EVOLUTION AND DEVELOPMENT OF MORPHOLOGICAL, ALLOMETRIC, AND LIFE HISTORY PATTERNS IN THE SPOTTED HYENA (*CROCUTA CROCUTA*)

By

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ABSTRACT

EVOLUTION AND DEVELOPMENT OF MORPHOLOGICAL, ALLOMETRIC, AND LIFE HISTORY PATTERNS IN THE SPOTTED HYENA (*CROCUTA CROCUTA*)

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My dissertation focuses on how maternal and ecological factors influence growth rate variation, the life history consequences of growth variation, and on the evolution and development of 'reversed' sexual size dimorphism (SSD) in spotted hyenas. My dissertation consists of four chapters following the introductory chapter. The first of these research chapters, Chapter 2, describes the ontogeny of morphological sexual dimorphism in spotted hyenas, and documents sex differences in rates of growth of overall both body size as well as growth rates of individual morphological traits. Chapter 3 documents the importance of litter size, intra-litter rank, maternal parity, prey availability, and maternal social rank on variation in growth rates and adult size, and the resulting life history and fitness consequences of variation in growth rates. Chapter 4 focuses on whether selection on overall body size or on specific components of body size is present among female hyenas using lifetime measures of fitness to identify putative evolutionary mechanisms maintaining the observed dimorphism. Finally, in Chapter 5 I address hypotheses for the role of selection in males and females as drivers of degree of sexual dimorphism. The hypotheses I test include that selection on size in males does not contribute to female-biased SSD, that smaller males have better reproductive success, and finally that both males and females have increased reproductive success with increasing size, but the fitness benefit of increasing size is greater for females. I also address the quantitative genetics of morphological traits by

estimating heritability; non-zero heritability is necessary for an evolutionary response to the observed selection. In its entirety, my dissertation results are consistent with the hypothesis that the difference in selection estimates between males and females contributes to the maintenance of sexual size dimorphism in the spotted hyena.

On the whole, the results of the analyses in my dissertation represents an important addition to our knowledge of morphology and life history in the spotted hyena, especially with respect to SSD. My dissertation also provides the first measures of selection using lifetime fitness in a large mammalian carnivore. Finally, my dissertation presents estimates of quantitative genetic parameters, which are rarely available for mammalian carnivores, or for large mammals in general.

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When I was a little kid I wanted to be a paleontologist. Sometimes when I consider the number of career and life detours I have taken since then, it amuses me to think of how similar my current work is to what I once imagined. Except with less dinosaurs and more time spent in front of a computer. Much of what I have now is the result of a few chance decisions that I apparently made correctly, good advice and support from a large number of people, and some hard work. I have many people to thank for the direction, advice, support, and inspiration I have received along the way, as well as the friendship that has helped me enjoy the journey.

In September 2003 I started my undergraduate research in the Ecology, Evolution and Behavior department at the University of Minnesota. Jacques Finlay, Carolyn Silflow, and Pete Lefebvre put up with my sometimes poor, and once dangerous (to me), skills as a laboratory assistant, and in Matthew Lavoie I found a boss who was also a friend to chat with while I should have been working. Throughout the first two years of my undergraduate work I explored a number of research interests, then in my junior year I took classes taught by Sharon Jansa, Anne Pusey, and Dave Stephens. Initially, it was in these courses that I became interested in evolutionary ecology. I began working with Sharon in her lab, and one day I asked Anne about opportunities to do an honors' thesis and she suggested I speak to Mark Bee. While Sharon inspired much of my interest in mammals and phylogenetics, and Anne my interest in behavioral ecology and the evolution of social behavior, Mark inspired my interest in science itself. I am forever indebted to Mark for showing me how cool science can be, while also making many of the hard parts of academia seem easy, tricking me into taking the next step to graduate school. And also for not leaving me in the swamp some dark night in revenge for accidentally breaking equipment.

When I was exploring opportunities for graduate work, Sharon suggested I contact Dr. Kay Holekamp. Sharon's recommendation was, "She's really smart!". Well, when I met Kay, I agreed. Kay also was interesting to talk to, I thought that we got along well, and she seemed like someone whose love of science would inspire me. And she has. Listening to Sharon, and contacting Kay about joining her lab was definitely the best decision of my career. I can't imagine having done what I've done over the last 6 years without her help, advice, and contributions. She has taught me so much about science. And if her schedule has sometimes made me feel a little like only a crazy person would want to be a professor, she has also really opened my eyes to how much one motivated person can accomplish. I also like to think that I have gained some of Kay's sense of adventure. I am not naturally a huge risk taker. But from driving alone across Kenya in a tiny Suzuki within weeks of my first visit, to independently learning how to solve a variety of research problems, to attending my first conference to give a talk, to taking on too much work and learning to fight my way through it, Kay's simple expectation that I just go do it motivated me to try a lot of things I might not otherwise have tried, and to work hard to succeed at them. Kay has been an awesome mentor, putting up with many, many, many meetings; terrible first drafts of papers, grants, and emails; and me barging into her office frantically ranting about my newest overly complicated problem. I will always be indebted to Kay for the inspiration, motivation, mentoring, and advice with which she has provided me, and I cannot thank her enough.

One of the great things about working at Michigan State is the wide variety of great mentors available. Even attending departmental seminars exposes you to a wide range of world class scientists. I would like to thank my graduate committee members in particular for the willingness to schedule (and reschedule) both personal and committee meetings, their advice, and their contributions both to my dissertation and to my preparedness for my future career. Ian Dworkin has been an always-helpful collaborator, mentor, and graduate committee member for me while I have been here. He has always been ready with advice, and willing to help out; I owe much of my quantitative knowledge to discussions with Ian and readings he suggested. Tom Getty has been a great graduate committee member and mentor, always ready with a thoughtful question to make me think a little harder about the work I've done. Kim Scribner I'd like to thank for the time he invested contributing to my quantitative and conservation genetics knowledge in the classroom, the laboratory, and during both individual and committee meetings. All three of my graduate committee members introduced me to ideas, books, and papers that have shaped my thinking and my career in important ways. Alex Shingleton and Barry Williams, although not on my committee, have both given important advice or help at different stages of my graduate career, and I am indebted to them for their help.

I have many lab-mates and friends to thank for making my time at MSU fun, helping me to stay sane, and helping me find ways to fill the not-so-empty days. First, I would like to thank Ben Dantzer and Jenn Smith for being great friends who I can have fun with, and still be amazed by professionally. Trying to live up to the quality and quantity of their work has kept me busy for 6 years. Second, I can't imagine life in Lansing or in Kenya without the many, many lab-mates I have had over the years, including Andy Flies, Katy Califf, Kate Shaw, Leslie Curren, Andy Booms, Greg Stricker, Jenn Smith, Wiline Pangle, Ryan Taylor, Ben Dantzer, Sarah Jones, Marc Wiseman, Dave Green, Julia Greenberg, Nora Lewin, Julie Turner, Kenna Lehmann, Eli Strauss, Suzanne LaCroix, Page Van Meter, Sarah Benson-Amram, Kevin Theis, and Jaime Tanner. Wiline, Kevin, Jaime, and Page all gave my some very good advice when I first got here, Kevin especially was a source of quiet, helpful advice. I would like to thank Greg for being a great friend and roommate for so long. Julia, Leslie, Greg, and Jenn were great office mates: 'headphones' on, late night work sessions in the big office, Dave eating lunch on my couch, and Greg bothering Pat by talking too loud; good times. Andy, Katy, Kate, Leslie, Andy: my 'sort-of lab cohort', since I didn't really have one, have been a great source of both fun and hyena discussion over the years, and I can't imagine the trip without you guys. Sarah, thanks for letting me and Darwin live with you guys, and for boxing with me. Nora, Julie, Kenna, and Eli, I'm glad the lab has a solid group of new(ish) members in you guys. I have a lot of other friends at MSU who were not in my lab, but whom I would have been miserable without for the last 6 years. I especially want to mention Austin Dreyer, Carlos Anderson, Nick Testa, and Lauri Torgerson-White. Lauri, Jenn Smith, and I had an awesome trip to Alaska, making my first professional conference ever one of the most awesome and unforgettable experiences ever. Austin, Carlos, and Nick: I might have finished grad school a semester earlier if I hadn't known you guys, but it would have been super boring. I can't imagine the next step without you guys.

My family have been awesomely supportive throughout my graduate career, and in everything leading up to it. My mom and dad both taught me about hard work through their examples, even though they still both seem to be better at it than I am. Both have taught me some important lessons, at times perhaps inadvertently, about how to balance my responsibilities with my interests, and my work with my life. My step-mom Sandy and my step-dad Richard are two people who didn't have to be my parents, but are. I can't really thank my parents enough for the support and guidance they've provided to make me who I am. I like to think that none of my parents are surprised by what I've accomplished, despite the fact that there were some times in my life when it didn't seem like I'd be ever accomplish much. But, I like to think that they were just waiting for me to find something interesting enough to hold my attention. My brothers and sisters, Travis, Jessie, Emily, and Zane are perhaps the people who I've most sacrificed my time with during graduate school, mostly because they will put up with it, and things are always just as fun when we hang out again. I'd also like to thank my fiancée Kelly's family, Jay, Karen, Jenni, and Lisa, who have become part of my family over the last nine years. The unwavering support of my family has meant a lot to me.

Kelly, my fiancée, has put up with a lot during my time in graduate school. She moved to Lansing with me for two years, and for a while worked at Potbelly selling sandwiches, to keep me in my sandwich habit. I was away for months at a time, and when she started medical school, I couldn't return the favor and go with her for a long time. She came to Kenya with me, braving poisonous snakes that luckily hated Andy more than her, hippo ambushes honking in the night, trains with seat-less toilets basically falling onto the tracks, upscale but abandoned resort restaurants in which we were the sole customers, overly friendly security guards, and traveling with Andy Flies, simply to spend time with me, and see what a place I had spent so much time in was like. Kelly may not have inspired my love of science, but she has been one of the main inspirations to get me to work hard enough to actually become a scientist. I can say definitively that I would not be here today without Kelly to support me, keep me company, make me laugh, remind me of the importance of working hard for something that I believe in, and make me feel guilty by comparison.

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General Introduction

General Introduction

Life history traits are closely tied to individual fitness (Blanckenhorn, 2000, Roff, 1992, Roff, 2002, Stearns, 1992, Badyaev, 2002, Bonner, 2006, Calder, 1984, Peters, 1983), and thus represent evolutionarily important phenotypic variation. The enormous diversity of life history variation in nature is produced by processes that are as yet poorly understood. Body size and growth rate are both critical life history traits that have a variety of ecological and fitness-related effects (Bonner, 2006, Calder, 1984, Stearns, 1983, Blanckenhorn, 2005). Both adult body size and growth rate are also considered 'central' life history traits, strongly influencing the optimal values for many other life history traits (Stearns, 1992, Roff, 1992, Roff, 2002). Body size, for example, explains as much 75% of the variance in life histories (Stearns, 1983, Read and Harvey, 1989, Dobson and Oli, 2007, Gaillard et al., 1989), and this covariation is thought to result from the effects of size on other life history traits (Stearns, 1992, Roff, 1992, Roff, 2002, Bonner, 2006, Calder, 1984). For individual species, ecological factors such as mortality rates also have strong effects on growth rate and adult body size. For example, body size and growth rate respond evolutionarily to increased extrinsic juvenile mortality rates from sources such as predation (Arendt, 1997), and adult body size responds to increases in extrinsic adult mortality from similar sources (e.g. Stearns et al., 2000, Trexler et al., 1992). However, evolutionary responses to these sources of selection alter the fitness surfaces not only for body size, but for other life history traits as well, creating a complex system of direct and indirect evolutionary effects that play out through developmental processes constrained by physiology and physics.

