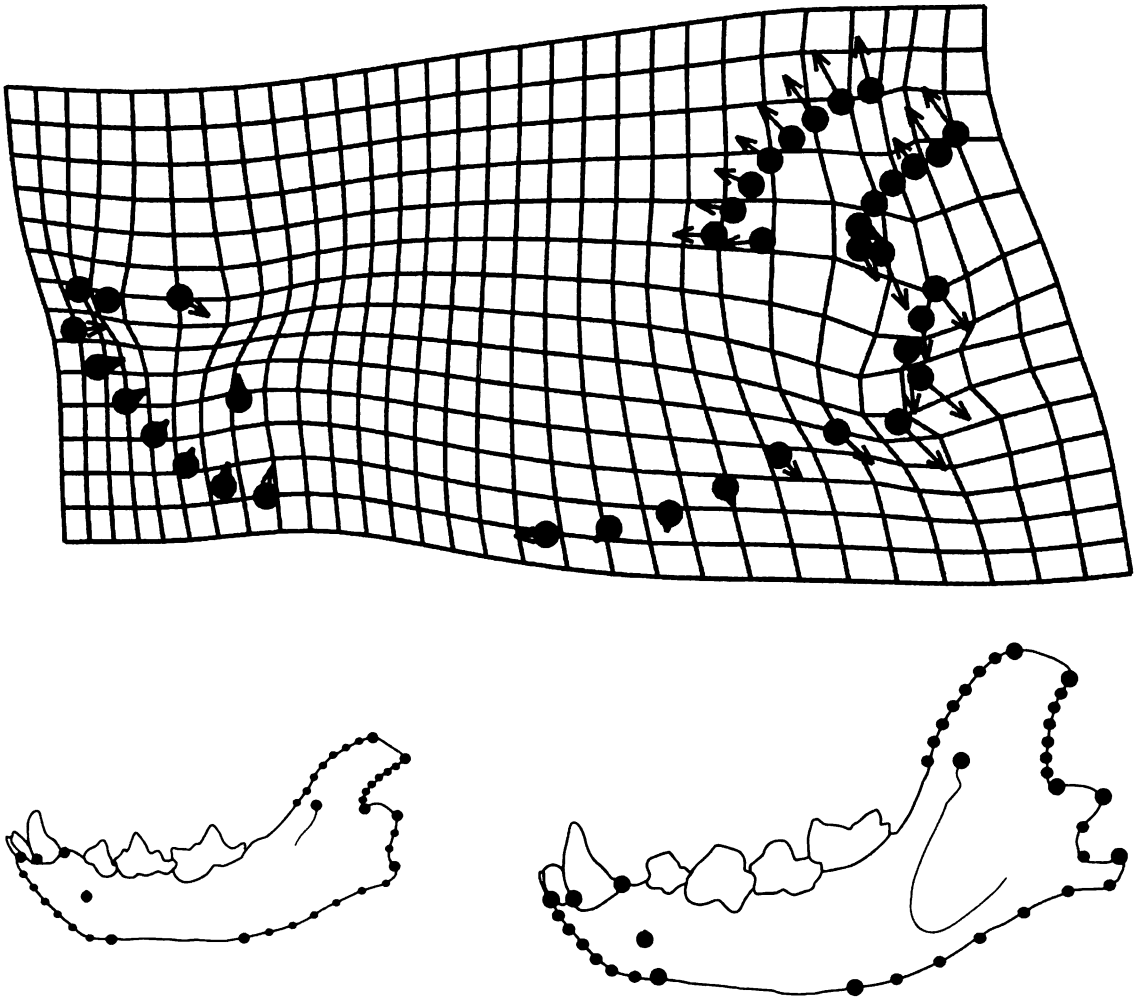


Figure 2.5. Deformation grid showing ontogenetic change in skull shape in the lateral mandible view. Vectors show the direction and magnitude of change from the youngest to the oldest specimens after centroid size is scaled to the same size for each specimen.

juveniles. We were unable to document changes in the tooth row since the teeth in juveniles and adults differ due to replacement of the deciduous dentition.

Although the regression analyzed shape change based on the assumption that it was linear throughout ontogeny, the trajectories of ontogenetic change in skull shape generated by the PCAs were actually curved in all three views (Figure 2.6 a, b, and c), with curvature in ventral and lateral views being particularly dramatic. For labeling purposes, specimens were divided into three age classes: cubs (two to 13 months of age), subadults(14 to 24 months of age), and adults (older than 24 months since this is when spotted hyenas are physiologically capable of breeding). In each PCA cubs differ from the other age groups.

The dominant linear trend was described in each view by PC1, which explained almost half of the variation in skull shape (45.9% in ventral view, 41.6% in lateral view, and 47.8% in the mandible). PC2 accounted for 12.4% of the variation in the ventral view, 12.3% in the lateral view and 14% in the mandible, which suggest substantial deviations from a linear trend. These components had a nonlinear relationship with age but were correlated with age such that PC2 increased from two months to 14 months and subsequently decreased from 14 months to 18 years of age indicating two distinct phases of

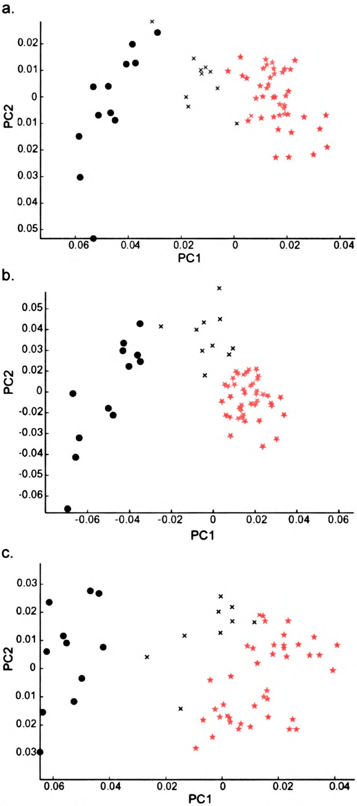


Figure 2.6. PCA plots for each view showing cubs (black circles), sub-adults (blue crosses) and adults (red stars); a) ventral, b) lateral and c) mandible.

skull ontogeny: two months to around the time of weaning (14 months), and weaning to adulthood.

To illustrate the differences in shape change between these two phases of ontogeny, we generated deformation grids for each phase for ventral (Figure 2.7) and lateral (Figure 2.8) views. Early in ontogeny (Figure 2.7a), changes in the ventral view were characterized by lengthening of the zygomatic arches and palate, and narrowing of the basicranium. In the second phase of ontogeny (Figure 2.7b) the zygomatic arches continue to lengthen, but here we also see a marked increase in bi-zygomatic arch breadth and a slight lengthening of the basisphenoid.

The differences in shape change between early and late phases of development were particularly interesting in the lateral view. Early in ontogeny (Figure 2.8a) there was a striking dorso-ventral flattening of the cranial region and a dramatic increase in the length of the rostrum relative to the other skull dimensions. Both are characteristic of ontogenetic patterns in many other mammals. Later ontogeny (Figure 2.8b) was characterized in hyenas by an increase in height and length of the caudal skull region, with development of the sagittal and nuchal crests.

Age at Maturity

Skull size (Figure 2.9), as indicated by centroid size, reached 95% of its asymptotic value around the time of sexual maturity or shortly thereafter in the ventral, lateral, and mandible views (at 26, 29, and 24 months respectively). The

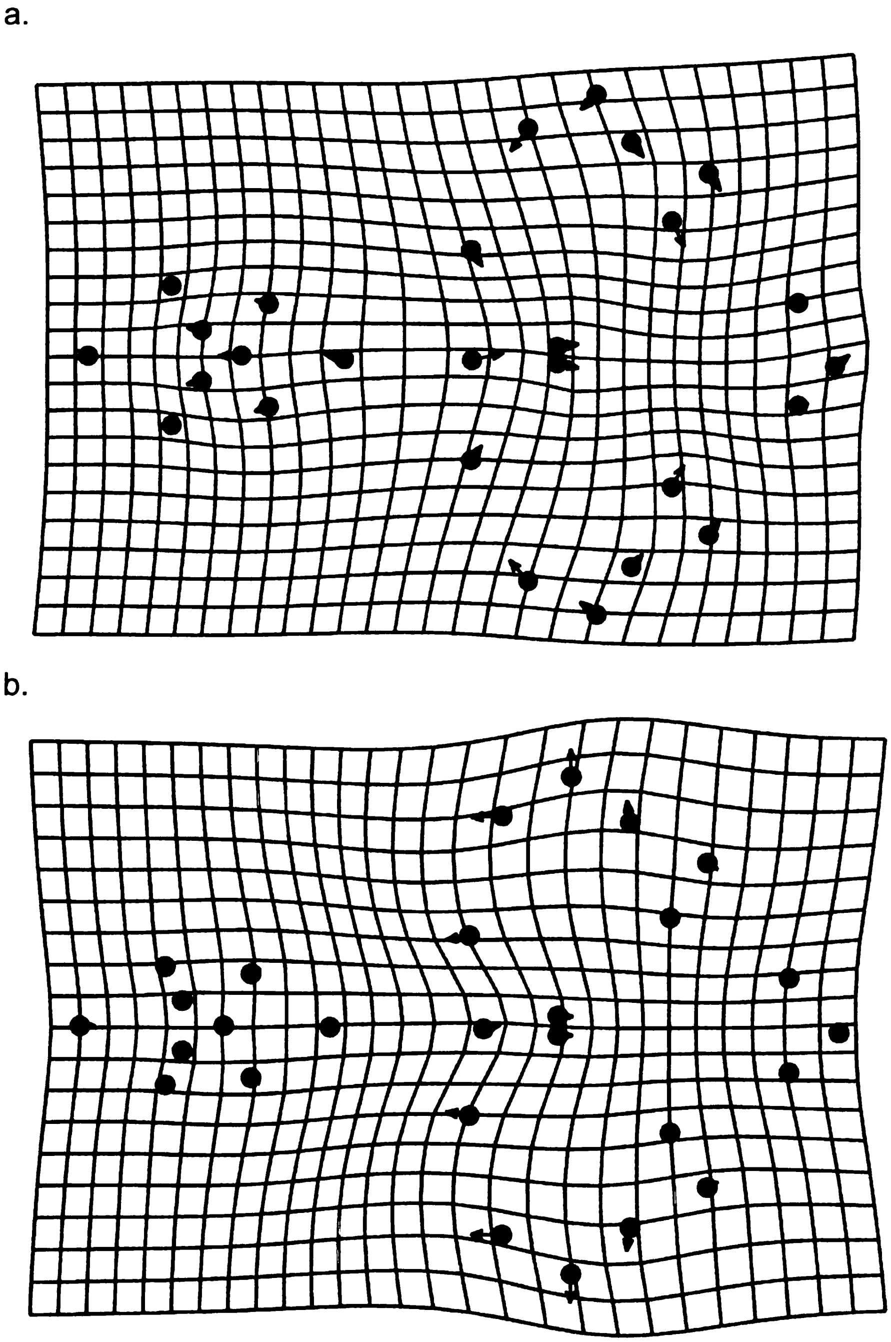


Figure 2.7. Deformation grids showing the two phases of skull development; a) individuals from two months until weaning (14 months), and b) from weaning until 18 years in the ventral view.

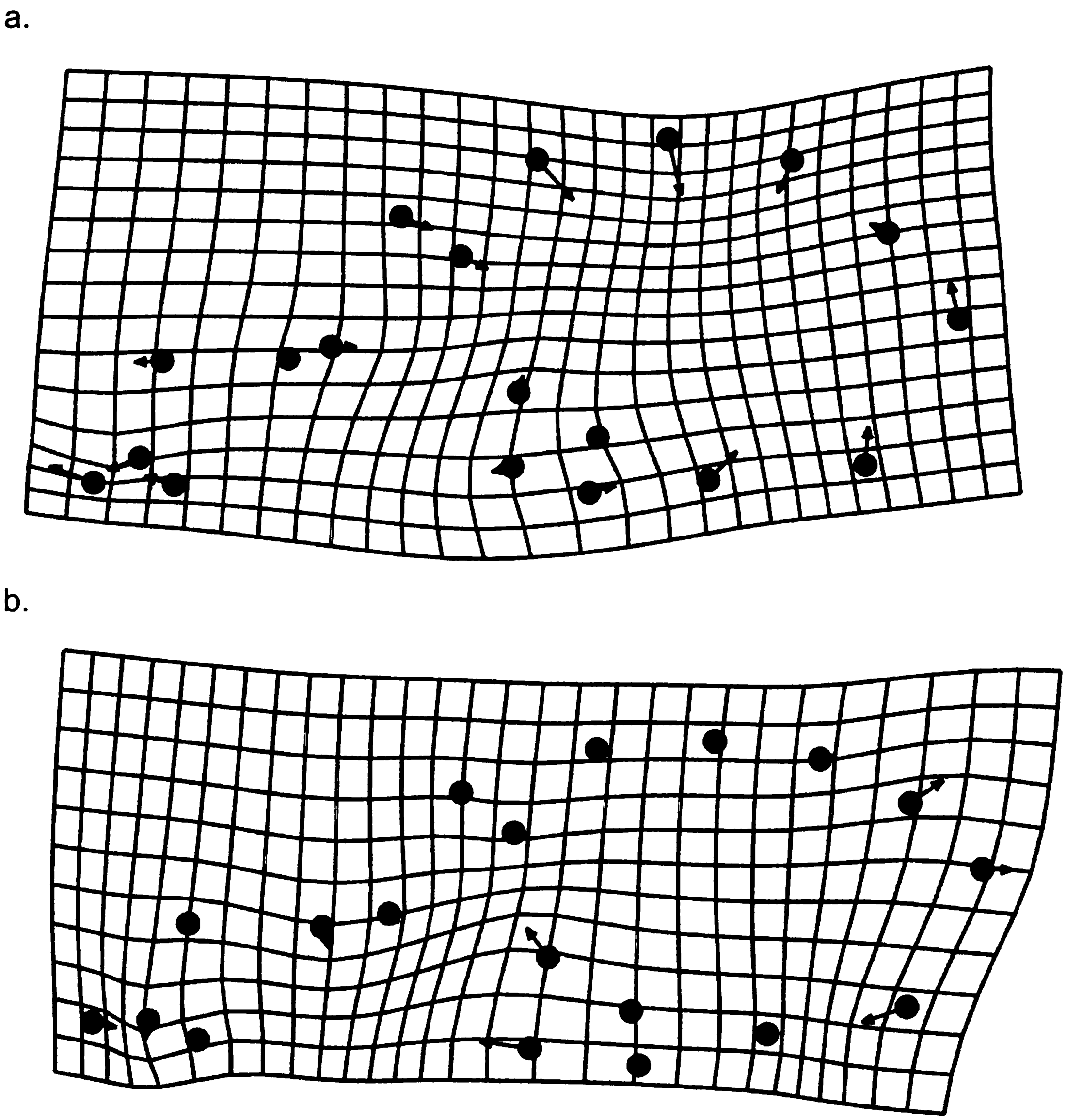


Figure 2.8. Deformation grids showing the two phases of skull development; a) individuals from two months until weaning (14 months), and b) from weaning until 18 years in the lateral view.