One of the most pervasive patterns in life history and morphology is that of dimorphism between the sexes in the form of differences in overall body size, sex differences in the sizes of various traits independent of body size (often considered 'shape'), and sex differences in the timing and form of life history traits. These dimorphic patterns are generally attributed, at some level, to different fitness optima for adult body size in the two sexes, and they are of special interest in part because they occur despite a strong genetic correlation between the sexes (Fairbairn, 2007). Among animals, degree of dimorphism ranges across many orders of magnitude; in most animals females are larger than males (Andersson, 1994, Fairbairn, 2007, reviewed in Fairbairn et al., 2007), whereas in mammals and birds, males are generally larger than females (Alexander et al., 1979, Darwin, 1871, Lindenfors et al., 2007, Szekely et al., 2007, Weckerly, 1998). There are many exceptions to these rules; and although the exceptions tend to occur within certain lineages (e.g. Lagomorpha and Chiroptera; Lindenfors et al., 2007), some species exhibit dimorphism exceptional for their taxonomic position (see Ralls, 1976 for examples in mammals). Spotted hyenas (*Crocuta crocuta*) represent one of the rare members of the order Carnivora to exhibit clear female-biased sexual size dimorphism (Ralls, 1976), as well as exceptional genital monomorphism and female social dominance over males (Frank, 1986, Hamilton et al., 1986, Kruuk, 1972). Both the genital monomorphism and the size dimorphism seen in spotted hyenas are unique among extant hyaenids; adults in the other three species exhibit 'normal' mammalian genitalia and sexual size monomorphism (van Jaarsveld et al., 1995, Holekamp and Kolowski, 2009).

Summary of Dissertation

My doctoral dissertation research, conducted at Michigan State University starting in 2007, focuses on how maternal and ecological factors influence growth rate variation, the life history consequences of growth variation, and on the evolution and development of 'reversed' sexual size dimorphism (SSD) in spotted hyenas. My dissertation consists of four chapters following the introductory chapter. The first of these research chapters, Chapter 2, describes the ontogeny of morphological sexual dimorphism in spotted hyenas, and documents sex differences in rates of growth of overall both body size as well as growth rates of individual morphological traits. Chapter 3 documents the importance of litter size, intra-litter rank, maternal parity, prey availability, and maternal social rank on variation in growth rates and adult size, and the resulting life history and fitness consequences of variation in growth rates. Chapter 4 focuses on whether selection on overall body size or on specific components of body size is present among female hyenas using lifetime measures of fitness to identify putative evolutionary mechanisms maintaining the observed dimorphism. Finally, in Chapter 5 I address hypotheses for the role of selection in males and females as drivers of degree of sexual dimorphism. The hypotheses I test include that selection on size in males does not contribute to female-biased SSD, that smaller males have better reproductive success, and finally that both males and females have increased reproductive success with increasing size, but the fitness benefit of increasing size is greater for females. I also address the quantitative genetics of morphological traits by estimating heritability; non-zero heritability is necessary for an evolutionary response to the observed selection. In its entirety, my dissertation suggests that the difference in selection estimates between males and females contributes to the maintenance of sexual size dimorphism in the spotted hyena.

On the whole, the results of the analyses in my dissertation represents an important addition to our knowledge of morphology and life history in the spotted hyena, especially with respect to SSD. My dissertation also provides the first measures of selection using lifetime fitness in a large mammalian carnivore. Finally, my dissertation presents estimates of quantitative genetic parameters, which are rarely available for mammalian carnivores, or for large mammals in general.

Summary of Chapter 2

Body size and growth rate are among the most important traits characterizing an organism, influencing niche occupancy, life history patterns, mortality rates, and many other fitness components. Sexual dimorphism in size and growth is a common phenomenon in animals, and in many animal taxa females are larger than males. In most mammals, however, males are larger than females. Spotted hyenas are one of the rare mammals that have been suggested to exhibit female-biased sexual size dimorphism. However, it has been argued that the evidence is equivocal regarding this reversal. This controversy may reflect variation among studies with respect to traits measured, methodology, or the ontogenetic stages at which individuals were sampled in these studies. Here I quantified ontogenetic size change in 651 individuals, the largest sample used in analyses of spotted hyenas to date. I measured 14 morphological traits as well as 4 linear combinations of these traits that provide multivariate estimates of size; these were used to examine growth patterns for males and females measured in a free-living population in Kenya. I demonstrate that female spotted hyenas are larger than males for most, but not all traits, and that females are larger because they grow faster, rather than exhibiting a prolonged period of growth. Early in life males and females appear to grow similarly. However, between weaning and reproductive maturity their multivariate ontogenetic trajectories diverge. Traits that mature before divergence of these ontogenetic trajectories are monomorphic, whereas traits that mature later are dimorphic. Furthermore, dimorphism is generally greatest in traits that cease development latest. I suggest hypotheses explaining these results. I also assess data obtained from captive hyenas to show that female-biased sexual size dimorphism occurs even when males and females are fed identical diets, allowing us to rule out a strictly environmental explanation for this dimorphism.

Summary of Chapter 3

Morphological growth rates are critical life history traits in their own right, reflecting multivariate life history patterns interspecifically, and both genetic and environmental variation intraspecifically. Growth rates also commonly have important consequences for the timing of life history events, as well as other fitness effects within populations. I present analyses of the role of maternal social rank, litter size, maternal parity, prey availability, and intra-litter rank on growth rate and adult size in spotted hyenas using a mechanistic growth model previously used in work on spotted hyenas. In addition, I estimate size-at-age residuals from a basic growth model for individuals under 24 months, and use these as predictors of the age at which different developmental milestones will be reached. Specifically, here I look at the effect of growth rate on age at weaning, age at dispersal, and age at parturition. I present strong evidence that subordinate twins grow more slowly than either singleton cubs or dominant twins, females with higher social rank bear offspring that grow more rapidly, as do mothers that have reared more than one litter. I do not find strong support for a role of any of the ecological factors examined here in influencing adult size. Finally, I find strong support for a role of higher growth rate in advancing weaning age, and some evidence that males exhibiting higher growth rates dispersed earlier. I did not, however, find evidence for a role of early-life growth rate in influencing age at first parturition of females.

Summary of Chapter 4

Size-related traits are common targets of natural selection, yet there is a relative paucity of data on selection among mammals, particularly from studies measuring lifetime reproductive success (LRS). I present the first phenotypic selection analysis using LRS on size-related traits in a large terrestrial carnivore, the spotted hyena, which displays a rare pattern of female-biased sexual size dimorphism (SSD). Using path analysis, I investigate the operation of selection to address hypotheses proposed to explain SSD in spotted hyenas. Ideal size measures are elusive, and allometric variation often obfuscates interpretation of size proxies. I adopt a novel approach integrating two common methods of assessing size, and demonstrate lifetime selection on size-related traits that scale hypoallometrically with overall body size. Our data support selection on hypoallometric traits in hyenas, but not on traits exhibiting isometric or hyperallometric scaling relationships, or on commonly-used measures of overall body size. Our results represent the first estimate of lifetime selection on a large carnivore, and suggest a possible route for maintenance of female-biased SSD in spotted hyenas. Finally, our results highlight the importance of choosing appropriate measures when estimating animal body size, and suggest caution in interpreting selection on size-related traits as selection on size itself.

Summary of Chapter 5

Intersexual variation in size, known as sexual size dimorphism (SSD), is a phenomenon of great interest in developmental and evolutionary biology. The presence of SSD suggests that fitness landscapes differ between males and females for morphological traits, and that this difference is reflected in sex-specific regulation of the same growth genes. Among most animals, females are larger than males, but among mammals, males are generally the larger sex. Spotted hyenas exhibit a number of sex-role reversed traits rare for their taxonomic position, among them female-biased SSD. Previous work suggested that selection on some traits for increased body size in females may contribute to this pattern, but did not address selection on males. Here I document selection in males, compare it to selection in females estimated using annual reproductive success as a common measure of fitness, and estimate quantitative genetic parameters to inquire what role selection plays in the evolutionary maintenance of female-biased SSD in spotted hyenas. I demonstrate that the difference between selection coefficients for males and females is correlated with the degree of dimorphism in specific morphological traits, and is thus consistent with an evolutionary explanation for dimorphism. Heritability estimates for most traits were low to moderate in magnitude, but nonetheless represent additive genetic variance on which selection can act.

General Methods

Study subjects.

Spotted hyenas are characterized by small litter sizes, slow life histories, female-biased sexual size dimorphism and unusual genital monomorphism (Holekamp et al., 1996, Kruuk, 1972, Watts et al., 2009, Hamilton et al., 1986). Spotted hyenas live in social groups called clans, consisting of up to 90 individuals including multiple females born in the clan and their young, as well as several adult immigrant males. Each clan is structured by a strict linear dominance hierarchy (Holekamp and Smale, 1990, Frank, 1986, Smale et al., 1993), and an individual's position in this hierarchy has profound effects on both survival and reproduction by mediating differential access to food at kills (Kruuk, 1972, Watts et al., 2009, Hofer and East, 2003). Here social rank assignments were based on the outcomes of dyadic agonistic aggressions (Holekamp and Smale, 1993, Smale et al., 1993).

Female spotted hyenas are physiologically competent to breed after 24 months of age, but first parturition usually occurs in the third or fourth year of life; the timing of first parturition varies greatly with rank (Holekamp et al., 1996, Hofer and East, 2003). Female hyenas are philopatric, whereas nearly all males emigrate and join neighboring clans after reaching reproductive maturity (Smale et al., 1997, Van Horn et al., 2003), which also occurs in males by 24 months of age (Glickman et al., 1992). Spotted hyenas live up to 19 years in the wild (Drea and Frank, 2003).

Here age is estimated either from tooth wear data (Van Horn et al., 2003), or from a known birth date (±7 days; Holekamp et al., 1996). Individuals are classified as juveniles either because they were under 8 months of age or because they had not yet been weaned; this usage varies among the chapters in this dissertation. Females are defined as adults after reaching 36 months of age or at their first conception, whichever occurred first; 36 months represents a conservative estimate of the age at which reproductive and morphological maturity is achieved among females in this species (Watts et al., 2009, Holekamp et al., 1996). All immigrant males are considered adults.

Study site

Spotted hyenas were studied in Kenya, with morphological, life history, and ecological data coming from the Masai Mara Game Reserve. The data were derived primarily from three social groups, or clans, called the Talek, Fig Tree, and Mara River clans. Data have been collected for over 23 years from the Talek clan, and for over 10 years from both the other clans, although some morphological measurements were taken from hyenas in the Fig Tree clan as early as 1992.

Morphological data and blood collection

Individual hyenas are routinely anaesthetized in the field as part of an ongoing research project: 6 cranial, 10 postcranial and 15 dental traits are measured, blood is drawn and collected in tubes from immobilized animals. After collection, blood samples are centrifuged, then serum and plasma are collected and frozen in liquid nitrogen.

Pedigree and fitness measures

Our pedigree was built upon a combination of known maternity (Holekamp et al., 1999), and assigned paternity based on genotypic data (see Engh et al., 2002 for details). Briefly, paternity was assigned based on 11 polymorphic autosomal microsatellite loci and one X-linked locus (Engh et al., 2002, Libants et al., 2000) when a 95% confidence level was met in the program CERVUS (Marshall et al., 1998). Maternity was assigned based on regular observation of nursing bouts, and confirmed using microsatellite data (Engh et al., 2002, Holekamp et al., 1999).