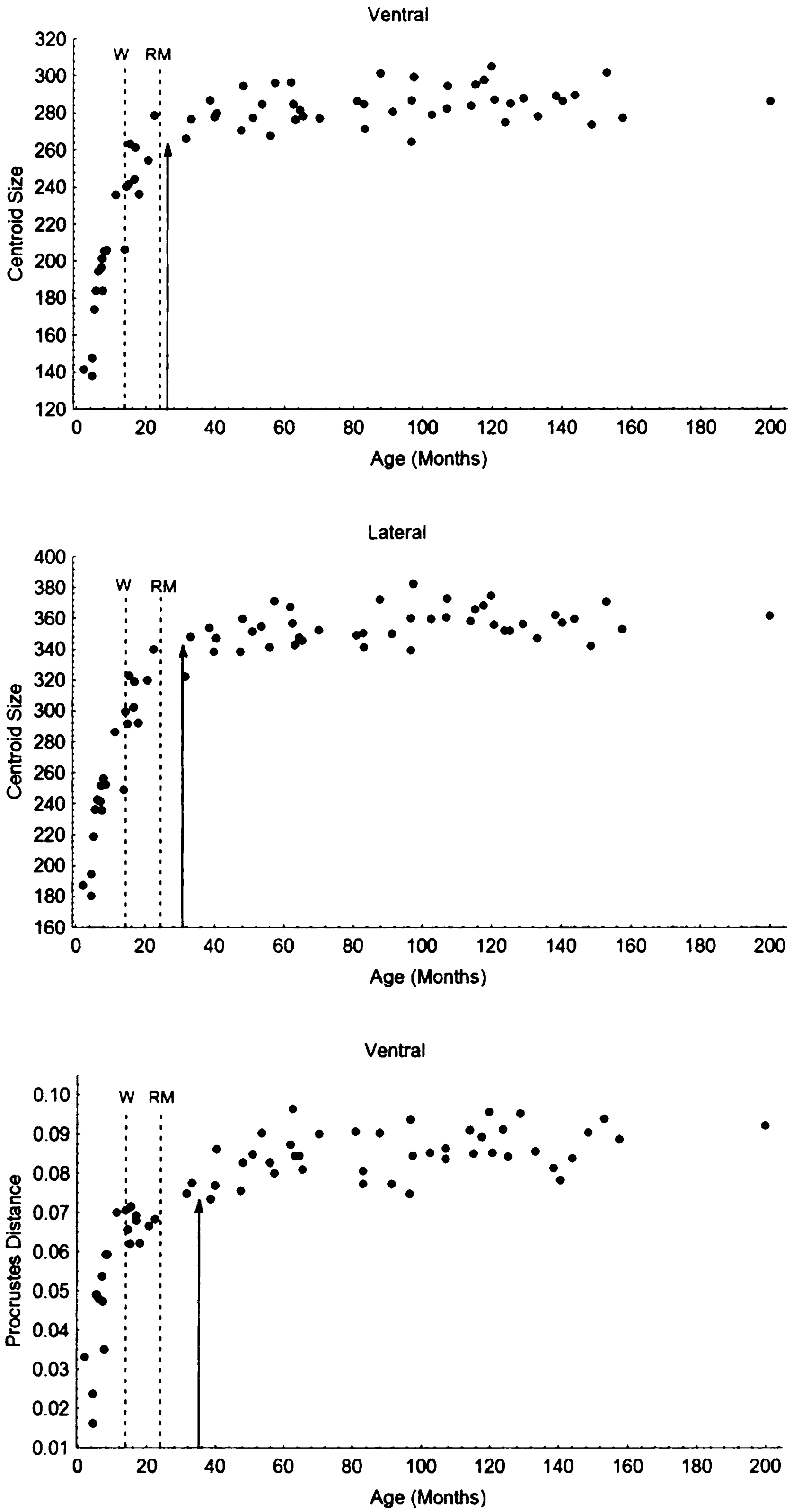


Figure 2.9. Ontogenetic changes in centroid size for each view. Arrows indicate 95% of asymptotic values from the monomolecular equation. Dotted lines indicate the average ages at weaning (W) and reproductive maturity (RM).

95% asymptotes for shape, as measured by Procrustes distance (of each individual from the average for the youngest age class), were much more variable (Figure 2.10). In all three views shape change was initially rapid, but change in the ventral view leveled off more gradually than did change in the other two, not reaching its 95% asymptote until 35 months of age (compared to 18 and 23 for the lateral view and mandible, respectively). Interestingly, there was a considerable individual variation observed even after adult size and shape were reached in all of the views examined (Figure 2.9 and 2.10).

Mechanical Advantage and Muscle Mass

Mechanical advantage of the temporalis (Figure 2.11a) increased rapidly before leveling off at 22 months of age, shortly before sexual maturity. In contrast, mechanical advantage of the masseter (Figure 2.11b) showed very high inter-individual variation and no clear pattern of ontogenetic change. Size of the jaw musculature, as reflected in zygomatic arch breadth, continued to increase until 33 months of age (Figure 2.11c), well past the age at which mechanical advantage of the temporalis reached an asymptote.

Regressions were performed to inquire whether age was a significant predictor beyond 95% of the asymptotic value for each measurement (centroid size, shape, mechanical advantage and muscle mass) and in each case, age was not a significant predictor.

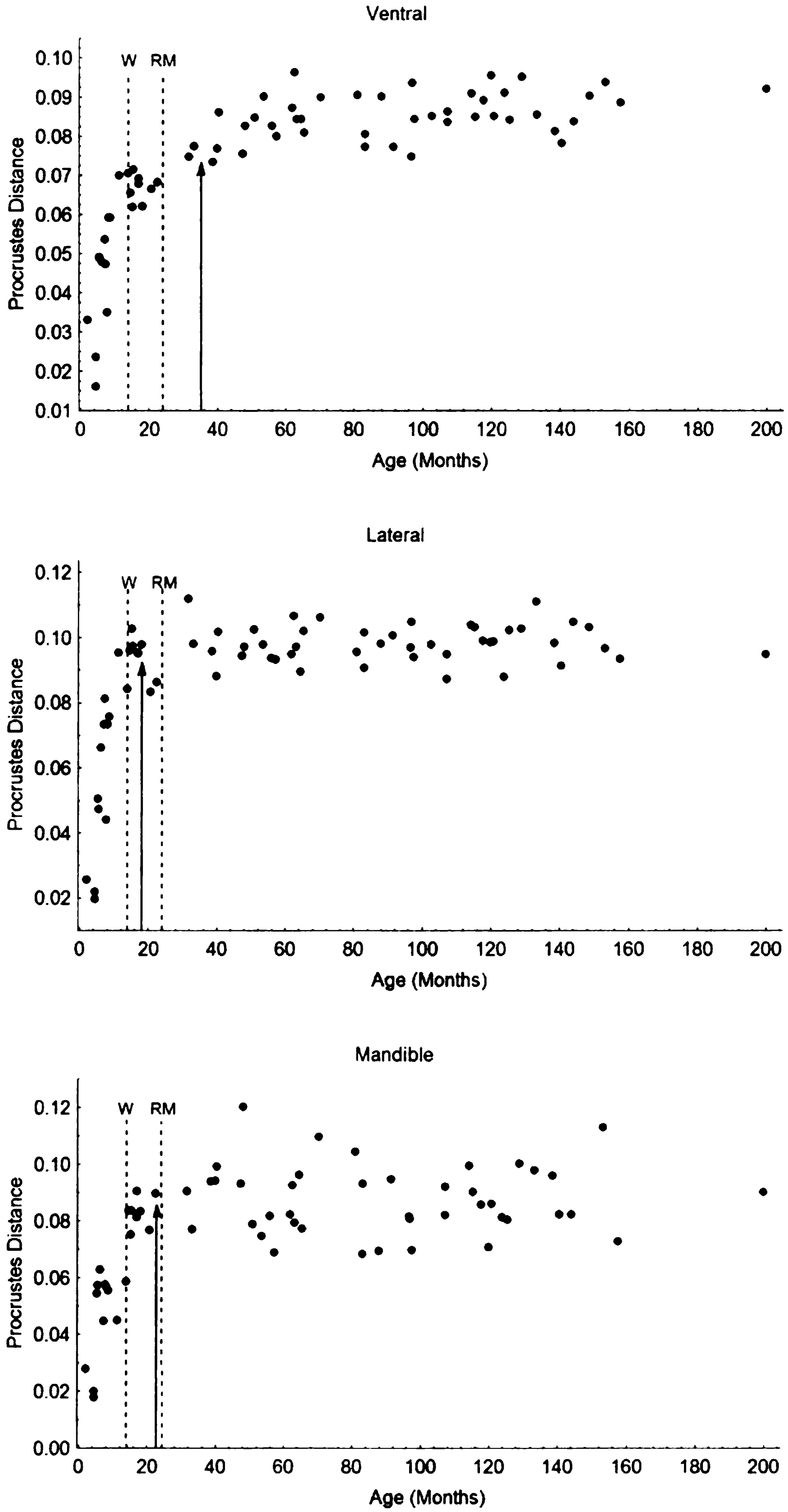


Figure 2.10. Ontogenetic changes in shape (procrustes distance) for each view. Arrows indicate 95% of the asymptotic values from the monomolecular equation. Dotted lines indicate the average ages at weaning (W) and reproductive maturity (RM).

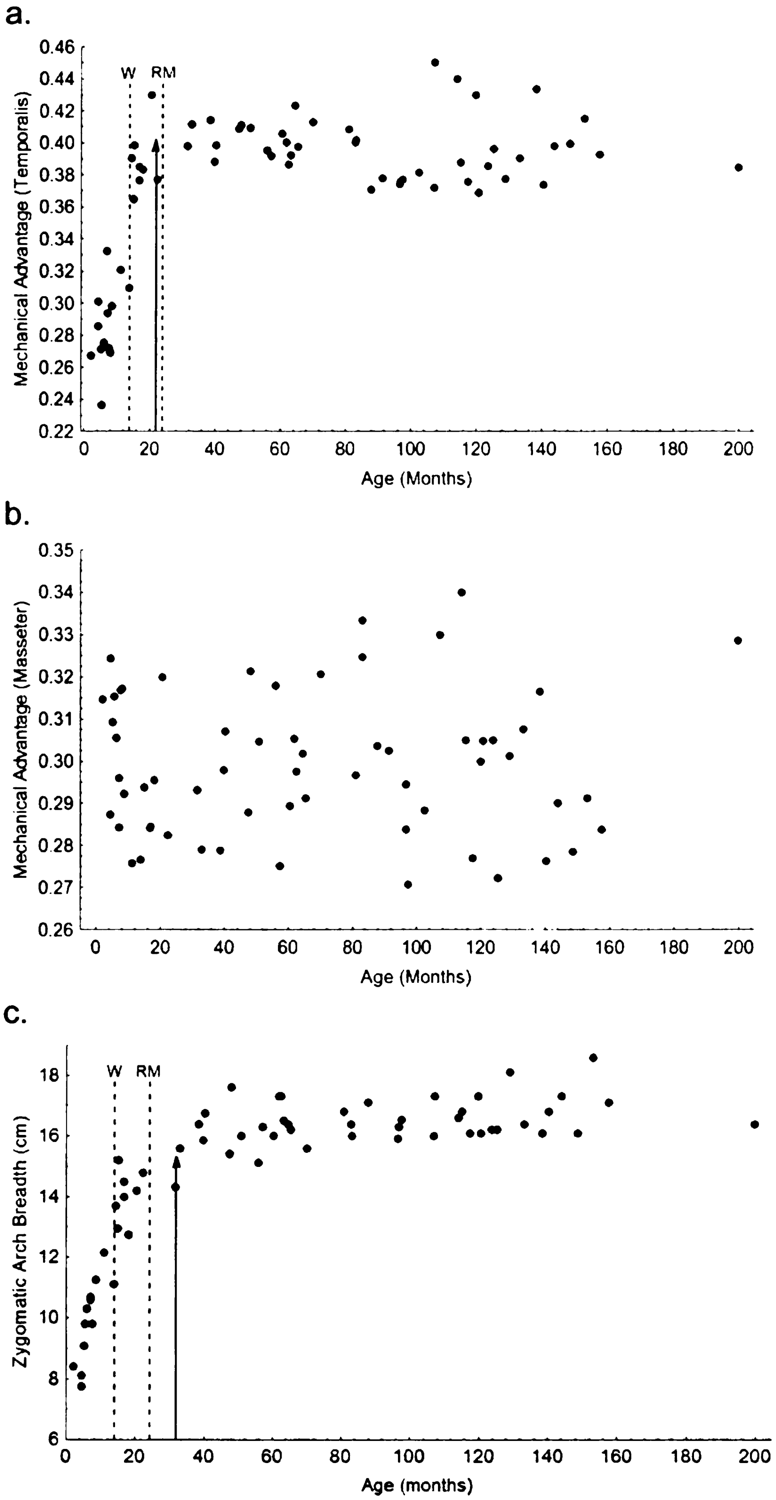


Figure 2.11. Ontogenetic changes in a) mechanical advantage of the temporalis, b) mechanical advantage of the masseter, and c) zygomatic arch breadth. The arrows indicate 95% of the asymptotic value as calculated from the monomolecular equation. Dotted lines indicate the average ages at weaning (W) and reproductive maturity (RM).

DISCUSSION

The ontogenetic changes in hyena skull morphology documented here reflect developmental changes in the associated musculature and thus also most likely in feeding performance, which is contingent upon morphology. The increase zygomatic arch breadth creates a larger space through which both the masseter and temporalis muscles can pass, and presumably corresponds to the increasing size of these jaw adductor muscles. Lengthening of the zygomatic arches (Figure 2.3) provides more muscle attachment area for the masseter. Similarly, the increase in height of the sagittal and nuchal crests, as shown in lateral view (Figure 2.4), results in increased muscle attachment area for the temporalis muscle, which is relatively massive in this species. Both the temporalis and masseter are importantly involved in jaw closure and bite force generation in all mammals, but the temporalis appears to play a disproportionately large role in bone-cracking hyaenids.

The increase in length of the rostrum during development also has implications for feeding performance. Jaw length dictates gape, which will determine the size of bones that individuals are able to crack open (Binder, 1998; Binder & Van Valkenburgh, 2000). As the upper jaw and mandible elongate (as seen in all three views), the out-lever increases in length yet the in-levers also lengthen with the increase in height of the coronoid process (Figure 2.4) and the increase in depth of the dentary, where the masseter inserts (Figure 2.5). The increase in the length of the in-levers relative to the out-lever results in improved mechanical advantage of the jaw musculature.

The age-related increase in mechanical advantage of the temporalis (Figure 2.11a) suggests that the length of the temporalis in-lever is growing with positive allometry relative to the out-lever. In contrast, the lack of a clear developmental pattern in mechanical advantage of the masseter (Figure 2.11b) suggests that the out-lever and the in-lever of the masseter are growing isometrically therefore there is no distinct pattern of change in the ratio of the in-lever to the out-lever. As the temporalis is the much larger of the two main adductor muscles in this species, it is not surprising that mechanical advantage is tied closely to the size of this muscle. However, although the masseter is not as prominent as the temporalis, the size of the masseter also changes during development.

The findings suggest that the traditional measurement of mechanical advantage of the masseter will not accurately predict its contribution to changes in feeding performance occurring during ontogeny. The estimate of muscle mass (zygomatic arch breadth) continued to increase until 33 months, well beyond the 22 months for the mechanical advantage of the temporalis (Figure 2.11). Therefore, by taking changes in muscle mass into account we may have a more realistic indicator of performance than the estimates of mechanical advantage alone. Performance is expected to increase with age as juveniles shift from a relatively easy diet comprised mainly of milk, to one that is more challenging after weaning. Perhaps the most interesting result here is that the transition from a young juvenile skull, with a feeding apparatus ill-suited for a durophagous diet, to an adult skull highly specialized for durophagy, continues long after nutritional independence from the mother.

On average, spotted hyenas in our free-living study population are weaned at 14 months of age (Holekamp et al., 1996). Thus, like other bone-cracking hyaenids, these animals are weaned quite late relative to other carnivores (Gittleman, 1989; Watts, 2007). Nevertheless, our data show clearly that the skulls of newly-weaned spotted hyenas are still far from adult size and shape (Figure 2.9, 2.10). Centroid size continues to increase past sexual maturity in each of the views examined in this study (Figure 2.9). Although centroid size reaches maturity at 26 months in the ventral view, shape in this view continues to change until 35 months of age, well into adulthood. This disparity may be due to the increase in breadth of the zygomatic arches along with the narrowing of the braincase.

In contrast shape change in the lateral view reaches maturity at only 18 months of age and centroid size continues to increase for another year after shape has reached maturity. This disparity is interesting, and might indicate that growth continues isometrically in the lateral view past 18 months of age. Alternatively, the early termination of change in shape in the lateral view relative to that in other views might be due to distortion from using 2D images to sample 3D shapes. Changes in shape beyond 18 months are apparent, particularly in the sagittal crest, when visually inspecting skulls side by side. Therefore, 3D morphometrics might better capture development of the sagittal crest.

In both the ventral and lateral skull views, we observed two distinct phases of ontogeny. The shape changes occurring in early ontogeny differed from the changes occurring later in ontogeny, and the shift from one phase to the other occurred around the time of weaning at 14 months. This bi-phasic growth might reflect changes in shape due to bone remodeling in response to the forces placed upon the feeding apparatus as young *Crocuta* start to eat more challenging foods after weaning. In any case, it is clearly inappropriate to treat ontogenetic development as a linear process in this species.

Our results show that maturation of skull size and shape in the spotted hyena is a complex process that continues well beyond the age of weaning. Based on our calculations of mechanical advantage, which derive strictly from skull size parameters, we would expect measures of feeding performance in this species to reach asymptotic values by around 22 months of age, when centroid size approaches maturity. However, our data show clearly that shape change continues in ventral views of the skull of the spotted hyena until 35 mo of age. This is not only long after weaning, at around 14 months, but is also long after spotted hyenas reach reproductive maturity at 24 months. Thus these animals are potentially capable of producing offspring nearly a year before development of the feeding apparatus is complete.

If feeding performance in these animals is affected by skull shape as well as by skull size, we would expect measures of feeding performance to reach asymptotic values at 30-35 months of age rather than at around 22 months, as suggested by size alone. In Chapter Three we investigate the relationship between the morphological measures presented here with direct measures of feeding performance in wild spotted hyenas.

Maternal investment in the spotted hyena is enormous compared to that seen in most carnivores (Hofer & East, 1993; Hofer & East, 1995; Holekamp et al., 1996; Holekamp & Smale, 1998a), and ontogenetic development of the physical and behavioral traits required for rapid ingestion of food seems unusually protracted in this species. Adult spotted hyenas are remarkably well equipped for the difficult tasks of capturing, killing, consuming, and defending their prey, but juveniles are handicapped during hunting and feeding by their small body size, weak chewing muscles, poor mechanical advantage of the jaws, and inexperience (Biknevicius, 1996; Binder, 1998; Binder & Van Valkenburgh, 2000). It would be surprising if these same traits did not handicap all young mammalian carnivores. However, extant *Crocuta* descended only 200,000 to 990,000 years ago from ancestors specialized for scavenging and durophagy (Lewis & Werdelin, 2000). Constrained ontogenetic development of a craniodental morphology competent for durophagy may have generated strong selection pressures during *Crocuta*'s recent evolutionary shift to feeding in large groups on live prey, favoring not only rapid development of the behavioral and morphological tools needed to cope with difficult foods, but also favoring mothers able to help their cubs during competitive feeding.