Throughout my dissertation I used multiple measures to estimate fitness. Two measures in particular were used or discussed in multiple chapters. These are lifetime reproductive success (LRS), defined as the total number of offspring produced that survived to weaning and annual reproductive success (ARS), defined as the number of cubs born in a given year. Accurate measurement of the fitness of mothers and their offspring accurately can be difficult, and it may not be clear whether to assign a particular component of fitness to the mother or to her offspring. Assigning the fitness component of cub survival to weaning as a maternal fitness component is common in mammalian studies. This practice has been criticized because selection theory emphasizes that the fitness of individuals in one generation (e.g. offspring) should not be assigned to individuals in another generation (e.g. parents), as this can result in incorrect predictions regarding evolutionary dynamics (Lande and Arnold, 1983). However, when the effect of the parental phenotype on a component of offspring fitness greatly overshadows the effect of the offspring phenotype and there is no genetic correlation between the parental trait of interest and the component of offspring fitness, then it may be beneficial to assign this component of fitness to the parent (Wolf and Wade, 2001). In earlier work, explicit consideration has been suggested for a genetic correlation between the parental trait and the component of offspring fitness of interest, based on researchers' knowledge of the species' biology and the traits in question (Wolf and Wade, 2001). In the case of spotted hyenas, there is no reason to suspect a direct genetic correlation between maternal size and offspring survival. In fact, offspring survival to weaning is dominated by the mother's ability to provide milk and protect her cubs (Hofer and East, 2003, Watts, 2007), and is strongly influenced by maternal social rank (Watts et al., 2009), which is learned and is not under genetic control (East et al., 2009, Engh et al., 2000). Thus, for the purposes of this dissertation, I assigned the fitness component of cub survival to weaning to the mother rather than to the offspring.

Writing style of this dissertation

The research comprising my dissertation has been a strongly collaborative effort. This is true both in a general sense with respect to data collection and archiving, as the nature of evolutionary ecology research in large animals requires data collection over many years and by many contributors, and in the more narrow sense that some of my chapters have had multiple collaborators who contributed critical pieces of the project. I could not have succeeded in this endeavor without my many contributors and collaborators. As such, throughout the remainder of my dissertation I will use the first-person plural rather than first person singular.

CHAPTER 2: Ontogeny of sexual size dimorphism in the spotted hyena

Swanson E.M., T. McElhinny, I. Dworkin, M. Weldele, S. Glickman, K.E. Holekamp. (*Submitted to Journal of Mammalogy).* Ontogeny of sexual size dimorphism in the spotted hyena (*Crocuta crocuta*).

Introduction

Intersexual variation in body size, or sexual size dimorphism (SSD), is common in animals, and has a variety of ecological and life history implications (Fairbairn et al., 2007, Fairbairn, 1997, Promislow, 1992). SSD is usually attributed to different fitness optima for body size in the two sexes, and is of interest because it occurs despite a strong genetic correlation between the sexes (Fairbairn, 2007). Female-biased SSD, in which females are the larger sex, is the norm in most taxa, and is often explained by invoking strong directional selection on overall size due to its correlation with fecundity in females (Andersson, 1994, Fairbairn, 2007, reviewed in Fairbairn et al., 2007). However, this pattern of SSD is rare among mammals, in which males are generally larger than females. The mammalian pattern is generally explained by invoking sexual selection, in part because positive selection on female size due to fecundity seems less common in mammals than in oviparous animals (Darwin, 1871, Alexander et al., 1979, Weckerly, 1998, Fairbairn et al., 2007, Ralls, 1976, Lindenfors et al., 2007).

One putative exception to the general mammalian pattern of male-biased SSD is the spotted hyena (*Crocuta crocuta*), a large mammalian carnivore that exhibits a variety of rare and unusual sex role-reversed traits. Adult female spotted hyenas are socially dominant to adult males (Smale et al., 1993, Frank, 1986), have preferential access to feeding sites (Kruuk, 1972, Frank, 1986, Mills, 1990, Tilson and Hamilton, 1984), are more aggressive than adult males (Van Meter, 2009, Szykman et al., 2003), and bear uniquely masculinized genitalia (Kruuk, 1972, Drea and Frank, 2003, Glickman et al., 1987, Matthews, 1939, Neaves et al., 1980, Skinner, 1976). In addition to these well-documented sex-role reversals, spotted hyenas are arguably the only terrestrial member of the order Carnivora that exhibit female-biased SSD (Ralls, 1976). Female-biased dimorphism in spotted hyenas was first described more than 70 years ago (Matthews, 1939), yet in the intervening years there has been considerable disagreement in the literature regarding whether or not females are truly larger than males in this species (see Table 2.1 for a list of prior studies and their findings). Although many studies document dimorphism in some (Matthews, 1939, Skinner, 1976, Hamilton et al., 1986, Henschel, 1986, Mills, 1990) or all (Kruuk, 1972, Neaves et al., 1980) morphological traits measured, others find that males and females are the same size for every trait measured (van Jaarsveld et al., 1988, Gottelli et al., 1994, Sillero-Zubiri and Gottelli, 1992).

Perhaps because of the controversy regarding whether or not females are larger than males, there has been virtually no empirical research on the evolutionary and developmental underpinnings of SSD in spotted hyenas, despite a great deal of evolutionary and developmental research on the other sex role-reversed traits expressed in this species (e.g. Smale et al., 1993, Glickman et al., 1987, Kruuk, 1972, Frank et al., 1990, Drea and Frank, 2003, Holekamp and Smale, 2000, Watts et al., 2009, Van Meter, 2009). Detailed documentation of the ontogeny of any trait represents an important first step toward understanding the developmental processes mediating its expression. Such documentation is also critical to our understanding of the evolution of SSD, as dimorphism in adult size cannot arise independent of development. Rather, the evolution of SSD occurs through alteration of sex-specific ontogenetic trajectories, and recognizing this represents an important step toward understanding evolutionary

Table 2.1. Published accounts of sexual dimorphism, or lack thereof, in wild *Crocuta crocuta*. 'BL' represents body length, 'CBL' represents skull condylobasal length, 'HBL' represents head-body length, 'SH' represents shoulder height, and 'ZB' represents zygomatic breadth. For van Jaarsveld (1988), total sample size was 30; numbers of males and females were not reported. Matthews (1939) did not perform statistical analyses, but did demonstrate that the median size of females was larger than that of males for HBL. Sample sizes for Matthews (1939) were not given in the original paper, but were determined by direct examination of the collected material (McElhinny, 2009). * represents traits where the difference between males and females corresponds to $p \leq 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$.

Females > Males	Females = Males	# of males	# of females	Location	Reference
HBL	CBL, ZB	63	40	Tanzania	Matthews (1939)
Mass**		12	8	Tanzania	Kruuk (1972)
BL*, CBL*, ZB**	Mass, SH	5	5	South Africa	Skinner (1976)
	Mass, HBL	13	12	South Africa	Whateley (1980)
Mass***		5	5	Kenya	Neaves et al. (1980)
Mass*, Girth*	HBL	25	18	Kenya	Hamilton et al. 1986
Mass*, Girth *	SH	8	6	South Africa	Henschel (1986)
	HBL, SH			South Africa	van Jaarsveld et al. (1988)
Mass***, Girth*	HBL	9	7	South Africa	Mills (1990)
	Mass, Girth, HBL	5	9	Kenya	Sillero-Zubiri and Gotelli (1992)

patterns of dimorphism among adults (Badyaev, 2002). Specifically, knowledge of the developmental program underlying SSD can shed considerable light on the mechanisms by which complex adaptations respond to selection, the evolutionary origins of dimorphism, the proximate neuroendocrine mechanisms mediating dimorphism, and the environmental factors influencing SSD.

Our goals in the current study were fourfold. First, we wanted to determine unequivocally whether or not female spotted hyenas are larger than males. Second, we wanted to understand the developmental route by which this occurs. Third, we wanted to understand why previous studies were unable to settle the question of whether or not spotted hyenas exhibit female-biased SSD. Finally, we compared captive individuals in which both sexes were fed identical diets to free-living hyenas to test whether the SSD is caused by differential food access; if so, we expected to find no sex difference among captive hyenas.

In order to accomplish our first and second goals, we measured sexual dimorphism in adult size, growth rate, and growth duration in 14 morphological traits using a large set of cross-sectional and longitudinal data collected during immobilizations of free-living spotted hyenas in Kenya. We also tested for dimorphism in overall 'body size', a common approach that is also more difficult than commonly appreciated. The two most widely-used approaches to quantifying overall body size involve either using univariate measures such as mass or body length, or using scores from the first principal component (PC) in a principal component analysis (PCA) of all size traits measured in each animal. Both of these approaches are problematic for reasons detailed in Swanson et al. (2011). Therefore, in addition to our univariate, 'trait-by-trait' approach to investigating sexual size dimorphism, we investigated dimorphism in the first PC axis from a PCA on all traits and also utilized a novel approach to defining size based on allometric relationships. This approach, which groups traits based on their multivariate allometric coefficients, was recently used to quantify size among adult female hyenas (Swanson et al., 2011).

To test for dimorphism we fitted growth models to the morphological data collected from the free-living hyena population. These are sigmoidal models that fit a growth rate parameter as well as an asymptotic parameter representing adult size. Sex was then fitted as a predictor of adult size and relative growth rate to simultaneously test for dimorphism in adult size and growth rate during ontogeny. Finally, to test the hypothesis suggesting that female-biased SSD in spotted hyenas results from differential food access mediated by female dominance over males, we analyzed a second, smaller data set collected from adult members of a captive colony where males and females experienced identical feeding and environmental conditions throughout the lifespan.

In relation to our third goal of addressing why previous studies have been unable to unequivocally determine whether or not female spotted hyenas are larger than males, we noted that many of the previous studies on both sides of this controversy had small sample sizes, which may have hampered researchers' ability to detect mild to moderate differences in size. We suggest this possibility because one of the well-known consequences of small sample size in statistical tests is low statistical power, the ability to detect a difference between two groups when the two groups are in fact different. We therefore performed bootstrapped power analyses using our data to test the hypothesis that previous studies have merely had insufficient statistical power to reliably find size differences between males and females. Finally, we suggest that the degree of dimorphism for different traits varies, and this also plays a role in determining researchers' ability to detect sexual dimorphism.

Materials and Methods

Study site and data collection from free-living spotted hyenas.—Spotted hyenas live in social groups, called clans, that have been observed to contain as many as 120 individuals ordered in a linear dominance hierarchy in which females and their offspring are dominant to breeding males (Frank, 1986, Smale et al., 1993). Breeding males are usually immigrants from other clans, but females are philopatric (Smale et al., 1997, Van Horn et al., 2003, Honer et al., 2007). Both sexes reach physiological sexual maturity at around 24 months (Glickman et al. 1992), but females often fail to give birth until after 36 months of age (Hofer and East, 2003, Holekamp et al., 1996). Free-living spotted hyenas live up to 19 years (Drea and Frank, 2003).