Spotted hyenas are unique among carnivores, and highly unusual among mammals, in that they exhibit female dominance over males. In light of the highly specialized bone-cracking morphology in this species, its prolonged period of offspring dependence, and the intensive feeding competition characteristic of this species, we hypothesize that developmental constraints on juvenile feeding capabilities, occurring in an environment characterized by intensive feeding competition, may have shaped the evolution of female dominance. Evidence of slow maturation of the feeding apparatus in *Crocuta* relative to that in other carnivores would suggest the operation of developmental constraints in young spotted hyenas.

Although our current results indicate that the feeding apparatus continues to change well into adulthood in spotted hyenas, further work comparing developmental patterns in skull size and shape among a large array of carnivore species is needed to determine whether an extended development is unique to bone-cracking hyaenids. Such a finding would provide evidence for developmental constraints on the feeding apparatus, which, together with intensive feeding competition, may have led to the evolution of female dominance in spotted hyenas.

CHAPTER THREE

EFFECTS OF AGE, MORPHOLOGY AND SOCIAL RANK ON FEEDING PERFORMANCE IN FREE-LIVING SPOTTED HYENAS (*CROCUTA CROCUTA*)

INTRODUCTION

Selection is likely to act upon individuals throughout their lives, until death or reproductive senescence. Therefore, selection should either reduce the length of the life history stages in which mortality risk is high, favor enhanced performance to improve chances of survival during risky life history stages, or both (Williams, 1966). Since juvenile mortality is high in most animals, selection for improved performance should be expected during early life stages (Carrier, 1996; Williams, 1966). Carrier (1996) predicted that juveniles, constrained by their small size, should compensate for their limitations by improving their performance relative to adults. Indeed, studies on reptiles and mammals have shown this to be the case for certain aspects of locomotor performance, in that juveniles are capable of running or jumping at higher speeds than adults (Carrier, 1996; Irschick, 2000; Herrel & Gibb, 2006). However, the few studies that have investigated ontogenetic variation in feeding performance have found that juveniles are limited in their feeding capabilities relative to adults, suggesting the operation of constraints (Binder, 1998; Binder & Van Valkenburgh, 2000; Thompson et al., 2003; Herrel & O'Reilly, 2006; Vincent et al., 2006). Schwenk and Wagner (2004) defined a constraint as any mechanism that limits the the response of characters to the selective pressures acting during particular life stages. In the case of feeding performance, pressures from competition might lead to the selection for rapid maturation of the feeding apparatus, and subsequently feeding performance. However, a limitation on this response such as delayed development of a highly specialized skull would represent a developmental constraint.

Bite force is a common measure of feeding performance (Binder & Van Valkenburgh, 2000; Herrel et al., 2001; Thomason et al., 1990; Wroe et al., 2004; Thomason, 1991) as it may determine the types of food that can be utilized by an individual. However, due to the difficulty of obtaining direct measures of bite force in large animals, many studies use indirect estimates derived from skull measurements (Wroe et al., 2004; Christiansen & Adolfssen, 2005; Christiansen, 2007; Thomason, 1991; Radinsky, 1981b; Radinsky, 1981a; Radinsky, 1982). In most mammals, bite force is likely to be largely determined by jaw muscle mass and mechanical advantage. Traditional approaches outlined by Maynard Smith and Savage (1959), and further developed by Greaves (1985) and Radinsky (1981b), model the jaw as a third class lever and use linear measurements from the dentary to calculate mechanical advantage of the jaw muscles, and maximum zygomatic arch breadth as an estimate of muscle mass. Calculation of mechanical advantage involves measuring the in-lever of the masseter and temporalis as the distance from the muscle insertion points on the mandible to the jaw joint. These distances are then divided by the out-lever, measured from the jaw joint to the bite point. Larger values of mechanical advantage and muscle mass are expected to reflect the ability to achieve greater bite forces.

Although these indirect measures have provided a great deal of insight into the morphological determinants of feeding performance, it would be preferable to obtain, when possible, direct measures of performance from live animals (Herring, 1985). The few studies directly quantifying feeding performance of wild animals have been primarily limited to reptiles (lizards and turtles: Herrel & O'Reilly, 2006; snakes: Vincent et al., 2006; alligators: Erickson et al., 2003). To date, all such studies of mammals have utilized captive populations (gray short-tailed opossum Thompson et al., 2003; Virginia opossum: Thomason et al., 1990; spotted hyena: Binder & Van Valkenburgh, 2000; Herring, 1985).

Here, we use both direct and indirect measures to investigate changes in feeding performance throughout development in free-living spotted hyenas (*Crocuta crocuta*). For large carnivores like hyenas, both capture and processing of food is extremely demanding, and juveniles may be particularly strongly disadvantaged during competition with adults. Where juvenile performance is poor relative to that of adults, provisioning or assistance from parents or other relatives might be expected until individuals have reached adult performance capabilities. Spotted hyenas are particularly interesting in this respect because they face extreme challenges during feeding, yet provisioning of young by parents is very rare (Holekamp & Smale, 1990).

Adult spotted hyenas have a highly durophagous diet and are capable of cracking open and consuming bones of large diameter. Associated with bone-cracking is a suite of traits in the feeding apparatus that allow hyenas to sustain and generate large bite forces. These include robust dentition, a vaulted forehead, a pneumatized sagittal crest, and enlarged jaw musculature (Joeckel, 1998; Werdelin, 1989; Van Valkenburgh, 2007). In addition to extreme durophagy, *Crocuta* also experience intensive feeding competition (Kruuk, 1972). Unlike most carnivores, spotted hyenas live in large social groups called clans that contain up to 80 individuals (Kruuk, 1972). Clans are structured by rigid linear dominance hierarchies, with social rank determining priority of access to food resources. In intensely competitive environments, like that characteristic of a spotted hyena clan, the ability to consume large quantities of food in a short period of time can be highly advantageous. Therefore juveniles should be disadvantaged during competition over food if they are unable to feed as quickly as adults.

The period of offspring dependence is unusually long for *Crocuta* and other bone-cracking members of the Family Hyaenidae relative to this period in other carnivores (Watts, 2007). Spotted hyena cubs are weaned, on average, at 14 months, but this can range up to 24 months of age (Holekamp et al., 1996; Kruuk, 1972). Yet, despite the late age at which *Crocuta* are weaned, it appears that individuals have still not reached adult performance levels by the time they attain nutritional independence from their mother. That is, in captive spotted hyenas, Binder and Van Valkenburgh (2000) found that bite force continued to increase well past puberty, which occurs in this species at around 24 months. Furthermore, the feeding tests they conducted using de-fleshed sheep femurs revealed that juveniles were at a disadvantage compared to adults when eating bone, and that there was a marked increase in feeding speed around 22 months of age. However, performance in this test was still improving in the oldest captive hyenas measured (30 months), so these earlier investigations did not establish when during ontogeny asymptotic performance was achieved. External linear measures of skull size from live captive hyenas leveled off at 20 months of age, shortly before the age of sexual maturity (Binder & Van Valkenburgh, 2000). They therefore attributed any improvements in performance after 20 months to increased size of the jaw musculature. In Chapter Two we used geometric morphometrics to quantify changes in skull size and shape in an ontogenetic series of *Crocuta* skulls from the wild (Figure 3.1), and found that skull size continued to increase until 26 months, but that skull shape continued to change until 35 months of age. In contrast to the pattern of change in skull shape, we found that mechanical advantage of the temporalis, estimated from traditional lever-arm models, reached maturity at 22 months of age. Using a traditional measurement of zygomatic arch breadth to estimate jaw muscle mass, we found that this continued to increase until 33 months of age. However, it remains to be determined which, if any, of these traditional estimates accurately reflect actual feeding performance.

Biknevicius (Biknevicius, 1996) proposed that the eruption of adult dentition, which is fully complete in spotted hyenas by 18 months

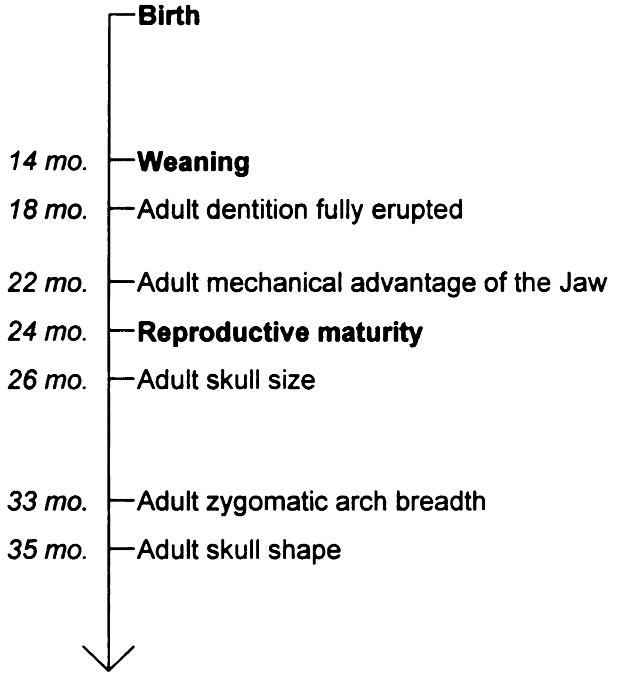


Figure 3.1. Timeline illustrating the age at maturity for morphological measures in relation to life history events (in bold).

(Van Horn et al., 2003), might be the main limiting factor affecting feeding performance in juveniles, and that weaning is delayed until tooth eruption is complete because this is when juveniles are capable of competing with adults at kills. If either mechanical advantage or skull size was the main factor limiting performance in young hyenas, then feeding performance should reach maturity at around 22 or 26 months of age, respectively. If skull shape or muscle mass was the main limiting factor, then feeding performance should continue to improve until 33 or 35 months of age, respectively when these morphological measures reach asymptotic values. Each of these morphological variables probably has important consequences for performance, and all of these variables are changing throughout early ontogeny. However, if feeding performance continues to improve beyond the time at which a particular morphological variable has ceased changing, this would suggest that other variables must subsequently be contributing to improved feeding.

Another factor likely to affect feeding performance in spotted hyenas is social rank, which is maternally inherited and therefore persists throughout life in females, and until dispersal in males. Higher ranking individuals in a clan feed first at a kill and have preferential access to the soft parts of a carcass such as viscera and muscle (Kruuk, 1972; Bearder, 1977). Consequently, the ability to generate large bite forces should be especially advantageous for lower-ranking hyenas as they are more likely to feed on relatively large amounts of bones. Furthermore the ability to feed quickly should be particularly helpful for lower-ranking hyenas as they may be displaced from food shortly after prey capture by higher-ranking conspecifics. If social rank influences feeding performance then lower-ranking individuals should feed faster than higher-ranking individuals of the same age.

The objectives of this study were threefold. First, we hoped to document ontogenetic changes in feeding performance as directly measured by both bite force generation and ingestion time. Second, we examined the relative roles of each of several morphological variables (dentition, skull size, skull shape, zygomatic arch breadth, or mechanical advantage of the temporalis) in limiting feeding performance during ontogeny. Finally, we tested the hypothesis that social rank influences feeding performance in this species.

MATERIALS AND METHODS

Study population

We conducted performance testing on known-age hyenas from a study population (Frank, 1986) that has been continuously monitored since 1988 in the Masai Mara National Reserve in southwestern Kenya. All individuals in the population were identified by their unique spot patterns and birth dates were estimated (to ± 7 days) based on the appearance of cubs when they were first observed above ground (Holekamp et al., 1996). We evaluated dominance relationships among mothers based on the direction of submissive behaviors (Holekamp & Smale, 1993); by convention, the highest ranking female was assigned a rank of one. Cubs were assigned the same social ranks as their mothers.

Direct Measurements of Bite Force

We obtained direct measurements of bite force using a bite force transducer (Kistler, Amherst, NY) presented to known-age hyenas in the study population between August 2003 and April 2005. The transducer was baited either with powdered milk for younger individuals, or meat for older individuals, and attached to our study vehicle. When an individual hyena voluntarily bit down on the probe, a piezoelectric plate embedded in the probe measured the force generated in Newtons, and this was recorded from a handheld charge amplifier. Similar devices have been used with captive hyenas at UC Berkeley (Binder, 1998; Binder & Van Valkenburgh, 2000), captive short-tailed opossums (Thompson et al., 2003), and with both captive and wild American alligators (Erickson et al., 2003).

Feeding Speed

Since both the ability to feed quickly and the ability to generate large bite forces are critical for feeding success in the competitive environment of spotted hyenas, we used feeding speed as another indicator of feeding performance. We measured ingestion time for two different types of food objects. First, from June 1989 until February 1996 we conducted 174 feeding tests by presenting 30g dog biscuits to 84 spotted hyenas of known age. Although, the dog biscuit is not an object that hyenas would naturally encounter in their environment, it allowed us to present a food item of identical size, shape and consistency to each individual tested, and to conduct tests on cubs as young as three months of age.

From July 2003 until June 2005 we also conducted 203 feeding tests on 43 individuals using de-fleshed goat femurs that were purchased from a local butchery. The bones used in performance tests varied in mass (70-118 grams), so the weight of each bone was recorded prior to testing. All femurs used in this study were fresh and presented to individuals within three or four days of preparation at the butchery. Although we did not know the last time at which each tested hyena had eaten, we never presented bones to individuals that had distended bellies, indicating recent ingestion of a substantial meal. Furthermore all individuals were tested when they were away from other individuals.

For both types of feeding tests, we measured ingestion time with a stopwatch. We only included the seconds during which each individual was actively processing the object; i.e., if they ceased chewing for any reason while food still remained (e.g., to engage in vigilance), we did not include those seconds in recorded ingestion time.

Analyses

In order to identify the age at which feeding performance ceased improving, we used nonlinear growth models to calculate asymptotic values. Using an IMP program, GrowChoice (Sheets, Canisius College, Buffalo, NY), eight different growth models were fitted to the data and Aikaike's Information Criterion (AIC) weights were calculated for each model. The monomolecular model (following Gaillard et al., 1997) had the highest AIC weight for the biscuit feeding data. The formula used was

$$x(t) = A\{1-\exp^{K(t_0-t)}\}$$

where x(*t*) is ingestion time at time *t*, *A* is the asymptotic adult value, *K* is the rate of approach to the adult value, and t_0 is the age at which growth begins (Gaillard et al., 1997; Zelditch et al., 2003). For the bone test data we used a Gompertz equation as parameterized by Fiorello & German (1997):

$$x(t) = A\exp^{-ke^{-bt}}$$

Here, *k* is the initial growth rate and *b* is the decay of the growth rate (Fiorello & German, 1997; Zelditch et al., 2003). We have reported age at maturity for each measure as 95% of its asymptotic value.

Although these models fit the curves well, there was a great deal of variation in both sets of performance data. Therefore, to further elucidate the causes of this variation, we used the breakpoint (the age at which 95% of the asymptotic value was achieved) to divide the data, and then constructed generalized linear mixed models (GLMM) that included both fixed and random effects for analysis of each set of linear data separately. Here age and social rank were modeled as fixed factors and the random factor of individual identity took into consideration repeated testing of some individuals at different ages to avoid pseuoreplication. For the bone tests, we also included the weight of the bone as a fixed factor since this varied among tests. Additionally, in order to determine the age at which feeding performance ceased to improve while taking the above predictors into consideration, we iteratively increased the lowest age (in months) represented in each subset of data until age was no longer a significant ($p \geq 0.05$) predictor of feeding performance in the model.

We compared the age at which feeding performance reached maturity in each test to the ages at maturity for mechanical advantage of the temporalis, skull size, skull shape, zygomatic arch breadth and eruption of the adult dentition. Mechanical advantage of the temporalis was measured as the distance from the dorsal tip of the coronoid process to the jaw joint, divided by the distance from the jaw joint to the third lower premolar. Zygomatic arch breadth was measured as the widest distance across the zygomatic arches. These measurements were obtained from 65 *Crocuta* skulls ranging in age from 2 months to 18 years (housed at the MSU Museum). Similarly, using geometric morphometrics, as described previously (Chapter Two), we calculated centroid size for each of the 65 specimens and examined shape variation (measured using Procruste's distances) among them. The monomolecular growth model described above was used to calculate 95% of the asymptotic value for each of these morphological measures.

Lastly, to determine if there were any effects of social rank on skull size and shape among adults, we performed a regression on skull centroid size and on skull shape variables (Procruste's distances) for adult females, using age and social rank as predictor variables. For these analyses we used centroid size and shape data from the ventral view calculated in the previous chapter from 11 adult females of known age and rank. All of the analyses were conducted with R software version 2.4.1 (R Core Development Team, 2005). The GLMMs were performed using the lme package in R.

RESULTS

Bite Force

Although we recorded 65 bites on the transducer from 31 hyenas, for a number of reasons we were unable to collect sufficient reliable bite force data to document ontogenetic changes in bite force. First, it was impossible to obtain measures from adult hyenas. Although juveniles would often become highly engaged in biting the transducer, the adults that approached it would usually remove the meat covering, test the transducer gently with their teeth, and then cease interacting with the device altogether. Second, it was clear that most juveniles biting the transducer were not biting down as hard as they could. In Figure 3.2, we present the bite force data collected from 31 individuals between the ages of 3.5 and 24 months. Interestingly, our largest bite force readings were from one individual at 12.2 and 13.1 months of age. At 13.1 months, this hyena had a bite force of 2682 N. Given how much greater this value is than all other values save one, it is unlikely that the other bites recorded reflected bite force maxima for those individuals.

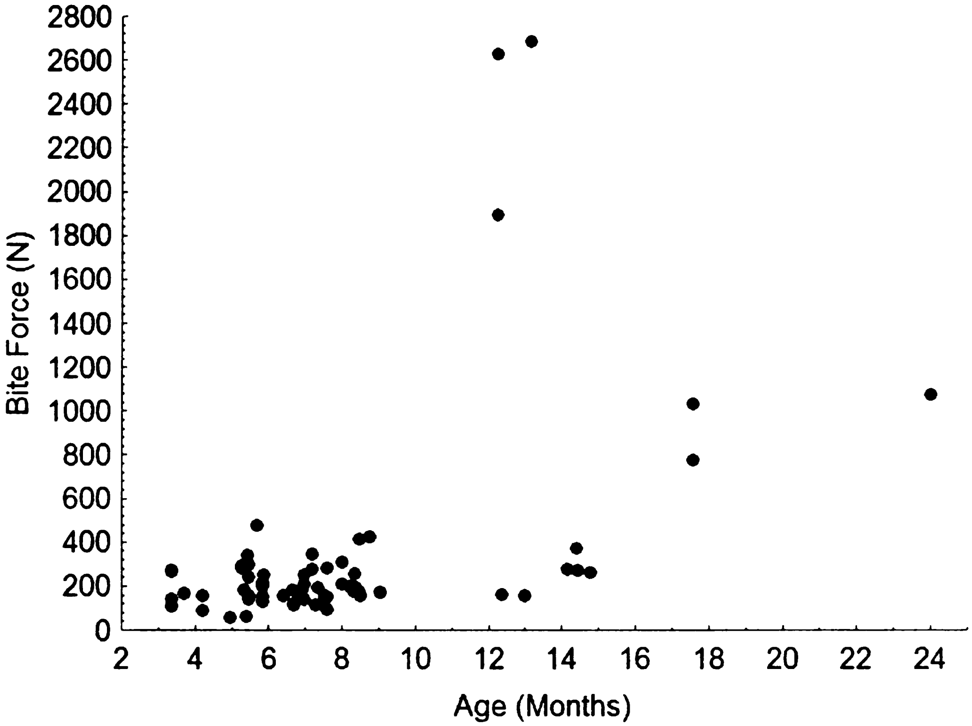


Figure 3.2. Measurements of bite force in Newtons (N) obtain with a piezoelectric transducer in a bite force meter available to wild hyenas.

Ingestion Time

Ingestion time measured in biscuit tests rapidly decreased during early ontogeny (Figure 3.3) and reached 95% of the asymptotic value at 19 months of age. Ingestion time measured from tests using goat femurs (Figure 3.4) followed a similar trajectory, reaching 95% of the asymptotic value at 20 months of age. However, when the ontogenetic data were subdivided into pre- and post-breakpoint subsets, age continued to be a significant predictor of performance well beyond 20 months (Figures 3.5 and 3.6). Specifically, ingestion time in the biscuit tests continued to decrease until 34 months of age (Figure 3.5c) and ingestion time in bone tests until 36 months of age. Thus, even though the most rapid and dramatic ontogenetic change in feeding performance in both tests occurred during the first 20 months of life, performance during ingestion of both soft (biscuits) and hard (bones) foods continued to improve significantly until late in the third year of life.

Table 3.1 shows the results of the mixed models for both biscuit and bone tests when social rank and individual identity were considered in addition to age. The GLMM for the biscuit tests revealed that, prior to 19 months, age was a significant predictor of ingestion time and that there was a trend for higher-ranking cubs to feed more quickly than lower-ranking cubs. After 19 months, age continued to be a significant predictor but there was no indication that rank influenced feeding speed. By 34 months, age was no longer significant, and social rank was highly significant. However, the direction of the influence of rank

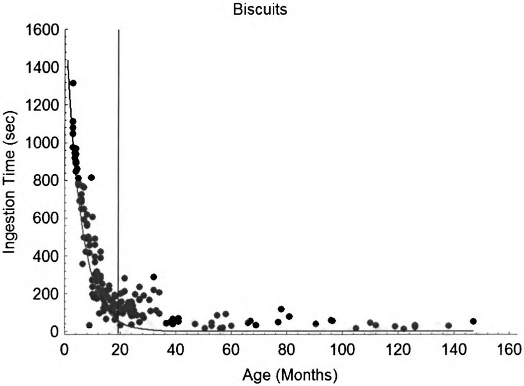


Figure 3.3. Ontogenetic change in the time required to consume a 30g dog biscuit during performance testing. The curved line represents the fit of the monomolecular growth model, and the straight vertical line indicates 95% of the asymptotic value achieved at 19 months of age.

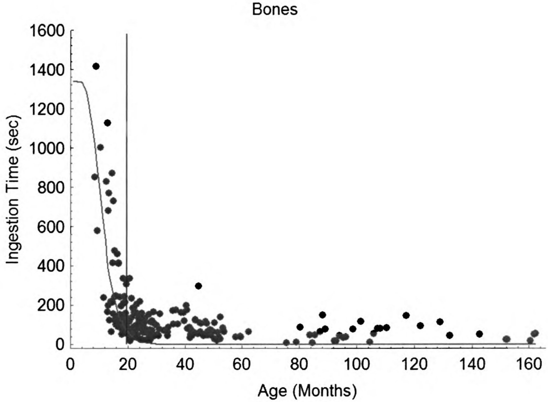
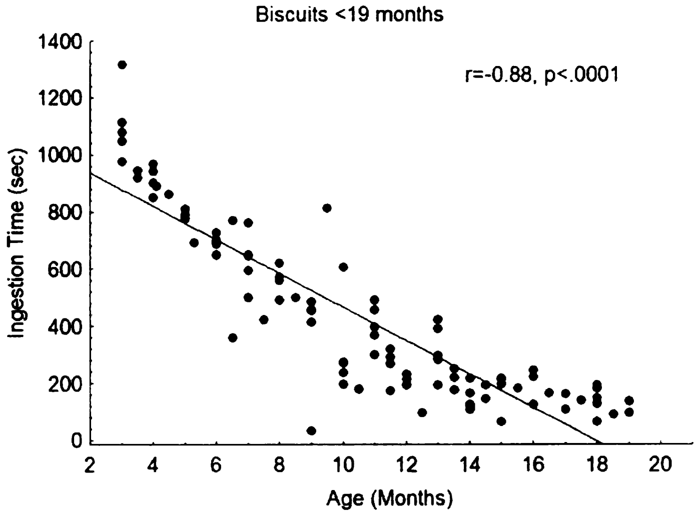


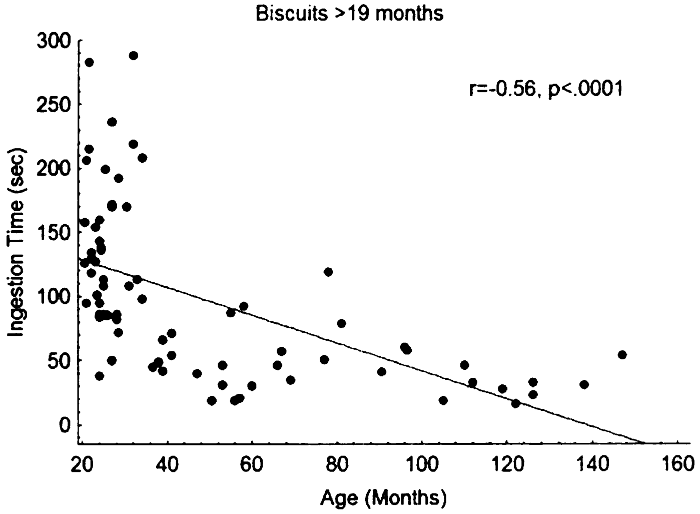
Figure 3.4. Ontogenetic change in the time required to consume a goat femur during performance testing. The curved line represents the fit of the gompertz growth model, and the straight vertical line indicates 95% of the asymptotic value achieved at 20 months of age.

Figure 3.5. Linear change in biscuit feeding performance for pre- and post-asymptotic subsets of data. Age is still a significant predictor of feeding performance in both pre- (a) and post- (b) breakpoint subsets. However, after 34 months (c), age is no longer a significant predictor of performance.

a.


Biscuits <19 months
r=-0.88, p<.0001
Ingestion Time (sec)
0
200
400
600
800
1000
1200
1400
2
4
6
8
10
12
14
16
18
20
Age (Months)

b.


Biscuits >19 months
r=-0.56, p<.0001
Ingestion Time (sec)
0
50
100
150
200
250
300
20
40
60
80
100
120
140
160
Age (Months)

c.

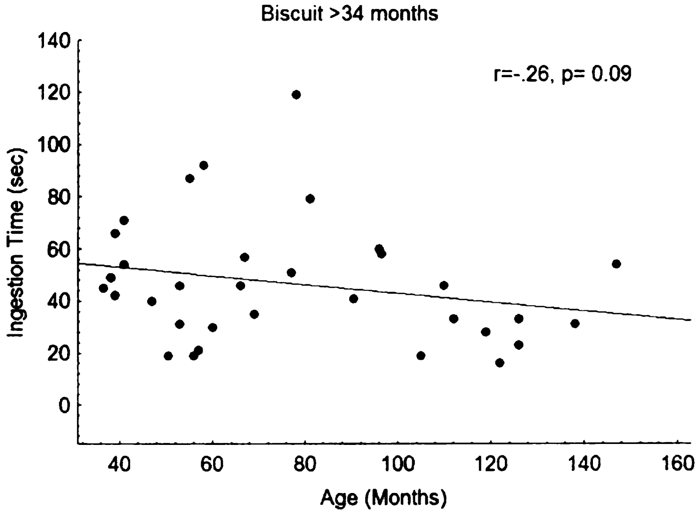
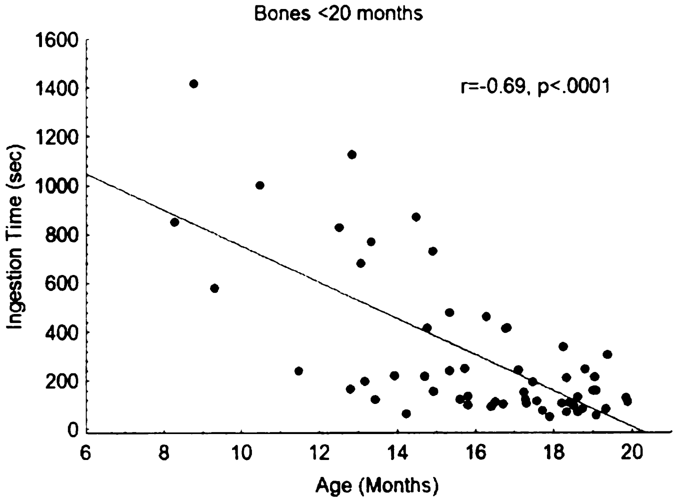
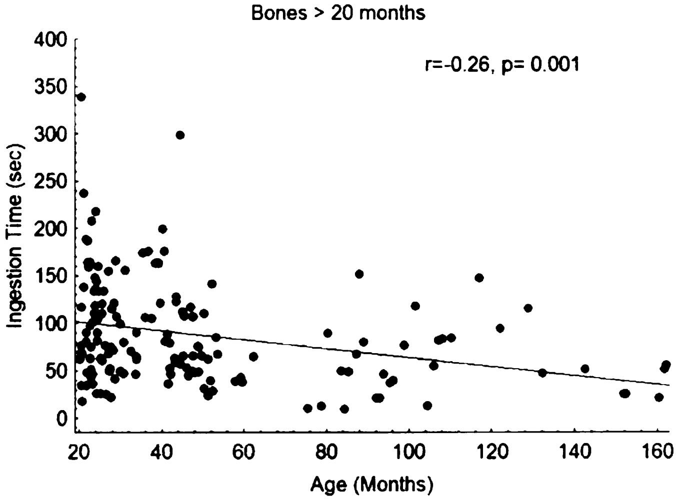

Biscuit >34 months
r=-.26, p= 0.09
Ingestion Time (sec)
0
20
40
60
80
100
120
140
40
60
80
100
120
140
160
Age (Months)

Figure 3.6. Linear change in bone feeding performance for pre- and post-asymptotic subsets of data. Age is still a significant predictor of feeding performance in both pre- (a) and post- (b) breakpoint subsets. However, after 36 months (c), age is no longer a significant predictor of performance.

a.


Bones <20 months

b.


Bones > 20 months

c.

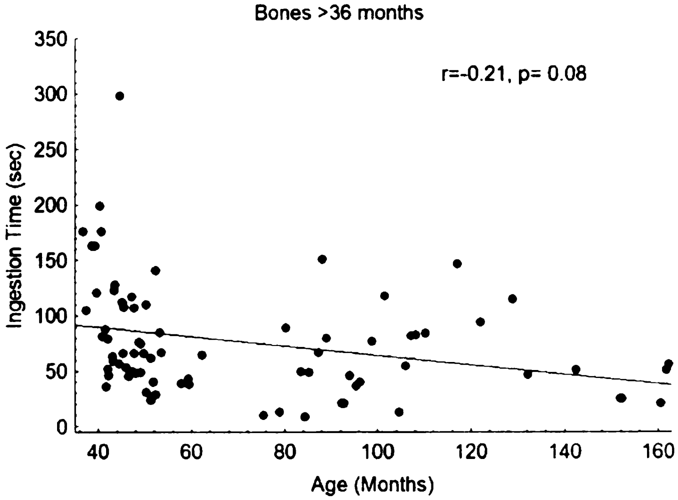

Bones >36 months

Table 3.1. Variables affecting feeding performance at different subsets of ages. Significant terms ($p<0.05$) are highlighted in bold font.

Feeding Test	*Age subset*	*Terms*	*b ± SE*	*t*	*df*	*p*
Biscuits	< 19	Age	-50.24 ± 6.02	-8.33	26	**<.00001**
		Rank	10.61 ± 5.71	1.86	26	.07
	> 19	Age	-1.05 ± 0.19	-5.60	39	**<.00001**
		Rank	-1.06 ±1.09	-0.97	39	0.34
	> 34	Age	-0.18 ± 0.11	-1.75	11	.1085
		Rank	-2.12 ± 0.59	-3.60	11	**.0042**
Bones	< 20	Bone wt.	8.5 ± 2.6	3.28	35	**.0024**
		Age	-80.18 ± 10.0	-8.01	35	**<.00001**
		Rank	-10.41 ± 10.0	-1.04	35	.3052
	> 20	Bone wt.	1.61 ± 0.38	4.17	111	**.0001**
		Age	-0.90 ± 0.32	-2.77	111	**.006**
		Rank	-4.23 ± 2.19	-1.93	111	.055
	> 36	Bone wt.	1.84 ± 0.49	3.74	58	**.0004**
		Age	-0.45 ± 0.25	-1.82	58	.07
		Rank	-0.87 ± 2.40	-0.36	58	.72

had changed from the pattern seen before 19 months, such that lower-ranking adult hyenas now had shorter ingestion times than did higher-ranking adults.

Bone test models included an additional variable, the weight of the bone, which was highly significant at all ages. Not surprisingly, heavier bones took longer to ingest. Furthermore, age continued to be a significant predictor of performance in this test until 36 months of age. Social rank was not significant in any of the GLMMs for bones.

Neither age nor social rank were significant predictors of skull shape in adult female hyenas. However, separate regressions of skull centroid size on age and rank for females older than 26 months of age (which is when skull size reached maturity), showed that age was not, but that social rank was, a significant predictor of centroid size (Figure 3.7). Lower-ranking females had larger skulls than higher-ranking females (n=11, r=0.69, p = .02).