Data were collected during 799 immobilizations of 651 individuals, including 351 females and 299 males. Of the 651 individuals, 551 were measured only once. The mean number of measurements of a single individual was 1.23, and the maximum number of times an individual was measured was 5. The mean age (in months) of measurement was 48.09 ± 41.79 (standard deviation (SD)); the youngest hyena was 0.20 months and the oldest was 207.58 months. Immobilizations were conducted in Kenya using Telazol (Fort Dodge Animal Health, Overland Park, KS; 6.5 mg/kg) administered via a lightweight plastic dart fired from a CO_2-powered rifle (Telinject Inc., Saugus, CA). All immobilizations were carried out in accordance with guidelines of the

American Society of Mammalogists (Sikes et al., 2011), and our protocols were also approved by the Institutional Animal Care and Use Committee at Michigan State University. Ages of most hyenas residing in their natal clans were determined based on known emergence dates from natal dens (+/- 7 days; Holekamp et al., 1996), and ages of other adults were based on patterns of tooth wear (± 6 months; Van Horn et al. (2003)). During each immobilization several morphological measurements were taken, including 4 cranial and 9 postcranial measurements, in addition to body mass. Measurements are shown in Figure 2.1, and a detailed description of each of these measures is given by Van Horn et al. (2003).

Allometric relationships among traits over ontogeny.—We used three approaches to quantify size in order to compare ontogenetic patterns of size change between male and female spotted hyenas. First, we computed the first principal component axis (PC1) from a PCA of all measured morphological traits except mass, and used this composite score as an estimate of overall size. Henceforth we refer to this composite score as overall body size. Second, we analyzed the ontogeny of each univariate trait separately. Finally, we adopted the approach suggested by Swanson et al. (2011), in which traits are grouped based on their multivariate allometric coefficients, estimated as the loadings of each individual trait on PC1. Morphological allometries represent the log-log ratios at which the size of specific traits increase with increasing overall body size. Multivariate allometric coefficients calculated in this way specifically represent the log-log ratio at which traits increase with increasing PC1. Traits that increase at log ratios greater than one are generally referred to as hyperallometric, traits that increase at log

Figure 1

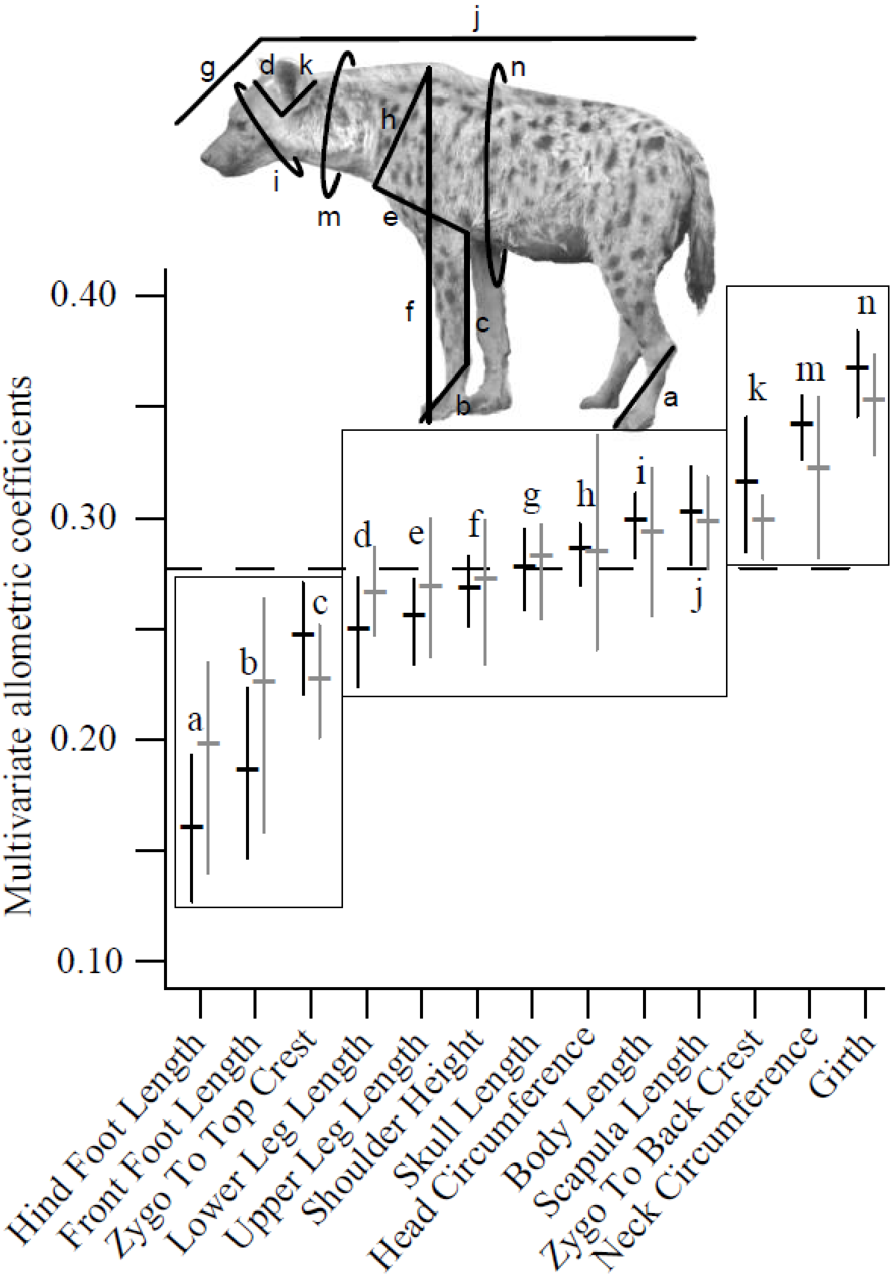


Figure 2.1. Bootstrap means (horizontal lines) and 95% CIs (vertical lines) for multivariate allometric coefficients for univariate morphological traits. Lines representing males are gray and lines representing females are black. Boxes around traits represent groupings for multivariate traits chosen by whether or not the allometric CI overlaps the isometric hypothesis (horizontal dotted line). Letters above each allometric CI correspond to measures on the hyena diagram. Any letters for which the corresponding morphological trait on the hyena diagram is ambiguous refer to the line directly below the letter.

ratios equal to one are called isometric, traits that increase at log ratios of less than one are called hypoallometric.

To obtain the multivariate size measures suggested by Swanson et al. (2011), we resampled our data with replacement 10000 times (Efron and Tibshirani, 1986), performed a PCA on the 13 log-transformed morphological traits (again excluding mass) for each resampled data set, and estimated 95% bootstrap confidence intervals (CIs) on the trait loadings (Tzeng and Yeh, 2002, Jackson, 1993). Following Swanson et al. (2011) we next assigned traits to groups based on whether or not their 95% CIs overlapped the expected value for isometry. The expected isometric value, or 'isometric hypothesis', is equal to $1/(p^{1/2})$ where p is the number of traits in the PCA, and is equivalent to the trait loadings in the hypothetical situation where every trait included in the PCA increases isometrically. Finally, we performed PCAs on each of the resulting groups, and used the PC scores associated with the first eigenvalue of each of these PCAs as a new multivariate trait, referred to respectively as the hypoallometric, isometric, and hyperallometric size traits. Although we recognize that the allometric variation is continuous, this method provides a useful basis for separation. These and all following analyses were carried out in R version 2.13.1 (R Development Core Team).

Sex differences in ontogenetic vectors.—One sex can only achieve greater size than another through a limited number of routes, and each route is suggestive of certain physiological mechanisms mediating the dimorphism. Specifically, members of one sex may achieve a larger size because they grow for a longer period of time, at a greater rate, or both (Altmann and Alberts, 2005, Leigh, 1996, Setchell et al., 2001, Derocher et al., 2005). If a dimorphism results from a disparity in growth rates between males and females, this disparity may be present throughout development, or it may occur in the form of a 'growth spurt', where growth rates differ between the sexes only during a limited period of time (Leigh, 1996). Growth spurts, particularly during the final stages of sexual maturation, are common among mammals, especially in some orders, such as Primates (Leigh, 1996). In addition to using the multivariate allometric coefficients to determine trait grouping, we estimated the angular difference between the allometric vectors (PC1 on all traits (see Pitchers et al., 2012)) calculated separately for males and females to test for overarching differences between the sexes with respect to the multivariate ontogenetic trajectory (Zelditch et al., 2003). We performed this analysis on resampled data in order to estimate 95% bootstrap CIs. Increases away from 0˚ in the angle between the ontogenetic vectors for males and females suggest divergence in growth rates among the sexes. We partitioned ontogeny into three separate time periods for comparison: individuals younger than 13.5 months, individuals between 13.5 and 24 months, and individuals between 24 and 36 months. 13.5 months is the mean weaning age in this population (Holekamp et al., 1996), 24 months is the age at which females are physiologically competent to breed (Matthews, 1939, Glickman et al., 1992), and 36 months represents a conservative estimate for complete morphological and reproductive maturity (Tanner et al., 2010) and the age after which females generally start to reproduce. Our intention with this analysis was to isolate the phases of development during which males and females exhibited notably similar or different ontogenetic vectors.

Sexual dimorphism and growth in free-living spotted hyenas.—To test for sexual dimorphism in adult size and relative growth rate, we fitted several flexible, commonly used sigmoidal growth models for each univariate variable, as well as for the hypoallometric, isometric, and hyperallometric multivariate traits, as well as overall body 'size'. The models we fitted included a saturating ‘monomolecular’ model (Gaillard et al., 1997), the Gompertz model of Zullinger et al. (1984), the Gompertz model as formulated by Fiorello and German (1997), the Von Bertanlaffy model (Zullinger et al., 1984), and the logistic model (Zullinger et al., 1984). Equations were parameterized as in Zelditch et al. (2003). Each of these models includes an asymptotic value representing adult size that is approached as age increases, a relative growth rate parameter representing the rate at which adult size is approached, and an age at which size is equal to 0 (see Figure 2.2 for an example of a growth curve with adult size for males and females modeled separately). Because we were fitting models to mixed cross-sectional and longitudinal data, we faced a potential issue of pseudoreplication, in which using multiple data points from the same individuals can bias results, or violate the assumptions of parametric statistical tests. To address the problem of bias, we first tested whether the combination of cross-sectional and longitudinal data resulted in a bias by estimating the variance in each morphological variable explained by individual identity. To do so we fitted models with the ‘nls’ and ‘nlme’ functions in R, using likelihood ratio tests calculated from model deviance to compare models fitted with or without a random effect of individual as a predictor of asymptotic adult size and relative growth rate. In no case was the model with the effect of individual a significantly better fit to the data, indicating that consistent similarities among measures on the same individuals do not explain a significant amount of variation in adult size or relative growth rate. This may be because individuals were virtually never sampled repeatedly