DISCUSSION

The difficulty we experienced obtaining bite force measures from adult hyenas in the wild highlights the impracticality of collecting such measures from wild carnivores, even those very well habituated to the presence of observers. Although, wild alligators can be stimulated to bite down with great force by touching their teeth with a transducer while the mouth is open (G. M. Erickson, Pers. Comm.), there is no way to elicit a comparable response from spotted hyenas. Future endeavors to collect such data will require greater creativity in

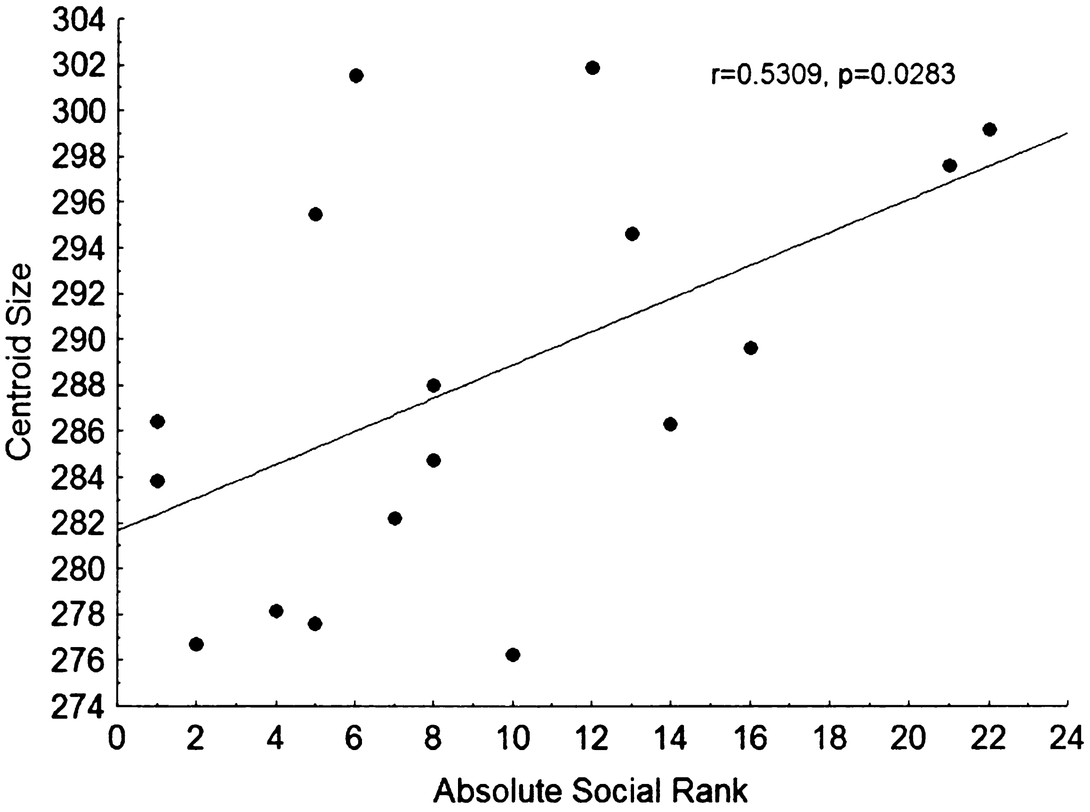


Figure 3.7. The relationship between social rank and skull centroid size in adult female spotted hyenas. By convention the highest ranking female is assigned a rank of 1.

obtaining bite force measures. The challenge of presenting an expensive and sensitive piece of equipment to a large carnivore in a way that might elicit a motivated response is difficult, to say the least. Even when individuals appear highly motivated to bite the transducer, it is difficult to determine whether values that have been recorded approach their maximum possible bite force. This difficulty is illustrated by the variation in bite force evident among young *Crocuta* (Figure 3.2). The strongest bite measured from a young hyena, 2682 N, was substantially stronger than most of the bite force readings obtained from adult hyenas in a captive colony (Binder & Van Valkenburgh, 2000). This suggests that previous bite force measures obtained from captive animals may have similarly failed to capture bite force maxima, and that they probably greatly underestimate the bite force capabilities of wild hyenas. It has been estimated that forces of between 7000-9000 N are required to crack open the limb bones of a wildebeest-sized ungulate (Stefen, 1997; Erickson et al., 1996; Meers, 2002; Therrien, 2005), a task spotted hyenas perform daily in many parts of Africa. Furthermore, spotted hyenas have been observed cracking open giraffe femurs and various bones from hippopotamus and rhinoceros (Deane, 1962), suggesting that they are capable of generating substantially more than 9000N of force during bone-cracking. All of these facts suggest that adult free-living *Crocuta* are capable of bite forces much greater than those reported here or earlier.

Our results from feeding performance tests measuring ingestion time were more comprehensive than those obtained with our bite force meter. These feeding performance tests allowed us to examine ontogenetic variation in feeding speed, and investigate factors limiting performance. Although the fitted growth models of raw performance data suggest that ingestion time reaches 95% of final adult values at around 20 months of age in spotted hyenas, a more accurate pattern emerges when additional variables influencing feeding performance are considered. Changes in feeding speed appear instead to continue changing well beyond 20 months and into adulthood, until between 33 and 36 months of age. This means that feeding performance in wild hyenas continues to improve, not just beyond weaning, but also for roughly a year beyond reproductive maturity.

Our performance data indicate that eruption of the adult dentition is not a primary contributor to improvement in feeding performance later in ontogeny. Dentition certainly determines the types of foods individuals can utilize, particularly among bone-cracking animals. The ability to crack open bone is expected to improve once the robust and specialized adult dentition is in place, yet there are clearly other aspects of development that contribute to improvement in feeding performance well beyond the age (18 months) at which tooth eruption is complete. Biknevicius (1996) suggested that, since the timing of weaning roughly coincides with the eruption of adult dentition, juveniles must be capable of effectively competing for food with conspecifics at this time. However, neither the results presented here nor the data from captive hyenas (Binder, 1998; Binder & Van Valkenburgh, 2000) support this hypothesis. Instead, it appears that hyenas are not able to compete effectively for food with older individuals via rapid feeding until 33 to 36 months of age, when feeding performance stops improving.

Mechanical advantage of the temporalis reached maturity at an earlier age than ingestion time (Figure 3.8). Our results suggest either that the traditional measures of mechanical advantage may not accurately reflect developmental changes in three-dimensional morphology, or that any improvements in feeding performance beyond the age at which mechanical advantage has reached 95% of the asymptotic value are due instead to changes in skull size, shape or muscle mass. Skull size reached maturity at 26 months of age, but increases in muscle mass (indicated by zygomatic arch breadth) and skull shape continued until 33 and 35 months of age, respectively. Therefore, it is most likely changes in these two aspects of the feeding apparatus that limit feeding speed until late in the third year of life. Additionally, experience gained by ingesting bones and other foods might also increase feeding speed in the third year.

One limitation of our data that prevents us from adequately testing hypotheses about the influences of morphological variables on feeding performance is that our performance measures and morphological measures were taken from different individuals. Ideally, in order to address these questions, both sets of measures would be taken together. However this was logistically impossible in our case, as behavioral measures could not be obtained from the individuals from which we obtained skulls.

In addition to the influences of morphology, social factors appear to influence variation in feeding performance during ontogeny. The results from the GLMMs on the biscuit data support our prediction that lower-ranking individuals feed faster than their higher-ranking conspecifics as adults. Rank-related

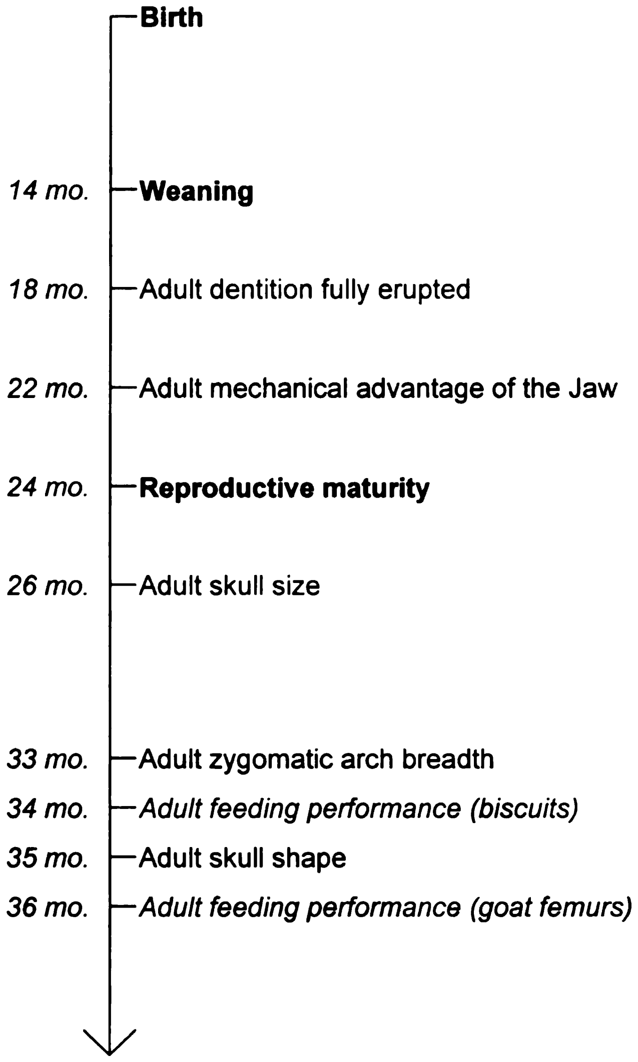


Figure 3.8. Timeline illustrating the age at maturity for feeding performance (in italics), for both dog biscuits and goat femurs, relative to life history events and to the age at maturity for morphological measures.

variation in feeding speed might reflect variation with respect to motivation level, morphology, or both. When we investigated rank-related variation in skull size (Figure 3.7), we found that lower-ranking females had larger skulls than higher-ranking females, which might contribute to the observed differences in feeding performance. One other hypothesis to explain the rank-related variation in skull size is that this reflects differential experience in feeding on bones. Lower-ranking hyenas are expected to feed on greater proportions of bone in their diet than are high-ranking individuals due to their relatively low priority of access to food. Skull and other bones remodel themselves in response to the stresses placed upon them (McGowan, 1998), and the greater stresses experienced by individuals that must feed on more bones might be reflected in increased skull size. Another possibility is that larger body size enhances survival among low-ranking female hyenas such that we are more likely to acquire skulls of larger than smaller individuals in this rank category among adults.

Interestingly, the trend seen in effects of social rank on feeding performance among hyenas younger than 19 months was in the opposite direction from that seen among adults. Higher-ranking sub-adults fed faster on biscuits than did lower-ranking sub-adults. Previous studies have shown that intra-litter rank and maternal rank affect growth rates among juveniles, with higher-ranked individuals growing faster than their lower-ranking peers (Wahaj & Holekamp, 2006; Hofer & East, 1996). The relatively large size of high-ranking juveniles might explain their ability to feed more rapidly.

The effect of social rank on performance was not as apparent from feeding tests using goat femurs as those using biscuits, perhaps due to the variation introduced by bone weight. There was a trend for lower-ranking hyenas to feed faster than higher-ranking individuals in the post-breakpoint linear dataset after 20 months of age, but this trend was not evident in the smaller subset of data encompassing individuals older than 36 months. Although goat femurs are undoubtedly more biologically meaningful objects than dog biscuits for performance testing, the variation in bone size and weight contributes additional error to the model. The goal of our study was to document ontogenetic changes in feeding performance, so we chose to use objects that even young hyenas could process. The trade-off, however, is that biscuits might not have been sufficiently challenging for adults, and thus not adequate for reflecting ontogenetic and social influences on feeding performance at older ages. Future work might utilize larger bones to address this issue. Presenting larger bones to adult hyenas might also provide a more accurate measure of their maximum bite force, as the force needed to crack open bones of particular diameters and material properties could be straightforwardly calculated. Nevertheless, spotted hyenas eat soft as well as hard foods and the data presented here clearly show that juveniles are handicapped relative to adults at eating both types of food.

Both the morphological variables measured in the previous chapter and the performance variables measured here indicate that juvenile spotted hyenas are at a disadvantage relative to adults with respect to feeding performance at the time of nutritional independence. In wild spotted hyenas, the phase of the life history during which risk of mortality is greatest occurs immediately after weaning (Watts, 2007). This likely reflects the fact that individuals at the time of weaning are neither equipped for, nor capable of, the levels of feeding performance attained by adults. Even after the age when individuals are first capable of reproducing, at 24 months of age, skull shape continues to change and ingestion time to increase, suggesting that there are developmental constraints on the feeding apparatus that limit performance well into adulthood. Although the period of offspring dependence is longer in spotted hyenas than in other carnivores, young hyenas after weaning are still poorly equipped to compete with adults. Given the limited feeding capabilities of young hyenas, we expect that females aid their offspring in other ways after weaning, perhaps by defending them while they feed. Indeed, individuals whose mothers survive past the individual's weaning age survive better than those whose mothers die shortly after weaning (Watts, 2007). This suggests that maternal presence aids in cub survival after weaning.

Spotted hyenas are unique among carnivores in that adult females are socially dominant to adult immigrant males in each clan. One explanation for the evolution of female dominance is that intensive feeding competition has selected for large, aggressive females who are better able than other females to provide resources to their dependent offspring (Frank, 1997). Although there is intense feeding competition in this species, there are other large carnivores, such as wild dogs and wolves, that experience similar levels of competition at kills (Creel & Creel, 2002; Schmidt & Mech, 1997). Thus selection pressures imposed by feeding competition alone do not seem sufficient to explain the evolution of female dominance. Alternatively, it may be that developmental constraints on juvenile feeding performance, *in addition to* intensive feeding competition, led to the evolution of dominant mothers capable of assisting their handicapped offspring. The data presented here, which indicate that development is a very slow and prolonged process in *Crocuta*, support this hypothesis. However, comparable studies on other carnivores are needed in order to determine whether the slow ontogeny of the spotted hyena is unique among carnivores.

CHAPTER FOUR

ONTOGENETIC VARIATION IN THE PLAY BEHAVIOR OF SPOTTED HYENAS (*CROCUTA CROCUTA*)

INTRODUCTION

Play is one of the most ubiquitous developmental processes known to occur in mammals, yet it also remains one of the most poorly understood. Because little is currently known about its functions, we adopt the structural definition of play suggested by Bekoff and Byers (1981), as "any post-natal motor activity that appears to be purposeless, in which motor patterns from other contexts may often be used in modified forms and temporal sequencing." Although this definition implies that play may serve no purpose, the frequency with which play occurs among juveniles, its wide taxonomic distribution (Fagen, 1981), and its costs with respect to energy expenditure and predation risk (Harcourt, 1991) suggest that play probably has important fitness consequences in the lives of mammals. Bekoff (1984) and Byers (1998) have argued that careful documentation of the occurrence of play during ontogenetic development in a wide array of species is a necessary prerequisite for evaluating alternative hypotheses that suggest specific functions of play. Among mammals, rates of play are frequently highest, and the forms of play most diverse, in primates and carnivores (Fagen, 1981). Thus study of play in members of these orders may provide useful insight into the adaptive significance of these behaviors. Here we report on play behavior in a highly gregarious carnivore, the spotted hyena (*Crocuta crocuta*).

Spotted hyenas live in complex social groups, called clans, which may contain up to 90 individuals (Kruuk, 1972). Every clan is structured by a strict linear dominance hierarchy, and an individual's position in the hierarchy determines its priority of access to food (Kruuk, 1972; Engh et al., 2002). Juvenile hyenas 'inherit' the social ranks of their mothers (Smale et al., 1993; Holekamp & Smale, 1993) via the same mechanisms as those operating in cercopithecine primates (Engh et al., 2000).

Female *Crocuta* give birth in an isolated natal den to litters of one, two, or rarely three cubs. Cubs remain at the natal den for the first 2-5 weeks of life (East et al., 1989; Kruuk, 1972). They are then brought to the clan's communal den where the cubs of multiple females are sheltered together in an underground burrow system. Cubs live at a communal den until 8 to 12 months of age, after which they cease using the den for shelter, and begin traveling around the clan's territory with their mother. The communal den serves as the social center of the clan and is periodically visited by all clan members. When older hyenas are present at the den, cubs emerge to nurse, rest and play.

As in other mammals, the play behavior exhibited by hyenas and other carnivores takes a variety of forms. These include play-fighting, sexual play, object play, and solitary locomotor play. Although it is widely recognized that each form of play behavior might have its own function, and thus offer unique benefits to the individual, it is not always clear whether such benefits are immediate for the developing animal or deferred until adulthood (Pellegrini & Smith, 1998). One of the goals of this study was to assess the various forms of play behavior exhibited by spotted hyenas in their natural habitat, and to document the relative frequency at which each form of play occurs throughout ontogeny. Like Byers (1998), we view this as a critical first step toward understanding the functional significance of each type of play.

Another goal of our study was to evaluate sexual dimorphism in play behavior. In many mammals (Bekoff, 1974), including humans (Hines, 2003), some forms of play appear strongly sexually dimorphic whereas others do not. In those species for which the physiological substrates of play have been examined, sexually dimorphic forms of play are mediated by pre- or early post-natal exposure to androgenic hormones or their metabolites (monkeys, Goy, 1996; Goy et al., 1988; Goy & Wallen, 1979; ground squirrels, Nunes et al., 1999; humans, Hines, 2003).

The spotted hyena is a particularly interesting species in which to examine sexual dimorphism in play behavior because many aspects of the behavior and morphology of female hyenas are heavily masculinized. For example, females are more aggressive than males (Szykman et al., 2003), and as adults, females are also socially dominant to males (Kruuk, 1972). In these respects, spotted hyenas are thus "sex-role reversed" relative to mammalian norms. Furthermore, the external genitalia of female hyenas are strongly virilized; the hypertrophied clitoris forms a large and fully erectile pseudopenis, and the fused vaginal labia form a structure resembling the scrotal sac of the male. Recent experimental work with captive hyenas has shown that, although androgens later modify the fine-structure of the phallus, these odd genitalia develop before androgens are present and therefore formation of the genitalia must be largely androgen-independent (Drea et al., 1998; Drea et al., 2002; Glickman et al., 2005; Glickman et al., 2006). However, the maternal ovary produces high concentrations of a weak androgen called androstenedione, which is transformed by placental enzymes into testosterone that reaches developing fetuses via the umbilical vein (Licht et al., 1998; Yalcinkaya et al., 1993). Fetal hyenas of both sexes are thus apparently bathed in androgens late in gestation.

Work with primates and rodents has shown that perinatal androgen exposure masculinizes social and sexual play behavior in juveniles, even in the absence of any circulating hormone during the period of ontogeny when play occurs (Goy & Wallen, 1979; Goy et al., 1988; Nunes et al., 1999; Pellis, 2002). Furthermore, the masculinizing effect of androgen on juvenile play behavior is separated in time from its masculinizing effect on genital differentiation (Wallen & Baum, 2002). In rhesus monkeys, for example, androgen exposure early in fetal development masculinizes the genitalia of the female but has no effect on juvenile play behavior; by contrast, androgen exposure late in gestation produces no genital masculinization, but strongly affects juvenile rough-and-tumble play and play-mounting (Goy et al., 1988). Similarly, in humans, girls affected with congenital adrenal hyperplasia (CAH), and consequently exposed to heightened levels of testosterone prenatally, exhibit more male-typical toy preferences and more masculine play behaviors than unaffected girls (Hines, 2003).

Interestingly, in both humans (Hines, 2003) and spotted hyenas (Dloniak et al., 2006), naturally-occurring variations in concentrations of maternal androgens during pregnancy are associated with variations in the sexually dimorphic play behavior of the offspring resulting from those pregnancies. That is, in both species, higher androgen concentrations in pregnant females are later associated with higher rates of some forms of male-typical play in female offspring. However, it is not currently known how many different forms of play are sexually dimorphic among young spotted hyenas, nor which forms of play might be influenced by prenatal androgen exposure. If androgens influence play in spotted hyenas as they do in primates and other mammals, then patterns of both social play and play-mounting in female hyenas should be similar to those of males. Pedersen et al. (1990) found that, among captive hyenas in same-sex groups, females engaged in more social play than did males. In the current study, we evaluated this possibility in wild spotted hyenas.

Our final goal was to test two hypotheses about particular classes of environmental variables known to influence rates of play in other mammals: energy availability and social context. Numerous studies on juvenile animals ranging from birds to primates have shown that rates of play decrease when energy supplies decline (Sharpe et al., 2002; Bateson et al., 1990; Nunes et al., 1999; Nunes et al., 2004; Pozis-Francois et al., 2004; Rajpurohit et al., 1995; Barrett et al., 1992; Baldwin & Baldwin, 1976). However, in some species the opposite relationship has been shown. For instance, by reducing energy availability, inducing an early onset of weaning increases rates of social and locomotor play in domestic cats (Martin & Bateson, 1985). Similarly, underfed rats play more than well-fed individuals (Loranca et al., 1999), and captive chimpanzees and bonobos engage in more social play before than after feeding; in these species play might function to reduce the intensity of aggression and competition around food (Palagi et al., 2004; Palagi et al., 2006).

Here we tested several predictions of an hypothesis suggesting that low levels of available energy are associated with relatively low rates of play in hyena cubs. Because intra-litter rank has a significant effect on cub growth rates (Hofer & East, 1996; Wahaj & Holekamp, 2006), we inquired whether rates of play behavior also varied here with intralitter rank. If so, we expected dominant cubs within twin litters to play at higher rates than their subordinate siblings, as they enjoy superior access to the mother's milk. Social dominance determines priority of access to food resources in this species, with high-ranking individuals enjoying better food access than low-ranking ones (Kruuk, 1972). Therefore, if the hypothesis is correct that energy affects hyena play, we expected that rates of play would be elevated in cubs with high-ranking mothers relative to those with lower-ranking mothers. Furthermore, after controlling for numbers of potential play partners, we expected singleton cubs, which have sole access to their mother's milk and therefore grow faster than twins (Hofer & East, 1996), to exhibit more play than cubs from twin litters. Additionally, if food availability affects rates of play, then play rates should be lower in all cubs during periods when food is scarce than during periods of prey abundance.

Finally, one important feature of the immediate social context that might influence rates of play is the number of potential play partners available to an animal (Poirier & Smith, 1974). The number of available partners can be quite variable for spotted hyena cubs, who live for several months at a communal den where offspring of two to several adult females may reside. If availability of play partners affects rates of play, we expected that individuals from large cohorts of cubs would play at higher rates than individuals from small cohorts. We emphasize that hypotheses suggesting energetic and social variables as determinants of play rates are not mutually exclusive, and that multiple variables might be operating concurrently to affect rates of play.

MATERIALS AND METHODS

Study Animals

We collected data from members of one large clan of spotted hyenas inhabiting the Talek region of the Masai Mara National Reserve in southwestern Kenya (Frank, 1986; Boydston, 2001). Adult and sub-adult hyenas were identified individually by their unique spot patterns. Young cubs, which have a solid black lanugo, were identified on the basis of distinctive scars, nicks on the ears and patterns of molt. Relationships between mothers and offspring were determined based on nursing associations, and birth dates were estimated (± 7 days) based on the appearance of cubs when they were first observed (Holekamp et al., 1996). Cub sex was determined from the dimorphic shape of the glans of the erect phallus (Frank et al., 1990).

Data collection

Repeated focal animal samples (Altmann, 1974), lasting on average 27 ± 2.11 minutes, were conducted on individual hyenas ranging in age from less than 1 month to adulthood. All occurrences of play were recorded during focal sampling. Ontogenetic data were collected between June 15, 1988 and August 14, 1998 from 24 males and 20 females, including 13 singletons and 31 animals from twin litters. Most cubs were sampled biweekly between birth and 30 months of age, with an average of 2.05 ± 0.21 hours of focal animal sampling for each individual during every two-month age interval. A total of 691 focal sampling hours were included in this component of the study. Den-dwelling cubs spend much of the day underground, typically entering the den two to three hours after sunrise and emerging again in the late afternoon, approximately two to three hours before sunset. Behavioral data were therefore collected mainly during daylight hours between 0600h and 0900h, and again between 1600h and 1930h.

The same two observers (K.E.H. and L.S.) collected all data included in the 2-4 month and play-mounting analyses. However, the larger ontogenetic study, which spanned a decade, included observations made by 16 observers who usually worked in pairs. All observers were blind with respect to the hypotheses tested here, and all were trained individually by K.E.H. for several months. Observers were allowed to collect data independently only once they could consistently and accurately identify all of the study animals as well as all play behaviors described below.

Play was consistently recognized by observers based on exaggerated movements, apparent purposelessness, specific initiation postures, and the absence of either aggressive posturing or vocalizations indicative of pain inflicted when bitten by another individual. We observed five types of play behavior: social play, romping, object play, play-mounting and non-nutritive chewing. *Social play* involved multiple individuals engaged together in chasing, wrestling, jumping or chewing on one another. *Romping* was identified as exaggerated running and jumping behavior exhibited by solitary individuals. *Object play* occurred when we observed an animal running with or tugging on sticks, bones, feathers or old scraps of skin. *Play-mounting* involved one cub approaching another from behind and placing its forepaws on the back of another cub to assume a posture like that exhibited by adult males during copulation; the phallus was often erect during play-mounting, but was not a necessary condition for us to record the occurrence of this form of play. If the animal being mounted did not pull away immediately, then the mounting animal typically also exhibited thrusting behavior, but no intromission. Lastly, one additional type of object play recorded here, seen mainly in carnivores, is *non-nutritive chewing.* This is defined as biting or gnawing on objects from which little or no energy or nutrients could be derived, including plants, sticks, or old dried pieces of bone (Power, 2000; Fagen, 1981). To determine whether non-nutritive chewing might simply reflect a process similar to the teething behavior seen in human infants, we examined non-nutritive chewing here in relation to patterns of tooth eruption and replacement in hyenas.

All types of play behavior occurred in bouts lasting from a few seconds to several minutes. In order to compare rates of play and examine effects of energetic and social variables on play rates, we recorded all play behaviors as states rather than discrete events. That is, if any play of a particular type occurred within a given minute of observation, then that 60-second interval was counted as a minute of play of that type. If a particular type of play occurred in multiple shorter bouts within a single minute, this was nevertheless counted as having occurred only once. Hourly rates of play behavior of each type were calculated for every individual in each two-month age interval from birth to 30 months of age. Rates were calculated by dividing the number of minutes in which each type of play occurred by the number of minutes in which that animal was observed as a focal animal during each age interval.

Although rates of play-mounting were recorded as states for purposes of comparison with other types of play, for more detailed analyses of play-mounting we also recorded each mount by a particular individual as a discrete event, such that it could be recorded as occurring multiple times within a given minute of observation. Each occurrence of play-mounting was recorded as a critical incident (Altmann, 1974) from 21 male cubs and 15 female cubs monitored throughout the first 8 months of life during all observation sessions at dens. An observation session began when we arrived at the den, and ended when observers left the den. Observation sessions at dens ranged in duration from five minutes to several hours. Cubs included in this analysis of play mounts as critical incidents were each observed at dens for an average of 117 ± 4 hours. These data were collected during 4212 hours of observation of all cubs individually.

In order to examine potential effects on play rates of social (cohort size) and energetic variables (prey density, litter size, intralitter rank and maternal social rank) we focused exclusively on the 2-4 month age interval because this proved to be the period during which the highest rates of play were observed. Of the original 44 cubs observed in the overall ontogenetic analysis, 40 (23 males and 17 females) were included in analyses focused exclusively on the 2-4 month age interval. We used a mean of 7.00± 0.48 focal animal samples per individual in this analysis (3.58± 0.23 focal samples per individual per month), such that each individual was observed for an average of 3.36 ± 0.22 hours during the 2-4 month age interval. The total observation time used for focal animal sampling in this component of the study was 134 hours.

We evaluated dominance relationships among mothers on the basis of the direction of submissive behaviors (Holekamp & Smale, 1993); by convention, the highest ranking female was assigned a rank of one. Cubs were assigned the same social ranks as their mothers. The hierarchy was then divided into equal thirds and individuals were assigned to one of three categories; high-, mid- and low-ranking. Throughout the study period bi-weekly prey censuses were conducted by counting all ungulates within 100m of each of two 4 km transects to monitor local prey availability in the Talek area. Prey availability was categorized as either high or low at each census based on whether the count was above or below the mean prey density for the entire study period. For each individual, its cohort size was calculated as the number of cubs born within six months of its birth date. Rank relations within litters were determined both by outcomes of dyadic aggressive interactions between littermates and by nursing positions. The dominant cub within a litter usually assumes a preferred nursing position against the mother's belly whereas the subordinate cub typically nurses while lying between the mother's hind legs (Wahaj & Holekamp, 2006).

Statistical Analyses

For the overall ontogenetic analysis we used non-parametric statistics to evaluate effects of age and sex on play rates because these data were not normally distributed. Within each age interval we compared rates for males and females, using the Mann-Whitney-U test statistic, and we examined changes across all age intervals with a Kruskal-Wallis ANOVA, followed by multiple comparisons among specific intervals using Bonferroni corrections. We used a Friedman test statistic to analyze variation in play rates throughout ontogeny for individuals for which we had data from all age intervals. For each sex we examined variation in the number of play mounts exhibited during the first eight months of life using the Friedman test statistic, followed by comparisons among specific intervals using Wilcoxon Signed Ranks tests. A Spearman's correlation coefficient was calculated to examine relationships between maternal social rank and rates of play-mounting. These statistical analyses were performed in STATISTICA 6.1 (Statsoft, 2002).

To test hypotheses about the effects of social and energetic factors on rates of play during the 2-4 month age interval, we used a generalized linear model (GLIM) with binomial error distributions and a logit link function to analyze each play type. A quasi-binomial distribution was used in order to correct for overdispersion in the model, and the significance of each term was evaluated using an *F* test (Crawley, 1993). The number of minutes during which a cub was observed engaging in a particular type of play throughout the 2-4 month age interval, and the number of minutes observed in which that type of play did not occur, comprised the binomial response variable in each model. This approach analyzes the proportion of minutes in which a specific type of play was observed, but weights the analysis based on the number of minutes observed (Venables & Ripley, 1999). The same predictor variables were initially used in each model, with cohort size as a continuous predictor and sex, litter size (singleton or twin), social rank (high, mid, low) and prey availability (high, low) as categorical predictors. Each model was then simplified by eliminating any variables that did not contribute to its explanatory power. Only individuals observed for at least 30 minutes during the 2-4 month age interval were included in this analysis. The GLIM analysis was conducted using R software version 2.1.1 (R Core Development Team, 2005).

Finally, to evaluate the effect of intra-litter dominance on rates of play during the 2-4 month age interval we performed Wilcoxon matched pairs analyses between dominant and subordinate littermates. Differences between groups were considered significant when $\alpha<0.05$ after Bonferroni correction, and mean values are presented as ± SE throughout.

RESULTS

Ontogenetic variation in rates of play

Social play was the type of play behavior most frequently observed, occurring on average during more than 12 minutes in each hour observed during the 2-4 month age interval (Figure 4.1a). The overall mean rate of social play between birth and 30 months of age was 4.50 ± 0.38 min/hr, but rates peaked during the second two-month age interval, and then declined (Figure 4.1a, Kruskal-Wallis H (18,268), F = 121.6, p < 0.00001). As we did not manage to sample every cub in every age interval, we performed a repeated-measures ANOVA on only the subset of cubs (n =13) sampled in every interval from 0-20 months, and we observed a pattern of results identical to that in our original analysis (Friedman test statistic = 41.46, Kendall coefficient of concordance = 0.39, df = 8, p < 0.00001). We found no sex difference in rates of social play (Mann-Whitney U = 204, p = 0.40). Interestingly, social play continued into adulthood at hourly rates much higher than those observed for any other form of play (Figure 4.1).

Romping occurred, on average, during 1.61 ± 0.20 min/hr between birth and 30 months of age. The highest rates of romping occurred during the first two months of life ($\bar{x}$ = 7.83 ± 1.97 min/hr), and dropped to near-zero values after 6 months of age (Figure 4.1b, Kruskal-Wallis H (18,268), F = 155.04, p < 0.00001).

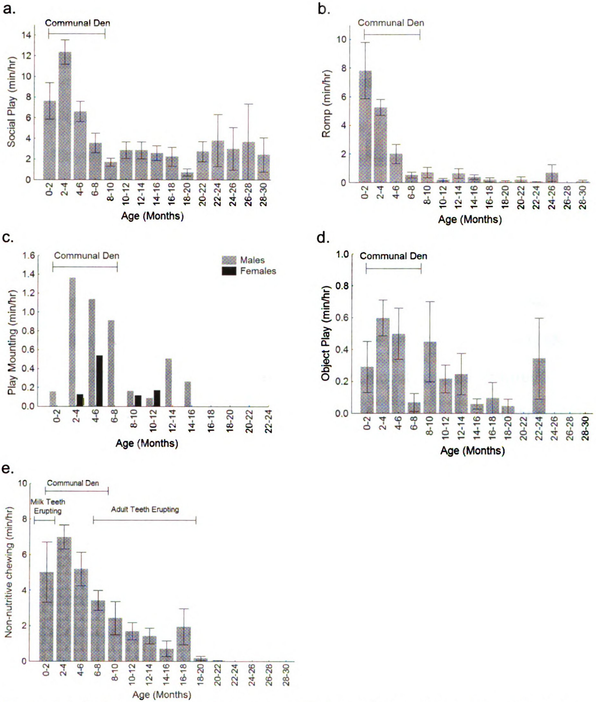


Figure 4.1. Ontogenetic variation in rates of (a) social play, (b) romping, (c) play mounting, (d) object play and (e) non-nutritive chewing in spotted hyenas. Age classes represent two month intervals (eg., 0-2 month interval includes individuals from 0.1 months to two months of age). Because no significant sex differences were found in other forms of play, separate rates for males and females are shown only in “c”. Horizontal bars indicate the period during which cubs live at the clan’s communal den on all graphs and on (e) periods of tooth eruption. Note that y-axis scales vary considerably.

Analysis of the subset of cubs sampled in every age interval produced the same pattern (Friedman test statistic = 46.98, Kendall coefficient of concordance = 0.45, df = 8, $p < 0.00001$). We found no sex difference in rates of romping (Mann-Whitney U = 190.5, p = 0.24).