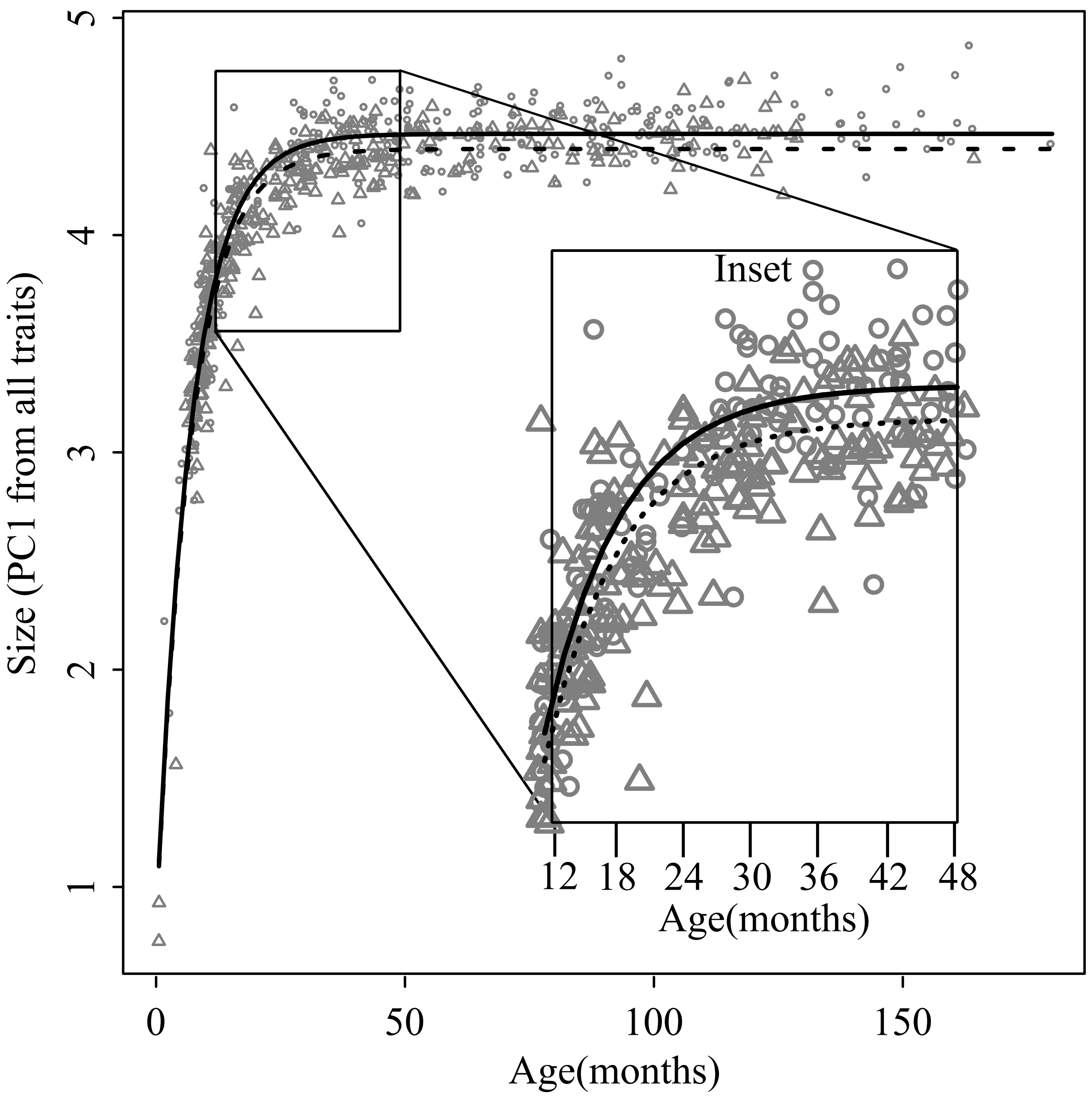


Figure 2.2. Plot of age versus size as estimated from PC1 taken from a PCA on all traits except mass (Size). Solid line plotted through data represents female growth curve taken from best-fitting monomolecular growth curve, and dashed line represents male growth curve from the best fitting monomolecular function. Data points for females are represented by circles and males by triangles. Inset is magnified between 12 and 48 months of age to better exhibit the dimorphic growth period.

during short time periods, so replicated measures within individuals were generally distributed widely over the lifespan. To further ensure that use of all our data, including replicated samples within individuals, did not affect our results, we re-estimated all p-values using the same parameter estimates, but using the total number of individuals as the sample size rather than the total number of immobilizations. Throughout we indicate when using this smaller sample size resulted in a difference. We then refitted the models using maximum likelihood estimation (MLE) with the ‘bbmle’ package in R (Bolker and R Development Core Team, 2012). Because the monomolecular model fit our data best for 11 out of 14 univariate traits compared (including mass), we present all results using the monomolecular model for the sake of comparison among results for different traits. However, we also repeated all tests using the best model for the three traits where a different model fit better. Specifically, the Von-Bertanlaffy curve fit better for skull length and upper leg length, whereas the logistic curve fit better for lower leg length. Repeating all relevant analyses using an alternative growth model had no effect on the results for any trait.

To test for differences in relative growth rate and adult size between males and females, we fit four separate monomolecular models. No sex difference in relative growth rate or adult size was fit for the first model. For the second model a sex difference was fit to adult size. The third model was fit with a difference in relative growth rate (the 'b' parameter in the monomolecular equation), and the fourth model was fit with a difference between the sexes in both adult size and relative growth rate. We again performed model selection using sample size-corrected Akaike’s Information Criterion (AICc).

Sexual dimorphism in adult captive spotted hyenas.—To determine whether size differences between the sexes have a genetic basis, or are instead merely a result of females' priority of access to food, we collected data from 32 captive adult spotted hyenas fed on uniform diets at the University of California - Berkeley Field Station for the Study of Behavior, Ecology, and Reproduction. Measurements were taken from 19 adult male and 13 adult female hyenas of similar age (t = -0.51, df = 30, p = 0.612), descended from animals collected at a site near our study area in the Masai Mara National Reserve. Individual hyenas were immobilized using a blowgun dart (Darts: Telinject Inc., Agua Dulce, CA; Blowgun: Addison Biological Laboratory, Inc., Fayette, MO) to administer a mixture of ketamine (10 mg/kg; 100mg/ml), xylazine (1 mg/kg; 100mg/ml), and atropine (0.045 mg/kg; 15 mg/ml), after which total mass, head-body length, and shoulder height were measured. Sedation was then reversed using yohimbine (0.075 - 0.12 mg/kg; 2 mg/ml; Lloyd Incorporated, Shenandoah, IA). We compared male and female size for mass, head-body length, and shoulder height using Student's t-tests.

Statistical power of previous studies.—To address the question of whether previous studies had insufficient statistical power to adequately address the question of sexual size dimorphism, we used our field data to perform power analyses. Using only data from wild adults older than 36 months, we separated males from females, resampled our data with replacement separately for the two sexes, and performed t-tests to assess our ability to find a significant difference in size between males and females for the trait in question at a variety of sample sizes. We started at a sample size of 5, and increased sample size by 1 until all 500 replicates for each run found the difference in size to be statistically significant at an α = 0.05. We replicated this process 10000 times and estimated 95% bootstrap CIs on the sample size for each sex at which 80% of the runs found a statistically significant difference in size for males and females. 80% was selected a priori as an estimate of statistical power, that is, the percent of times one would expect to find a statistically significant difference in size between males and females. We only performed these power analyses for traits that our growth models found to differ significantly between adult males and females in the wild; these included mass, the distance between the widest point on the zygomatic arch and the back of the sagittal crest (henceforth 'zygo to back crest'), zygomatic arch to the top of the sagittal crest (henceforth 'zygo to top crest'), skull length, body length, head circumference, girth, neck circumference, shoulder height, upper leg length, and scapula length.

Results

Allometric relationships.—We compared the bootstrap CIs for the multivariate allometric coefficients for the univariate variables to determine where they fell in relation to the isometric hypothesis. The allometric CIs for front foot length, hind foot length, and zygo to top crest fell below the isometric hypothesis, while CIs for lower leg length, upper leg length, scapula length, body length, skull length, head circumference and shoulder height overlapped the ‘isometric hypothesis’ (indicated by the dashed line in Figure 2.1). The allometric CIs for girth, neck circumference, and zygo to back crest all fell above the isometric hypothesis. Ontogenetic allometries did not generally correspond with static allometries (which describe variation among individuals in the same ontogenetic stage, rather than over development) from earlier work (Swanson et al., 2011), but in a few cases they did, namely the hyperallometric traits. Based on their multivariate allometric CIs, we grouped together the hypoallometric traits falling below the isometric hypothesis, and will refer to PC1 from this group as the hypoallometric trait (Figure 2.1). We also grouped the traits overlapping the isometric hypothesis; we refer to PC1 from a PCA on these traits as the isometric size trait. Finally, we grouped the traits falling above the isometric hypothesis, and we refer to PC1 from a PCA on these traits as the hyperallometric trait.

Sex differences among allometric vectors.—Allometric CIs across the entirety of ontogeny generally differed little between males and females, although hyperallometric traits did appear to be slightly more hyperallometric, and hypoallometric traits slightly more hypoallometric among females (Figure 2.1). When comparing angles between male and female allometric vectors over specific periods of ontogeny, however, it is apparent that, as individuals approach sexual maturity, the angle increases, suggesting that some traits are growing differently in males and females during this period (Figure 2.3). It seems likely that this represents the period of dimorphic growth.

Sexual dimorphism and growth among free-living hyenas.—To assess the ontogeny of SSD we fitted several different growth models that capture key parameters such as maximum growth rate and adults size. The monomolecular model fit best for 11 out of 13 univariate models, and for all four of the growth models using multivariate traits (see Appendix A; Table A.1). For lower leg length, upper leg length, and skull length, the three traits for which monomolecular was not the best model, there were no qualitative differences between any results obtained by using the monomolecular versus the best model, so we present results from the monomolecular to maintain consistency regarding the meaning of the parameters discussed.