Play-mounting was the only type of play for which we found a significant sex difference (Figure 4.1c, Mann-Whitney U = 94, p = 0.0005), with males engaging in this activity at much higher rates than females. Play-mounting occurred at lower rates throughout development than did either social play or romping (males: $\bar{x}$ = 0.30 ± 0.07 min/hr; females: $\bar{x}$ = 0.05 ± 0.02), but there was significant variation among age classes (Figure 4.1c, Kruskal-Wallis H (18, 268), F= 66.78, $p < 0.00001$). For males, a peak in mounting rates occurred during the second age interval, at 2-4 months of age (Figure 4.1c). Mounting behavior vanished from the play repertoire much earlier than did other forms of play; it was never observed after 12 months of age among females, nor after 16 months of age among males. Male spotted hyenas pass through puberty at around 24 months of age (Holekamp & Smale, 1998b).

Object play occurred at the lowest rates of any play type observed ($\bar{x}$ = 0.25 ± 0.03 min/hr). Although we observed significant variation among age classes in rates of object play for all individuals, and also for those individuals sampled in every age interval (Figure 4.1d, Kruskal-Wallis H (18, 268), F= 148.34, $p < 0.00001$; Friedman test statistic = 25.96, Kendall coefficient of concordance = 0.25, df = 8, $p < 0.0011$), the ontogenetic pattern in object play was less clear than for other forms of play. Rates of object play appeared to be largely determined by availability of suitable objects in the immediate environment of each focal cub at the time it was sampled. We found no significant difference between males and females in rates of object play (Mann-Whitney $U = 204$, $p = 0.39$).

After social play, the mean rate of non-nutritive chewing was the highest of the various types of play observed between birth and adulthood ($\bar{x} = 2.61 \pm 0.24$ min/hr), with cubs 2-4 months old spending a mean of seven minutes of every hour observed engaged in this activity. We documented significant ontogenetic variation in rates of non-nutritive chewing (Figure 4.1e, Kruskal-Wallis H (18, 268), $F = 148.34$, $p <0.00001$; Friedman test statistic = 39.53, Kendall coefficient of concordance = 0.38, df = 8, $p < 0.00001$). Chewing rates were highest between two and four months of age ($\bar{x} = 6.98 \pm 0.68$ min/hr), and then declined to nil by 24 months of age.

To inquire whether higher rates of non-nutritive chewing reflected teething, we examined rates of this form of play in temporal relation to eruption of both deciduous (milk) teeth and adult dentition. Although *Crocuta* cubs are born with fully erupted deciduous incisors and canines (Frank et al., 1991), the deciduous cheek teeth erupt during the first several weeks after birth, and this process is complete by two months of age (Figure 4.2). There is then a four-month hiatus before the adult teeth start to erupt at six to seven months of age, followed by a period during which both milk and adult teeth are present concurrently. The adult teeth are fully erupted by 18 months of age (Van Horn et al., 2003).

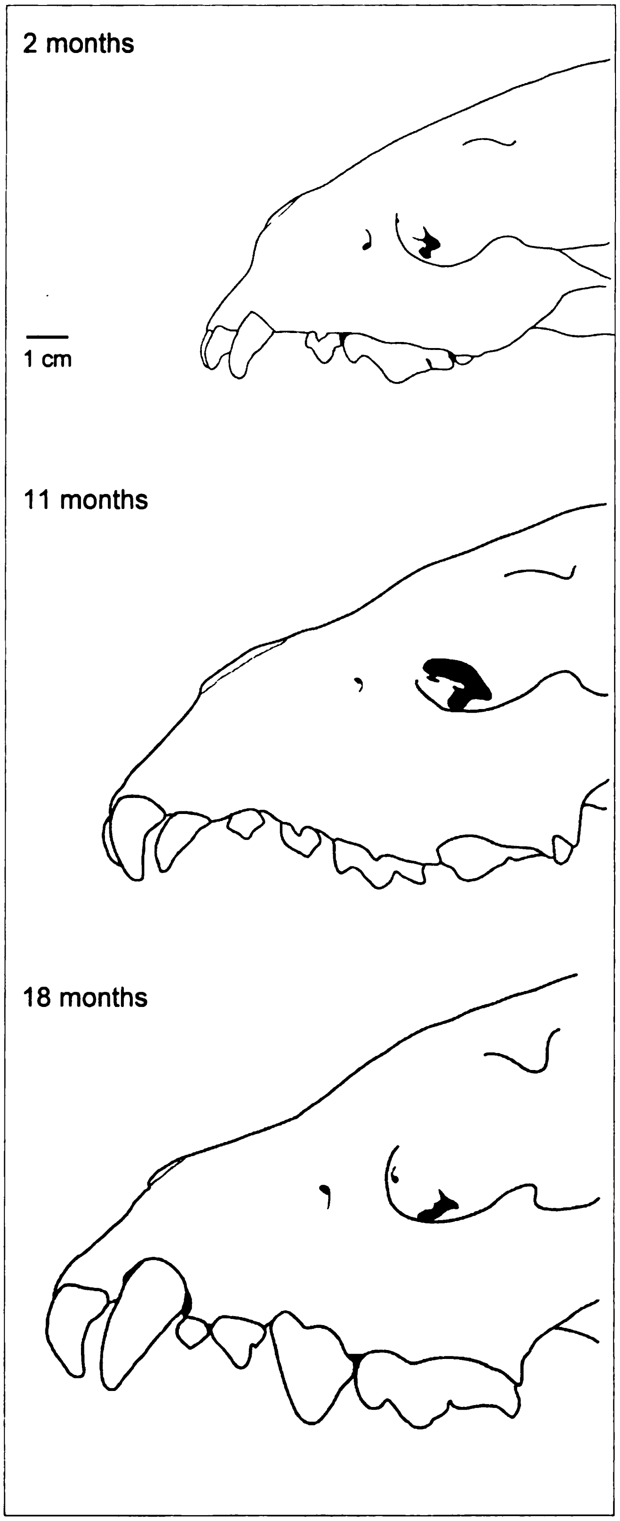


Figure 4.2. The eruption of the deciduous dentition (shaded) is complete by two months of age. Adult teeth (white) begin to erupt around six months but are not fully erupted until 18 months of age.

The highest rates of non-nutritive chewing occurred during the period (2-4 months of age) between eruptions of deciduous and adult teeth; rates declined significantly before initial eruption of the adult teeth (Figure 4.1e, multiple comparisons with corrected p-values: 2-4 months vs. 1-2 months, $p < 0.000001$; 2-4 months vs. 4-6 months, $p < 0.00001$; 2-4 months vs. 6-8 months $p < 0.0001$). This pattern was largely inconsistent with the hypothesis that this behavior represents teething. Furthermore, non-nutritive chewing was observed, albeit at low rates, at 18-22 months of age, after all adult teeth have fully erupted (Figure 4.1e).

Analysis of play mounts as events

Mean rates of play-mounting were significantly higher among males than females during all but the youngest age interval (Figure 4.3a), during which 14 of 15 females exhibited no mounting at all but one female engaged in this behavior at an extremely high rate. Interestingly, as an adult, this female (RV) never reproduced and appeared to be infertile, so may have been exposed *in utero* to an unusually high concentration of androgens relative to other females. Yalcinkaya et al. (1993) suggested that early exposure of female spotted hyenas to androgens appears to severely modify ovarian histology, and it may be that this occurred in RV and impaired such physiological processes essential for female reproduction as oogenesis or ovulation. Among females, rates of play-mounting were generally low, and did not vary significantly with age (Friedman

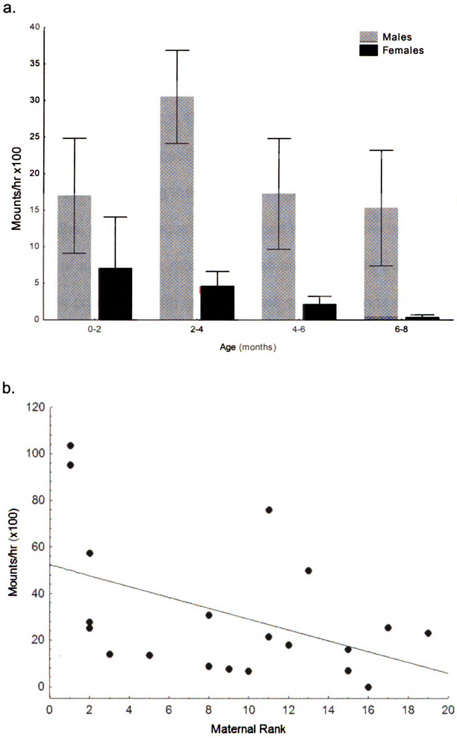


Figure 4.3. Rates of play-mounting when individual mounts were recorded as events a) among males and females throughout the first eight months of life, b) among males between 2-4 months of age, as a function of their mothers' social ranks.

test statistic= 4.220, Kendall coefficient of concordance= 0.094, df=3, $p > 0.05$; Figure 4.3a).

Among males, rates of play-mounting varied significantly with age (Friedman test statistic = 15.857, Kendall coefficient of concordance = 0.252, $p < 0.001$; Figure 4.3a). Specifically, play-mounting rates were relatively low from 0-2 months, peaked during the 2-4 month age interval, decreased between four and six months, and subsequently remained relatively low between six and eight months of age. Finally, mounting rates among males 2-4 months of age were positively correlated with maternal rank such that sons of high-ranking females engaged in this behavior at significantly higher rates than did sons of low-ranking females (Figure 4.3b; Spearman's R= 0.389, F= 4.306, $p < 0.05$).

Modulating influences on play at 2-4 months of age

Table 4.1 presents mean rates of play of each type during the 2-4 month age interval, and shows how mean values for each play type were affected by each categorical predictor variable. Table 4.2 summarizes results from analyses using generalized linear models, indicating which independent variables had significant or near-significant effects on rates of play of each type. Because play-mounting was strongly sexually dimorphic, we included only males in the 2-4 month GLIM of this type of play. There was a trend for more romping to occur among males than females, but sex was not a significant predictor of romping rates (Table 4.2, GLIM, $F_{1,38} = 5.29$, p= 0.09), nor was any other independent variable included in the final model for this form of play.

Table 4.1. Mean rates (min/hr) and SE by categorical predictor of each play type for *Crocuta* cubs 2-4 months of age. Significant ($p < 0.05$) predictors from generalized linear models are highlighted in bold font.

Play Type	$\bar{x}$	Sex		Litter Size		Social Rank			Prey	
		Male	Female	Singleton	Twin	High	Mid	Low	High	Low
Social	12.5±1.1	13.4±1.7	11.3±1.2	13.4±1.5	12.2±1.4	**15.8±2.3**	9.8±1.4	11.5±1.4	8.8±2.0	**13.5±1.2**
Chew	6.9±0.7	7.3±0.9	6.3±0.9	8.8±1.62	6.2±0.8	7.5±1.3	6.1±1.2	6.9±1.2	6.6±2.0	7.9±0.7
Romp	5.5±0.6	6.5±0.9	4.1±0.5	6.6±1.15	5.1±0.6	6.9±1.2	4.5±0.9	4.8±0.7	4.8±1.0	5.6±0.7
Mount	0.9±0.2	**0.9±0.2**	0.1±0.0	1.2±0.45	0.9±0.3	1.3±0.4	0.9±0.6	0.6±0.2	0.3±0.2	**1.2±0.3**
Object	0.6±0.1	0.6±0.2	0.6±0.2	**1.0±0.34**	0.5±0.1	0.6±0.3	0.5±0.2	0.7±0.2	0.6±0.6	0.8±0.1

Table 4.2. Factors associated with rates of play in 2-4 month old spotted hyena cubs (n=40), identified in generalized linear models. Only significant terms (*), and terms that indicate trends, are presented.

Play Type	Effect	Model Term	*F*	df	*p*
Social	Low > High	Prey Category	4.79	1,32	0.04 *
	High > Mid & Low	Rank category	3.27	2,36	0.05 *
	Larger > smaller	Cohort size	3.23	1,38	0.08
Romp	M > F	Sex	5.29	1,38	0.09
Play Mount (males only)	Low > High	Prey Category	5.29	1,18	0.03*
Object	Singleton > Twin	Litter Size	4.71	1,38	0.04*
Non-nutritive Chewing		All N/S			

Singleton cubs engaged in object play at higher hourly rates than did cubs from twin litters (Tables 4.1 & 4.2, GLIM, $F_{1,38} = 4.71$, $p = 0.04$), but litter size did not appear to affect rates of any other play type. None of the independent variables examined here had any appreciable influence on rates of non-nutritive chewing during the 2-4 month age interval. Similarly, intralitter rank appeared not to affect rates of any form of play, as we found no significant effects of this variable (Wilcoxon Matched Pairs, *social play*: n = 15, T = 55, p = 0.78; *romp*: n=15, T= 53, p = 0.69; *object play*: n=15, T = 19, p = 0.12; *non-nutritive chewing*: n=15, T = 47, p = 0.73). Maternal dominance status had a significant effect on rates of social play, with high-ranking cubs engaging in more play than either mid- or low-ranking animals (Figure 4.4; Table 4.2, GLIM, $F_{2,36} = 3.27$, $p=0.049$). Unexpectedly, social play occurred more frequently during periods of low prey availability than during periods when prey were abundant (Tables 4.1 & 4.2, GLIM, $F_{1,32} = 4.79$, $p=0.04$). Similarly, rates of play-mounting among males 2-4 months of age were higher during low than high prey periods (Table 4.2, GLIM, $F_{1,18} = 5.29$, $p = 0.03$). We observed a trend towards higher rates of social play in cohorts of large size (Table 4.2, GLIM, F 1, 38 = 3.23, p = 0.08).

DISCUSSION

Play appears to be a significant component of the behavioral repertoire of wild spotted hyenas, particularly during early development. Drea et al. (1996) examined the emergence of play behavior in captive *Crocuta* cubs from twin

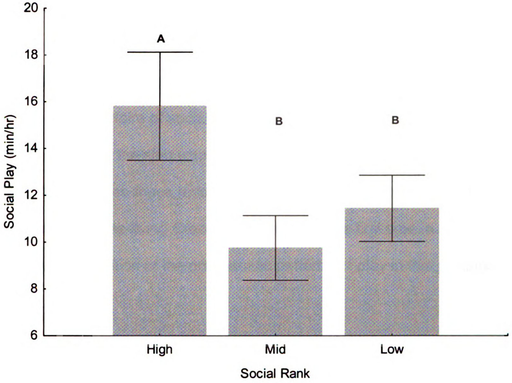


Figure 4.4. Rates of social play among cubs 2-4 months old in relation to maternal social rank category. Letters indicate significant difference among maternal rank categories.

litters in the first four weeks of life, a period during which wild cubs are seldom seen above ground, and found that rates of social play increased over these first few weeks. These authors hypothesized that early social play promotes the development of a repertoire of social behavior that prepares young cubs to become integrated into the clan when they are moved to the communal den (Drea et al., 1997). When these results from captivity are combined with the data presented here from free-living *Crocuta*, we have, for the first time, a comprehensive description of the ontogenetic patterns of play in this gregarious carnivore.

Factors modulating rates of play

Our results were mixed with respect to the potential effects of availability of energy and social partners on rates of play by hyena cubs. We documented a trend toward higher rates of social play among cubs that grew up with the largest cohorts of peers. This suggests, but cannot confirm, that partner availability influences patterns of play in spotted hyenas. Similarly, although the influence of available energy on play rates was suggested by our finding that rates of all five play types were higher in singletons than in twins, none of these differences were statistically significant. Cubs of the highest-ranking females engaged in social play at significantly higher rates than did their lower-ranking peers (Figure 4.4). This result is consistent with the notion that energy availability affects play rates, but an alternative explanation is that it reflects a preference among clan members for high-ranking play partners. Indeed, although partner preferences have not yet been examined in young hyenas, adult females prefer to associate with high-ranking females (Holekamp et al., 1997). More detailed analysis of play initiation will be needed to evaluate kin and rank-related partner preferences during play among free-living *Crocuta*.

We found that prey density was actually inversely related to rates of play among hyenas. Although this was surprising, there are at least two possible explanations. First, prey availability is relatively high throughout the year in the Masai Mara National Reserve (Ogutu & Dublin, 2002), where our Talek study site was situated. It is therefore possible that, even during periods of low prey abundance, energy resources are sufficient to support high rates of play among Talek cubs. Indeed, fluctuations in prey abundance do not appear to affect cub survival in this study population (Watts, 2007). Alternatively, our time frame for looking at energy availability may have been too broad, and may therefore have obscured relationships between food intake and play. A finer-grained analysis of play in temporal relation to nursing bouts for individual cubs might reveal a different pattern from that observed here. Nevertheless, neither of these explanations could account for our observation of significantly higher rates of play during low than high prey periods.

Some studies of other species have reported higher rates of play during periods of low than high food availability (Martin & Bateson, 1985; Loranca et al., 1999; Palagi et al., 2004; Palagi et al., 2006). It has been suggested that play under these conditions may function to reduce stress associated with competition for limited food resources which might conceivably be the case among Talek hyenas. However, this seems unlikely among hyenas because, when we observe den-dwelling cubs who are energetically stressed after their mothers die, play is one of the first activities to disappear from their behavioral repertoire. Lastly, it is possible that female *Crocuta* spend more time in search of food during periods of prey scarcity, and less time at the den, than when prey are abundant. Therefore, increased rates of social play during the former periods might reflect more "free" time available to cubs lacking opportunities to nurse. This pattern appears to occur in juvenile vervet monkeys (*Cercopithecus aethiops*) in which play rates are elevated when mothers are absent (Govindarajulu et al., 1993). We are currently examining the relationship between prey availability, den attendance and other aspects of maternal care (Stricker et al., unpublished data).