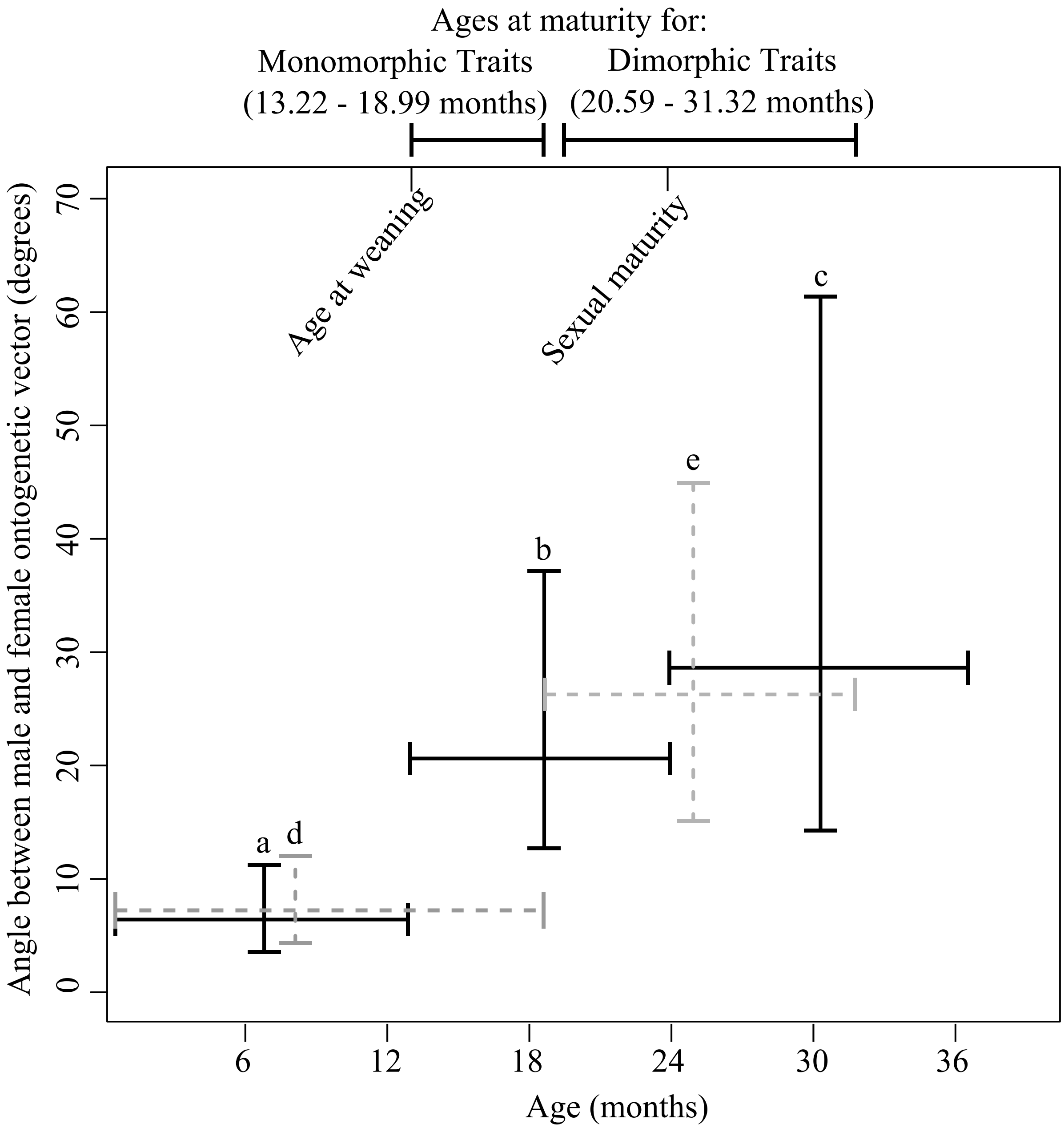


Figure 2.3 Angles between ontogenetic vectors for males and females calculated from PC1 on all traits for each sex individually. Vertical bars on the plot represent 95% bootstrap CIs (vertical bars) from 10,000 bootstrap replicates of estimation of the ontogenetic angle, horizontal bars show the age ranges themselves. Black bars with solid lines (a, b, and c) represent the a priori choices for age ranges (0 - 13.5 months, 13.5 - 24 months, and 24 - 36 months respectively), and gray bars with dashed lines represent post hoc choices for age ranges based upon our proposed 'monomorphic' growth period (d; 0 - 18.99 months) and 'dimorphic' growth period (e; 18.99 - 31.23 months).

Adult females were larger than adult males for ten of 13 univariate traits (Table 2.2; Table 2.3), as well as three of four multivariate traits, including the isometric and hyperallometric traits and total size (Table 2.2; Table 2.3). For the three monomorphic univariate traits (hind foot length, front foot length, and lower leg length), the best model was the monomolecular model with no difference between males and females fitted for relative growth rate or adult size. For the hypoallometric trait, the best model was the model with sex fitted as a predictor to adult size, but the difference was not significant at $\alpha = 0.05$ (Table 2.3). For traits that we identify as dimorphic, perhaps the better question is how much sexual dimorphism is there, and what is the biological significance of this dimorphism, questions we address in the discussion. Of the three monomorphic univariate traits, front foot length and hind foot length were in the hypoallometric group, whereas lower leg length was the most hypoallometric trait in the isometric trait group (Figure 2.1). All of the skeletal measures in our study reach 95% of their adult size before 32 months. Mass, the only measure that does not mature by this point, reaches 95% of its adult value at 45 months (Table 2.3).

Sexual dimorphism among adult captive spotted hyenas.—As with the free-living hyenas, female hyenas from the captive colony were heavier than males (females: 55.6 ± 2.0 kg, males: 49.0 ±1.3 kg, $t_{30} = 2.89$, $p = 0.0071$), and longer (head-body length - females: 131.4 ± 1.2 cm, males: 127.2 ± 1.1 kg, $t_{30} = 2.24$, $p = 0.0193$). In contrast to our results from the free-living population, however, we were unable to detect dimorphism in shoulder height (females: 82.0 ± 0.54 cm, males: 81.7 ± 0.4 cm, $t_{30} = 0.53$, $p = 0.5996$). Interestingly, for the free-living population, shoulder height exhibited

Table 2.2. dAICc values for sex model selection for multivariate and univariate morphological traits. dAICc values of >2 are generally considered to represent evidence that the model does not fit the data as well. Generally this is used to determine whether a parameter is a useful addition to a model given the complexity it adds. Columns represent: [1]a model denoting no difference in either adult size or relative growth rate, [2]a model denoting a difference in adult size between the sexes, but no difference in relative growth rate (Rel. GR), [3]a model denoting dimorphism in relative growth rate (Rel. GR), but not adult size, and [4]a model denoting a difference in both adult size and relative growth rate (Rel. GR). Bolded cells represent the 'best' model, or the model which most closely fits the data.

	No trait[1]	**Adult size**[2]	**Rel. GR**[3]	**Adult size and Rel. GR**[4]
Size	21.6	**0**	17.8	1.7
Hypoallometric size	1.7	**0**	2.8	2
Isometric size	20.3	**0**	14.3	2
Hyperallometric size	29	**0**	22.8	1.5
Zygo To Top Crest	9.6	**0**	6	2
Zygo To Back Crest	5.2	**0**	4.9	1.8
Skull Length	12.8	**0**	11.6	1.1
Head Circumference	51.1	**0**	36.7	1.2
Girth	33	**0**	27.7	0.2
Lower Leg Length	**0**	1.9	1.7	3.8
Upper Leg Length	2	**0**	0.5	1.3
Scapula Length	7.8	**0**	7.5	1.8
Shoulder Height	3.4	**0**	4.2	1.9
Front Foot Length	**0**	1.8	2	3.7
Hind Foot Length	**0**	1.6	1.4	3.4
Neck Circumference	36	**0**	28.6	0.7
Body Length	23.4	**0**	10.3	1.2
Mass	78.5	0	52.9	1.1

Table 2.3. Model parameters for ‘best’ fitted models. Asymptote refers to the estimate for adult size for females (± standard error), whereas 'Male effect' is the difference in adult size estimated for males compared to the estimated adult size for females (± standard error). 'z' is the test statistic for the comparison of male and female adult size and 'p' is the associated p-value. 'b' represents the relative growth rate of each trait and 't0' the estimated age at onset of development of the trait. Age at maturity for each trait is the age in months at which the predicted size for the trait is equal to 95% of the asymptotic value, or adult size. Units for mass are kilograms (kg), and units for all other univariate traits are centimeters (cm). Letters preceding trait names correspond to Figure 2.1. Asterisks designate p-values no longer significant at α ≤ 0.05 after correcting z-scores for reduced sample sizes.

Trait	**Female size (asymptote)**	**Male effect**	**z**	**p**	**b**	**t0**	**n**	**Age at maturity**
All size	4.466±0.011	-0.070±0.014	-4.91	<0.001	0.142±0.003	-1.447±0.222	621	19.65
Hyperallometric Size	2.139±0.008	-0.058±0.010	-5.64	<0.001	0.115±0.004	-1.831±0.366	659	24.22
Isometric Size	3.269±0.007	-0.046±0.010	-4.77	<0.001	0.142±0.003	-1.262±0.197	649	19.83
Hypoallometric Size	2.099±0.006	-0.015±0.008	-1.93	0.053	0.206±0.006	-0.544±0.33	669	14
Mass	59.386±0.467	-5.721±0.612	-9.35	<0.001	0.069±0.003	1.865±0.310	631	45.28
k. Zygo To Back Crest	17.392±0.082	-0.283±0.104	-2.70	0.007	0.078±0.004	-7.085±0.873	679	31.32
g. Skull Length	30.103±0.098	-0.493±0.127	-3.87	<0.001	0.089±0.003	-6.076±0.516	686	27.58
n. Girth	83.937±0.374	-2.938±0.488	-6.01	<0.001	0.099±0.004	-2.833±0.474	681	27.43
i. Head Circumference	53.097±0.152	-1.453±0.195	-7.45	<0.001	0.094±0.003	-5.369±0.458	683	26.5
m. Neck Circumference	50.607±0.222	-1.808±0.288	-6.27	<0.001	0.099±0.004	-3.565±0.547	678	26.69
d. Zygo To Top Crest	13.001±0.065	-0.291±0.085	-3.43	<0.001	0.102±0.007	-6.035±0.989	682	23.33
j. Body Length	98.170±0.353	-2.368±0.464	-5.09	<0.001	0.100±0.003	-4.255±0.409	690	25.7
h. Scapula Length	28.458±0.103	-0.430±0.136	-3.14	0.002	0.113±0.004	-2.911±0.367	687	23.6
f. Shoulder Height	78.431±0.207	-0.645±0.276	-2.33	0.019	0.130±0.003	-2.013±0.262	678	21.03
e. Upper Leg Length	25.458±0.097	-0.262±0.130	-2.01	0.044*	0.131±0.005	-2.282±0.333	688	20.59
c. Lower Leg Length	25.911±0.077	-	-	-	0.146±0.004	-1.526±0.041	687	18.99
b. Front Foot Length	19.157±0.066	-	-	-	0.190±0.007	-1.210±0.264	687	14.56
a. Hind Foot Length	23.395±0.066	-	-	-	0.207±0.007	-1.255±0.209	688	13.22

perhaps the least dimorphism of those univariate traits exhibiting statistically significant differences in male and female size as adults (Figure 2.4; Table 2.3).

Statistical power of previous studies.—Our analysis suggested that the sample size required to achieve sufficient power to detect a statistically significant difference in size between males and females varies greatly depending on the trait of interest. For example, reliably detecting dimorphism in body mass in our power analysis required about 14.4 individuals in each sex (Bootstrap standard deviation (SD): 0.683). Other traits required larger samples, ranging from 33.85 individuals to more than 350 individuals of each sex to achieve a statistical power of 0.80 (Figure 2.4). Upper leg length was an extreme case, requiring over 2500 individuals in each sex to reach a power of 0.80 and representing barely detectable dimorphism. Even with very large sample sizes, the traits for which dimorphism appears to be of the greatest biological significance are the traits for which it is easiest to detect statistical significance.

Discussion

Our results demonstrate that, when large sample sizes are available, female-biased SSD is clearly apparent for most morphological traits in the spotted hyena, as well as for composite measures of body size (Table 2.3). However, there is marked variation among traits regarding both the degree of dimorphism, and the sample size required to reliably detect that dimorphism (Figure 2.4). Some traits, such as hind foot length, front foot length, and lower leg length, do not appear to exhibit any dimorphism at all even when statistical power is enormous (Table 2.2; Table 2.3). Previous

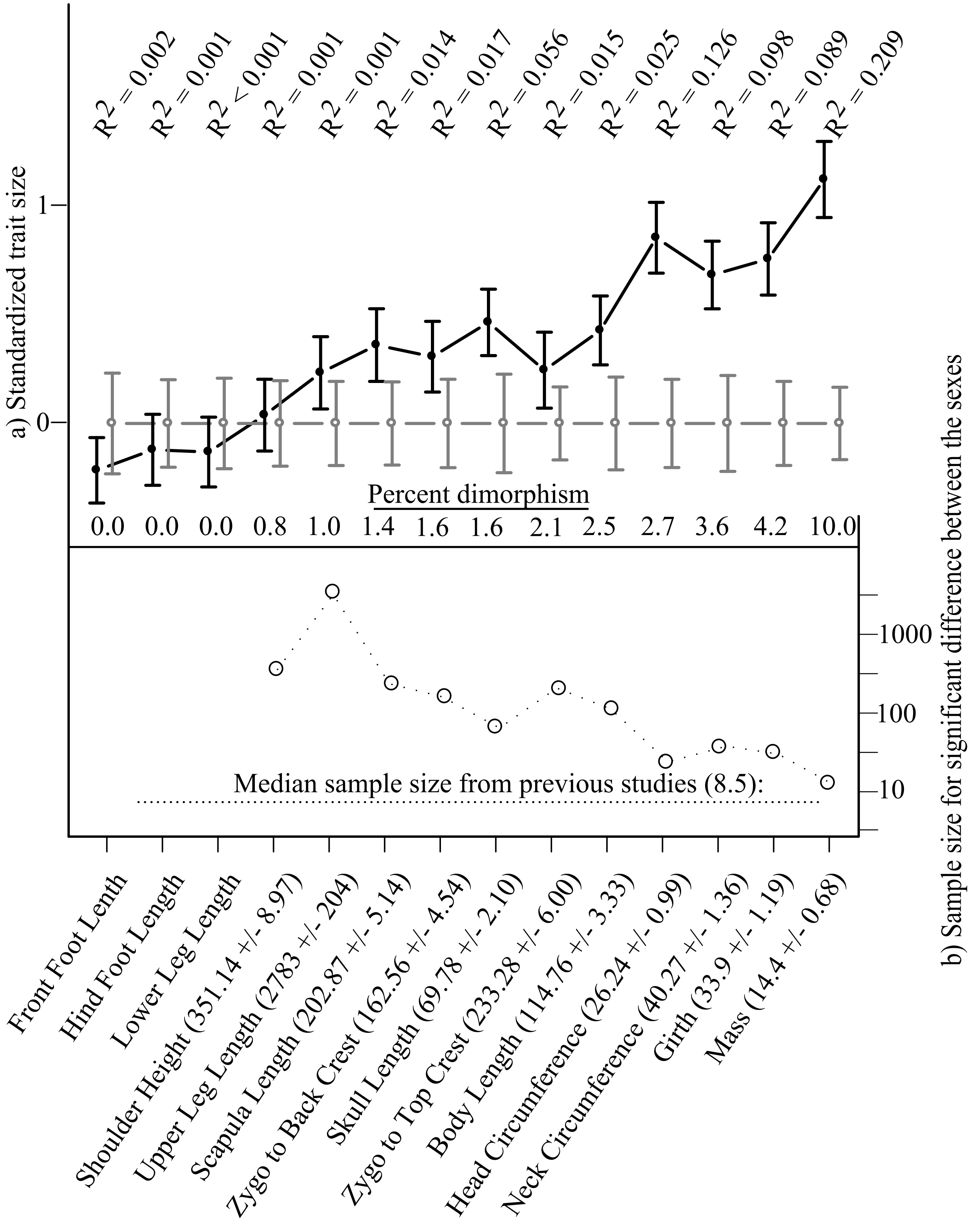


Figure 2.4. Size differences between males and females and sample sizes required to detect the differences. a) Size differences between males and females shown using

Figure 2.4 (cont'd)

standardized line plots (using the 'sciplot' package in R (Morales et al., 2011)) of male and female size distributions for each univariate trait. Females are in black and males in gray. Data for each trait are mean-centered to the male mean for that trait, and standardized by dividing all data for the trait by the pooled standard deviation of the trait calculated for each sex separately and weighted by sample size for each sex (Nakagawa and Cuthill, 2007). For this figure we only used data from immobilizations for which measurements from every trait are available (n = 261). Circles represent means, error bars represent 1.96*SEM (95% CIs) using pooled standard errors. R-squared values given above each traits represent the percent variance in the trait explained by the sex difference between males and females among adults over 36 months. b) Statistical power required to reliably detect a significant difference between males and females. Hollow circles represent the sample size at which 0.80 power is reached for each variable. The secondary y-axis (right-hand axis) is on a log base 10 scale, with actual sample sizes given in x-axis labels with bootstrap standard deviations. Median sample size from previous studies is represented by horizontal dotted line.

disagreement regarding whether or not spotted hyenas exhibit female-biased SSD can be explained in part by variation in the degree of dimorphism among different traits. Thus, earlier studies that chose to measure more dimorphic traits may have been more likely to suggest that female spotted hyenas are larger than males.

However, variation in degree of dimorphism clearly cannot explain all of the differences in the results presented in the literature. Body length and skull length, for example, both exhibit clear, if moderate, dimorphism in our data set. Only 1/3 of previous studies however, found dimorphism in skull length (CBL in Table 2.1), body length (BL in Table 2.1), or the combination of the two, head-body length (HBL in Table 2.1). This suggests that other factors contribute to the variation observed among previous studies with respect to SSD in the spotted hyena. In fact, our power analyses suggest that the majority of the controversy over whether spotted hyenas exhibit female-biased SSD largely results from insufficient power in most previous studies. In only one study in Table 2.1 was a combined sample size greater than 50 available for the two sexes, and in that case, no statistics were actually calculated (Matthews, 1939). Most studies, in fact, measured fewer than 10 individuals of each sex (Table 2.1; median = 8.5), which, given the results of our power analyses, is insufficient to detect sexual dimorphism in spotted hyenas for even the most strongly dimorphic traits (Figure 2.4). Even body mass, the trait exhibiting the greatest degree of dimorphism of all those we measured (Figure 2.4; Table 2.3), required on average of 14.4 (± 0.683 bootstrap SDs) individuals of each sex to reliably find a significant difference. Interestingly, the monomolecular growth models we fitted to our data appear more likely to detect statistically significant differences in adult size between males and females than t-tests comparing only adults (see Appendix A - Table A.2). We assume this is due to the inclusion of large numbers of nearly mature individuals exhibiting partial but incomplete dimorphism that cannot be included in simple two-group comparisons of fully mature adults. This suggests that fitting such models in general may be a good practice when the data are available for species in which the sexes diverge to some extent before complete maturity, even to answer questions that directly involve only adults, such as the degree of SSD among adults.

Because female spotted hyenas have higher priority of access to food than do males, it might be argued that the adult size difference is mediated strictly by sex differences in nutrition. This is not likely to be a critical factor in determination of sexual size dimorphism in nature because young hyenas of both sexes retain the same ranks as their mothers as long as they remain the natal clan, so diets of males and females don’t differ until males disperse, and male hyenas rarely disperse before the end of the growth period; the mean age of emigration in our study area is 42.1 +/- 10.5 months (SD) (Van Horn et al., 2003). All of the skeletal measures in our study reach 95% of their adult size before 31.6 months, 1 standard deviation younger than the mean age of dispersal. In addition, comparison of male and female hyenas fed identical diets in captivity demonstrates that captive females are both longer (head-body length) and heavier than captive males. Shoulder height was monomorphic in the captive population, but our power analysis suggested that enormous sample sizes would be required to reliably distinguish a sex difference in this trait. Thus, it seems likely that the failure to discern dimorphism in shoulder height among captive hyenas simply reflects a lack of sufficient statistical power in our sample. These results also suggest that female-biased SSD in spotted hyenas has a genetic basis, and is not simply a result of better nutrition for free-living females, because it persists in a lab population where feeding conditions are identical for both sexes.

One key consideration here is the biological importance of this SSD; females are hardly any larger than males for some traits, and other traits, such as lower leg length, hind foot length, and front foot length, do not appear to be dimorphic at all. However, traits associated with overall length exhibit fairly clear dimorphism, and traits associated with 'robustness', such as head circumference, neck circumference, and girth, exhibit marked dimorphism (Figure 2.4; Table 2.3). Mass, in particular, exhibits notable dimorphism (see Table 2.3 and Figure 2.4). The functional significance of this dimorphism is not fully clear in this species, as we know little about the performance consequences of body size in spotted hyenas with reference to hunting, intraspecific fighting, or interspecific interactions with other large predators. The mass difference alone, however (about 10% of adult size), is large enough to suggest functional consequences regardless of the role of other morphological traits in hunting or fighting.

Interestingly, for all traits measured here, male and female spotted hyenas appear to grow at the same rate relative to their respective adult sizes (Table 2.3). In other words, there is no difference between the age at which male and female hyenas cease growth, so any sex differences in measured traits must result from differences in absolute growth rate. Thus our data supported the hypothesis that females are larger because they grow more rapidly, rather than for a longer period of time.

Our results suggest that the higher growth rates in females do not occur continuously, but rather as a 'growth spurt', with male and female growth patterns diverging as they approach sexual maturity. The greater angle between allometric vectors for males and females in later than earlier development indicates that growth differences between males and females arise sometime after weaning. Interestingly, if we repeat the allometric angle analysis, using 0 - 18.99 months and 19.0 - 31.32 months as our time periods, we find essentially the same result (gray dashed lines in Figure 2.3). 0 - 18.99 months represents the time period from the onset of growth to the age at maturity of the last-maturing sexually monomorphic trait, lower leg length. 18.99 - 31.32 months represents the time period from age at maturity of the earliest maturing sexually dimorphic trait, upper leg length, to age at maturity of the latest maturing sexually dimorphic trait excluding mass, zygo to back crest. For the time period of 0 - 18.99 months we found an estimate of 6.41 degrees with a 95% bootstrap CI of 3.53 - 11.21. Because we use the absolute value of the dot product of the two vectors, these angle measures cannot overlap 0, so one can only make relative comparisons among them. For the time period from 18.99 - 31.32 months we found an angle of 25.46 degrees with a 95% bootstrap CI of 14.27 - 44.11. The 95% CIs do not overlap here, suggesting that the developmental trajectory differs most between males and females from about 19 months to 24 months of age.

Our recent work suggested that certain morphological traits were under positive selection among females in this population, and that the observed SSD could potentially result from this selection (Swanson et al., 2011). If this were the case, one would expect the traits under selection, namely body length, shoulder height, lower leg length, head circumference, and scapula length, to be among the most dimorphic traits. Some of the traits under selection, such as body length and head circumference, are indeed among the most dimorphic traits and support this hypothesis. Others however, such as shoulder height, are weakly dimorphic, and yet others, such as lower leg length, are not dimorphic at all. We propose a hypothesis to explain this observation, suggesting that the endocrine factor mediating the response to selection on morphological traits is a systemic developmental factor that affects all morphological traits in some similar manner, leading to a genetically correlated response. If true, this would suggest that variation in dimorphism likely results from some process other than simple variation in growth rate, which would likely be influenced somewhat similarly for each morphological trait. Our results here provide some evidence for this hypothesis. Specifically the degree of dimorphism among measured traits was significantly correlated with age at trait maturity using a non-parametric Spearman rank correlation (n (sample size) = 13, R_S = 83.06, p = 0.002) but not with average absolute postnatal growth rate for different traits (n = 13, R_S = 336.81, p = 0.808). This suggests that the degree of dimorphism in different traits depends only on how long the trait continues to develop after the onset of divergent growth, and while certainly not definitive, offers support for our hypothesis. It is interesting that, although SSD in hyenas occurs because females grow faster than males, the degree of dimorphism observed in specific traits is related to the duration of growth rather than to growth rate.