Possible functions of play in spotted hyenas

Although our goal was not to test specific hypotheses about the functions of each type of play, the ontogenetic patterns we observed here suggest some ways in which play might enhance fitness in spotted hyenas. In the wild, social play occurred more frequently and continued until much later in life than did any other form of play (Figure 4.1). The highest rates of social play occurred when cubs were 2-4 months old, during which time individuals engaged in vigorous social play for roughly 20% of each hour observed. This pattern suggests that the benefits derived from this form of play must be considerable in order to outweigh the energetic costs associated with it. These high rates of social play were observed shortly after cubs were brought to the clan's communal den, when

Crocuta cubs must learn not only the identities of their group members, but also their own places in the social hierarchy (Holekamp & Smale, 1993).

The ontogenetic pattern observed here suggests that social play facilitates the acquisition of social knowledge and integration into the clan (Gomendio, 1988). It seems unlikely that adults would continue to engage in this activity if no benefits were accruing from it. Indeed, the persistence of social play behavior from the natal den into adulthood suggests that the benefits from this form of play probably continue to accrue long after cubs become independent of the den. Although adults were observed playing away from the den, their play rates may largely reflect interactions with den-dwelling cubs when older focal individuals returned to the den to visit. It may be that the older individuals are gaining information about new members of the clan by playing with them, or that they are honing their own parental skills during their extensive playful interactions with young cubs.

Rates of romping were highest during the first two months of life, but subsequently decreased. The first months of life are critical for developing locomotor skills, and it is likely that romping enhances the development of coordination and movement in very young cubs, as predicted by the 'motor training hypothesis'(Spinka et al., 2001). The gradual decrease in romping after two months of age may also reflect a transition from individual play to social play that accompanies transfer from the natal den to the communal den between two and five weeks of age. That is, to the extent that play facilitates development of coordination and other motor skills, these benefits may be attained mainly from vigorous social play, rather than solitary romping, after the first few weeks of life.

Social play, play-mounting and non-nutritive chewing all reached their peaks during the 2-4 month age interval, shortly after cubs were moved to the clan's communal den. However, these rates of play were not merely a response to living at the communal den, as they dropped to adult levels before cubs became independent of the den. Furthermore, captive cubs play at high rates during the first few weeks of life, even in the absence of a communal den (Drea et al., 1996). If these forms of play facilitate acquisition by cubs of new information about their social and physical environments, the fact that play rates drop even before wild cubs leave the den suggests that the ability to capture new information via playful interactions is declining. We found that solitary object play occurred quite infrequently throughout ontogeny, but the rarity of this play type may merely reflect a shortage of suitable objects ("toys") at the den, or the abundance of playmates there.

Non-nutritive chewing is not likely to be primarily associated with tooth eruption or alveolar development in spotted hyenas, as the highest rates of this form of play were observed during an age interval when no tooth eruption is occurring (Figure 4.1e). Additionally, the skull and jaw muscles of a spotted hyena continue to develop even after 32 months of age (Tanner, unpublished data), whereas rates of non-nutritive chewing drop dramatically after 18 months, and this behavior is no longer observed after 22 months of age. Although we are unable to rule out the possibility that non-nutritive chewing might stimulate musculo-skeletal development early in life, its occurrence certainly does not correspond well with the period of most pronounced change in the morphology of the feeding apparatus. Another possible function of non-nutritive chewing is that it allows individuals to explore their immediate environment. Spotted hyenas, like other cursorial carnivores, are limited in their abilities to manipulate objects with their forelimbs when compared with primates or even with more dexterous carnivores such as bears or raccoons (Glickman & Sroges, 1966). Hyenas may therefore rely heavily on their mouths for gaining information about objects encountered in their environments. Thus it is not surprising that the rates of this activity are highest after individuals have moved to a new environment when they are transferred to the clan's communal den.

Sex differences in play and their implications

The variation observed here among the different types of play suggests that the mechanisms underlying their development might also vary. Although we found that rates of play-mounting were higher in male than female hyena cubs, rates of social play did not differ between the sexes. By contrast, in many other mammalian species including humans, males typically engage in higher rates of both of these forms of play than do females (Table 4.3). In a study of captive spotted hyenas, Pedersen et al. (1990) found that females engaged in more social play than males when they were monitored in same-sex groups, but these investigators observed no sex differences when youngsters were in mixed-sex groups (Pedersen et al., 1990). The results from the mixed-

Table 4.3. Sex differences in play behavior in various mammal species. Female-dominated species are indicated in bold font.

	Species	**Social / Rough-and-Tumble Play**	**Sex Play**	**Source**
Carnivores	**Spotted hyena (wild)**	M = F	M > F	Present study
	Spotted hyena (captive)	M < F (same sex groups) M = F (mixed sex groups)	----	(Pedersen,J., et. al 1990)
	Domestic cat (*Felis cattus*)	M = F	----	(Barrett, P. & Bateson, P. 1978)
	Coyotes, wolves and Beagles (*Canis spp.*)	M = F	M > F	(Bekoff, M., 1974)
	Ferret (*Mustela furo*)	M = F	M > F	(Biben, M., 1982; Stockman, et. al 1986)
	Galapagos fur seal (*Arctocephalus galapagoensis*)	M > F	----	(Arnold, W. & Trillmich, F., 1985)
Rodents	Belding's ground squirrel (*Spermophilus beldingi*)	M = F	M > F	(Nunes, S. et. al, 1999; 2004)
	Richardson's ground squirrel (*Spermophilus richardsonii*)	M = F	M > F	(Pasztor, T. J., et. al, 2001)
	Golden Hamsters (*Mesocricetus auratus*)	M > F	----	(Vieira, M. L. et. al, 2005)
	Rats (*Rattus norvegicus*)	M > F	----	(Pellis, S. M., 2002)
Ungulates	Cuvier's gazelle (*Gazella cuvieri*)	M > F	M > F	(Gomendio, M., 1988)
	Lambs (*Ovis aries*)	M > F	M > F	(Orgeur, P., 1995)
Primates	Japanese monkey (*Macaca fuscata*)	M > F	----	(Koyama, N., 1985)
	Rhesus Macaque (*Macaca mulatta*)	M > F	M > F	(Goy, R. W. et. al 1988; Wallen, K., 1996)
	Ring-tailed lemur (*Lemur catta*)	M = F	M > F	(Gould, L., 1990)
	Humans (*Homo sapiens*)	M > F	----	(Pellegrini A. D. & Smith, P. K., 1998; Scott & Panksepp, 2003)

sex groups in captivity are most easily comparable to those from the current study, as both male and female cubs were present at communal dens in the wild. In a study on another female-dominated species, the ring-tailed lemur (*Lemur catta*), Gould (1990) similarly found no sex differences in social play, but did observe dimorphic sex play (Table 4.3).

The lack of sexual dimorphism in social play is intriguing given the sex-role reversal seen in both lemurs and spotted hyenas with regard to social dominance. A popular hypothesis for the function of social play in general, and rough-and-tumble play in particular, is that it serves as practice for aggressive encounters in adulthood (Maestripieri & Ross, 2004; Spinka et al., 2001; Smith, 1982). Therefore, the higher rates of rough-and-tumble play observed among the males of many other mammalian species have been attributed to the male's greater need to practice for male-male combat in adulthood (Pellis et al., 1997; Smith, 1982).

Although overall rates of aggressive behavior are higher among adult female than male *Crocuta* (Szykman et al., 2003), both sexes engage in considerable aggression, particularly in the context of intrasexual interactions during feeding competition, and when members of neighboring clans encounter each other. Therefore, the absence of a sex difference in social play within female-dominated species such as spotted hyenas and ring-tailed lemurs is consistent with the hypothesis that social play provides practice for agonistic encounters in adulthood. However, sexual monorphism in social play has also been found in some other carnivore species studied in captivity, including cats, ferrets and various canids, all of which hunt terrestrial vertebrate prey (Table 4.3). Therefore, rough-and-tumble play among young carnivores may provide practice for skills necessary in pursuing and capturing prey as well as for adult combat; presumably, development of these skills would be just as important for females as for males in predatory species (Fagen, 1981).

Copulation represents an unusually difficult challenge for the adult male spotted hyena (Drea et al., 2002). First, he must be tolerated by the large, socially dominant and highly aggressive adult female. Then he must insert his erect phallus into her flaccid one, which is positioned far more anteriorly than is the vaginal opening in other carnivore species. To achieve successful mating in adulthood, the male hyena may thus need practice earlier in life, and the communal den may offer the male his only opportunity for such practice. We found that male hyena cubs engaged in play-mounting at rates considerably higher than those exhibited by females (Figure 4.3a). Dimorphic sex play is seen among juveniles of a variety of mammalian species (Table 4.3), and these sex differences are promoted by differential exposure to testosterone earlier in development (Goy, 1996).

Both male and female *Crocuta* cubs are exposed to androgens late in gestation (Yalcinkaya et al., 1993; Licht et al., 1998). Although prenatal treatment with anti-androgens can de-masculinize certain aspects of the genitalia and nervous system of the spotted hyena (Drea et al., 1998; Drea et al., 2002; Forger et al., 1996; Fenstemaker et al., 1999), it is not currently known whether, or to what extent, such treatment also affects the sexually dimorphic patterns of play observed here among young hyenas. Three mechanistic hypotheses might account for the patterns observed here. First, sex differences in play may be androgen-independent in this species, although this seems unlikely because it would make spotted hyenas unique among mammals in this regard. Second, it may be that the critical period for sensitivity of key neural substrates to testosterone does not occur during prenatal development in this species, but instead occurs during the first month after birth, when testosterone concentrations are higher in male than female cubs (Frank et al., 1991). Third, there may be a sex difference in prenatal testosterone exposure or receptor density in this species that has not yet been detected.

The rates of play-mounting observed here were affected by maternal rank as well as by offspring sex. Specifically, at 2-4 months of age, sons of high-ranking mothers mounted other cubs at higher rates than did sons of lower-ranking mothers. Interestingly, this pattern was apparent before the ages at which maternal rank begins to influence dominance relations among cubs (Holekamp & Smale, 1993). Mounting rates among young male *Crocuta* are correlated with maternal androgen concentrations late in gestation, and these, in turn, vary with maternal rank such that sons of high-ranking females are exposed to higher androgen concentrations *in utero* than are sons of low-ranking females (Dloniak et al., 2006).

Conclusions

The patterns of development described here are consistent with the idea that some forms of play confer immediate benefits whereas other forms confer benefits later in life. Here it appeared that most benefits conferred to hyenas by romping and non-nutritive chewing were probably largely immediate, whereas those conferred by play-mounting were most likely delayed until later in life; furthermore, it appeared that social play might confer both immediate and delayed benefits. The five types of play we documented in hyenas pale in comparison to the broad array of human behaviors that fit the definition of play adopted here (from Bekoff & Byers 1981). Nevertheless, as appears to be true of hyena play, some forms of human play may help children cope with specific challenges encountered during one or more early stages of development, whereas others may have long-lasting consequences for individual welfare in adulthood. Just as social play may help young *Crocuta* cubs become integrated into the clan, there also appears to be a relationship between rough-and-tumble play in male children and measures of social competence (Pellegrini, 1995). Although it is not known to what extent play reorganizes the individual's behavioral phenotype, anecdotal evidence from humans indicates that early play deprivation is often closely associated with pathological behavior in adulthood (Brown, 1998). This suggests that play importantly affects structural or functional aspects of brain development that enhance the individual's ability to cope effectively with environmental complexity in general, and with specific stressors in particular (Siviy, 1998).

Finally, the patterns observed here highlight the fact that mammalian play must be heterogeneous in its regulatory mechanisms as well as in its form and function. We observed a great deal of variability among the different forms of hyena play in regard to their patterns of occurrence during development. This suggests that the genetic and neural substrates regulating expression of one form of play are not necessarily the same as those regulating expression of other forms. For example, we found that patterns of temporal variation differed strikingly between non-sexual social play and play mounting. Furthermore, we found that one of these two forms of social play, both of which are sexually dimorphic in many other mammals, is 'masculinized' in female spotted hyenas (non-sexual social play) but the other (play mounting) is not. These data suggest that genetic mechanisms and neural circuits regulating developmental patterns differ between these two forms of social play. Although the specific motor patterns involved and the exact patterns of sex differences in hyena and human play are distinctly different, overall it appears that the general principles concerning the functions of play, as well as the heterogeneous nature of the mechanisms regulating its development and its expression, may be surprisingly similar in these two distantly related species.

APPENDIX

Table A.1. List of MSU skull specimens used in Chapter Two.

Catalog Number	Age (mos)	Catalog Number	Age (mos)
36093	2.00	36558	57.19
36089	4.37	35857	61.83
36088	4.40	36074	62.40
36087	5.13	36550	63.13
36166	5.47	36168	64.39
36579	6.13	36553	65.27
36086	7.00	36160	70.04
36159	7.00	36570	80.87
36157	7.20	36163	82.97
36075	7.23	36079	83.09
36155	7.53	36569	87.80
36085	8.00	36009	91.22
35863	8.57	35854	96.57
35861	10.00	35853	96.71
36010	11.13	36165	97.40
36549	13.80	36161	102.50
36090	14.37	35856	107
36578	15.00	897BFT	107.17
36167	15.27	486ECO	113.98
36162	16.73	36083	115.20
36081	16.87	36551	117.50
36577	18.00	225VGS	119.85
799HUM	20.53	36566	120.65
35859	22.23	36580	123.65
36576	31.60	36084	125.23
36556	32.93	36008	128.87
36156	38.58	35852	133.16
36082	39.85	36094	138.36
36164	40.35	36552	140.35
35858	47.33	36011	143.83
36581	47.97	36080	148.58
36567	50.83	36571	153.00
36568	53.47	36078	157.53
35855	55.90	36077	199.73

Table A.2. Description of landmarks for each view used in Chapter Two.

Ventral Landmarks	
1	Juncture between incisors on the premaxilla
2	Premaxilla-maxilla suture there it intersects the medial edge of the canine
3	Posterior-most point of the incisive foramen
4	Posterior-most premaxilla-maxialla suture on the palate
5	Anterior-most point on the foramen magnum
6	Palatal foramen
7	Maxilla-palatine midline suture
8	Midline suture between the left and right palatine
9	Maxilla-palatine suture on the posterior edge of the palate
10	Medial edge of the maxilla-jugal suture
11	Lateral edge of the jugal-squamosal suture
12	Anterior lateral edge of the glenoid fossa
13	Medial edge of the glenoid process
14	Medial edge of the jugular foramen
15	Anterior edge of the external auditory meatus
16	Lateral and posterior edge of the basisphenoid

Lateral Landmarks	
1	Anterior-most upper edge of the I3
2	Anterior-most upper edge of the canine
3	Posterior-most upper edge of the canine
4	Upper edge of the infraorbital foramen
5	Upper edge of the lacrimal foramen
6	Uppermost tip of the zygomatic process
7	Upper edge of the jugal-squamosal suture
8	Ventral edge of the jugal – squamosal suture
9	Poster-most edge of the pterygoid
10	Anterior-most upper edge of the external auditory meatus
11	Anterior-most upper edge of the occipital condyle
12	Posterior edge of the nuchal crest
13	*Ray on the dorsal edge of the cranium
14	*Ray on the dorsal edge of the cranium
15	*Ray on the dorsal edge of the cranium
16	*Ray on the dorsal edge of the cranium
17	Tip of the post-orbital process
18	Anterior-most edge of the nasal-premaxilla suture
19	Posterior-most edge of the premaxilla-jugal suture

Mandible Landmarks	
1	Anterior edge of I4
2	Anterior edge of the canine

3	Posterior edge of the canine
4	Upper edge of the mental foramen
5	Dorsal apex of the curve on the coronoid process?
6	Posterior edge of the coronoid process
7	Anterior edge of the mandibular condyle
8	Posterior edge of the mandibular condyle
9	Dorsal tip of the articular process
10	Posterior edge of the mandibular symphysis
11	Dorsal edge of the masseteric fossa
12	Ventral apex of the curve of the dentary
13	* Semi-landmark
14	* Semi-landmark
15	* Semi-landmark
16	* Semi-landmark
17	* Semi-landmark
18	* Semi-landmark
19	* Semi-landmark
20	* Semi-landmark
21	* Semi-landmark
22	* Semi-landmark
23	* Semi-landmark
24	* Semi-landmark
25	* Semi-landmark
26	* Semi-landmark
27	* Semi-landmark
28	* Semi-landmark
29	* Semi-landmark
30	* Semi-landmark
31	* Semi-landmark
32	* Semi-landmark
33	* Semi-landmark
34	* Semi-landmark
35	* Semi-landmark
36	* Semi-landmark
37	* Semi-landmark
38	* Semi-landmark
39	* Semi-landmark

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