We also note here that we cannot falsify the hypothesis suggesting that the evolutionary explanation for at least some of the observed dimorphism is historical in nature and not a result of observable contemporary selection. Hypotheses concerning evolutionary history are notoriously difficult to falsify in specific cases. We propose instead that future investigations into relevant anabolic hormones such as growth hormone and insulin-like growth factor might be fruitful, as both these hormones play important roles in mitogenesis and cell growth (Kappeler et al., 2008, reviewed in Dantzer and Swanson, 2012, Zapf and Froesch, 1999, Froesch et al., 1985). The question of the physiological mechanism mediating SSD in spotted hyenas is especially interesting because there are a number of ways in which the 'masculinized' endocrine profiles of female spotted hyenas might mediate anabolic growth in a sex-specific manner. Gonadal steroids generally have sex-specific profiles and can also influence the release of growth hormones (e.g. Veldhuis et al., 1995, Muniyappa et al., 2007, Mauras et al., 1996). One interesting factor that might mediate growth rates in spotted hyenas is androstenedione, a testosterone precursor that can have anabolic effects of its own (Chen et al., 2004). Circulating androstenedione concentrations are very high in female spotted hyenas during infancy, and they subsequently remain higher in females than males throughout development, though levels decline in females across ontogeny (Glickman et al., 1987, Glickman et al., 1992). It is especially telling that the divergence in ontogenetic trajectories for males and females occurs sometime between weaning (13.5 months) and sexual maturity (24 months), suggesting that neuroendocrine factors responsible for increased growth in females are somehow related to pubertal changes that result in adult sexual characteristics in this species (Figure 2.3). We suggest it is possible that androstenedione levels might act in a non-additive manner with other hormones to produce higher growth rates, or have organizational effects that set up conditions for faster growth during pubertal development. Addressing these, and related, physiological hypotheses should help us understand the mechanisms mediating

SSD in spotted hyenas and suggest specific hypotheses concerning the role that evolutionary history plays in contemporary patterns of SSD.

APPENDIX

Supplementary Materials for Chapter 2

Table A.1. Model comparison using AICc for different base growth models for univariate and multivariate size traits.

Univariate Mass Model

	AICc	df	dAICc	weight
Monomolecular	4068.1	4	0	0.917
Von Bertalanffy	4073.4	4	5.3	0.064
Gompertz	4077.2	4	9.1	0.010
German Gompertz	4077.2	4	9.1	0.010
Logistic	4090	4	21.9	< 0.001
Quadratic	4396.9	4	328.8	< 0.001
Linear	4721.3	3	653.2	< 0.001

Univariate Zygo To Top Crest Model

	AICc	df	dAICc	weight
Monomolecular	1941	4	0	0.234
Von Bertalanffy	1941.2	4	0.2	0.211
Gompertz	1941.3	4	0.3	0.198
German Gompertz	1941.3	4	0.3	0.198
Logistic	1941.8	4	0.8	0.158
Quadratic	2069.9	4	128.9	<0.001
Linear	2214.4	3	273.5	<0.001

Univariate Zygo To Back Crest Model

	AICc	df	dAICc	weight
Monomolecular	2156.9	4	0	0.393
Von Bertalanffy	2158	4	1.1	0.226
German Gompertz	2158.6	4	1.8	0.163
Gompertz	2158.6	4	1.8	0.163
Logistic	2160.8	4	4	0.054
Quadratic	2417.3	4	260.4	<0.001
Linear	2660.4	3	503.5	<0.001

Univariate Skull Length Model

	AICc	df	dAICc	weight
Von Bertalanffy	2479.5	4	0	0.226
German Gompertz	2479.6	4	0.1	0.214
Gompertz	2479.6	4	0.1	0.214
Monomolecular	2479.6	4	0.1	0.211
Logistic	2480.5	4	1	0.135
Quadratic	2900.4	4	421	<0.001
Linear	3214	3	734.6	<0.001

Table A.1 (cont'd).

Univariate Head Circumference Model

	AICc	df	dAICc	weight
Monomolecular	3089	4	0	0.443
Von Bertalanffy	3090.4	4	1.4	0.226
Gompertz	3091.2	4	2.2	0.149
German Gompertz	3091.2	4	2.2	0.149
Logistic	3094.2	4	5.2	0.034
Quadratic	3573.1	4	484.1	<0.001
Linear	3887.1	3	798.1	<0.001

Univariate Body Length Model

	AICc	df	dAICc	weight
Monomolecular	4269.4	4	0	0.332
Von Bertalanffy	4270.1	4	0.7	0.234
German Gompertz	4270.6	4	1.2	0.182
Gompertz	4270.6	4	1.2	0.182
Logistic	4272.5	4	3.1	0.069
Quadratic	4686.1	4	416.8	<0.001
Linear	4944.3	3	674.9	<0.001

Univariate Neck Circumference Model

	AICc	df	dAICc	weight
Monomolecular	3566.3	4	0	0.335
Von Bertalanffy	3567	4	0.7	0.232
Gompertz	3567.5	4	1.2	0.181
German Gompertz	3567.5	4	1.2	0.181
Logistic	3569.4	4	3.1	0.071
Quadratic	3864.8	4	298.5	<0.001
Linear	4108.6	3	542.3	<0.001

Univariate Girth Model

	AICc	df	dAICc	weight
Monomolecular	4279.5	4	0	0.572
Von Bertalanffy	4281.6	4	2.1	0.196
German Gompertz	4282.8	4	3.3	0.108
Gompertz	4282.8	4	3.3	0.108
Logistic	4286.7	4	7.2	0.015
Quadratic	4621.9	4	342.4	<0.001
Linear	4868	3	588.5	<0.001

Table A.1 (cont'd).

Univariate Front Foot Length Model

	AICc	df	dAICc	weight
Monomolecular	2357.2	4	0	0.255
Von Bertalanffy	2357.6	4	0.4	0.213
Gompertz	2357.8	4	0.5	0.194
German Gompertz	2357.8	4	0.5	0.194
Logistic	2358.4	4	1.1	0.144
Quadratic	2518.7	4	161.5	<0.001
Linear	2592.9	3	235.7	<0.001

Univariate Lower Leg Length Model

	AICc	df	dAICc	weight
Logistic	2502.2	4	0	0.294
German Gompertz	2502.8	4	0.7	0.212
Gompertz	2502.8	4	0.7	0.212
Von Bertalanffy	2503.2	4	1	0.176
Monomolecular	2504.2	4	2.1	0.105
Quadratic	2915	4	412.9	<0.001
Linear	3093.6	3	591.4	<0.001

Univariate Upper Leg Length Model

	AICc	df	dAICc	weight
Von Bertalanffy	2540.4	4	0	0.223
Monomolecular	2540.5	4	0	0.220
Gompertz	2540.6	4	0.1	0.209
German Gompertz	2540.6	4	0.1	0.209
Logistic	2541.4	4	1	0.138
Quadratic	2893	4	352.6	<0.001
Linear	3087.6	3	547.2	<0.001

Univariate Scapula Length Model

	AICc	df	dAICc	weight
Monomolecular	2585	4	0	0.232
Von Bertalanffy	2585	4	0.1	0.227
German Gompertz	2585.2	4	0.2	0.208
Gompertz	2585.2	4	0.2	0.208
Logistic	2586.2	4	1.2	0.126
Quadratic	2974	4	389	<0.001
Linear	3231.1	3	646.1	<0.001

Table A.1 (cont'd)

Univariate Shoulder Height Model

	AICc	df	dAICc	weight
Monomolecular	3508.8	4	0	0.429
Von Bertalanffy	3510.1	4	1.2	0.231
Gompertz	3510.9	4	2.1	0.153
German Gompertz	3510.9	4	2.1	0.153
Logistic	3513.9	4	5.1	0.033
Quadratic	4111.7	4	602.8	<0.001
Linear	4372	3	863.1	<0.001

Univariate Hind Foot Length Model

	AICc	df	dAICc	weight
Monomolecular	2368.6	4	0	0.241
Von Bertalanffy	2368.9	4	0.3	0.210
German Gompertz	2369	4	0.4	0.196
Gompertz	2369	4	0.4	0.196
Logistic	2369.5	4	0.9	0.157
Quadratic	2502.3	4	133.7	<0.001
Linear	2575.2	3	206.6	<0.001

Hypoallometric size model

	AICc	df	dAICc	weight
Monomolecular	-1383	4	0	0.22
Von Bertalanffy	-1382	4	0.1	0.205
German Gompertz	-1382	4	0.2	0.198
Gompertz	-1382	4	0.2	0.198
Logistic	-1382	4	0.4	0.178
Quadratic	-1171	4	211.2	<0.001
Linear	-1084	3	298.6	<0.001

Isometric size model

	AICc	df	dAICc	weight
Monomolecular	-922.2	4	0	0.287
Von Bertalanffy	-921.7	4	0.5	0.22
Gompertz	-921.4	4	0.8	0.189
German Gompertz	-921.4	4	0.8	0.189
Logistic	-920.4	4	1.8	0.115
Quadratic	-226.6	4	695.6	<0.001
Linear	77.2	3	999.3	<0.001

Table A.1 (cont'd).

Hyperallometric size model

	AICc	**df**	**dAICc**	**weight**
Monomolecular	-925.1	4	0	0.307
Von Bertalanffy	-924.4	4	0.7	0.218
German Gompertz	-924	4	1	0.185
Gompertz	-924	4	1	0.183
Logistic	-922.9	4	2.1	0.106
Quadratic	-503	4	422	<0.001
Linear	-225	3	700	<0.001

Table A.2. Results from t-tests on univariate male and female size traits. These data generally correspond to the plots in Figure 2.4 in the main text, but include all individuals measured for each trait individually, rather than only including individuals for whom all traits were measured.

	t	**d.f.**	**p**
Mass	7.771	228	**<0.001**
Zygo To Back Crest	2.152	263	**0.032**
Skull Length	4.004	269	**<0.001**
Girth	5.065	262	**<0.001**
Head Circumference	6.166	264	**<0.001**
Neck Circumference	5.314	261	**<0.001**
Zygo To Top Crest	2.027	264	**0.0437**
Body Length	1.751	268	**0.008**
Scapula Length	1.942	266	0.053
Shoulder Height	1.297	262	0.196
Upper Leg Length	0.573	266	0.567
Lower Leg Length	-0.086	267	0.931
Front Foot Length	-0.732	267	0.464
Hind Foot Length	-0.588	266	0.557

CHAPTER 3

Growth rates in spotted hyenas: socio-ecological determinants and life history consequences

Introduction

Growth rates represent critical life history traits and fitness components that, together with mortality rates and adult body size, profoundly influence intra- and inter-specific variation in ecology and life history (Case, 1978, Charnov, 2004, Zullinger et al., 1984). Interspecific variation in growth rates and growth trajectories influences the broader life history patterns of species, such as their position on the 'fast-slow' life history continuum (Gaillard et al., 1989, Promislow and Harvey, 1990, Read and Harvey, 1989, Dobson and Oli, 2007, Bielby et al., 2007), and reflect fundamental ecological factors like juvenile mortality (Stearns, 1992, Oli, 2004), adult mortality (Gasser et al., 2000) and maternal investment per offspring (Pontier et al., 1993). Many important fitness-related ecological activities, such as foraging and reproduction, require individuals to approach a minimum size. That is, variation in growth rate can affect individuals' ability to hunt (MacNulty et al., 2009), disperse (Nunes et al., 1998, Nunes et al., 1999), escape predation (Urban, 2007, Arendt, 1997, Werner and Gilliam, 1984, Day et al., 2002), or recruit into the breeding population at younger ages (McDonald et al., 2005, Arendt, 1997, Roff, 1992, Stearns, 1992).

Both the familial and extrinsic environments play crucial roles in shaping early morphological development in animals. Realized offspring growth rates are commonly influenced by a number of ecological factors such as local food abundance and climatic variables (Altmann and Alberts, 2005, McAdam and Boutin, 2003a). Familial variables such as the number of littermates competing for food resources provided by parents, and the rank relationships among those littermates, also commonly affect growth rates (e.g. Altmann and Alberts, 2005, Nunes et al., 1998, Scheel et al., 1977). In gregarious species growth rates may also be affected by other social variables such as dominance status. Dominance status may be especially critical in mammals whose societies are organized by linear dominance hierarchies, as social rank is these species commonly determines priority of access to food and other resources. Thus, in species exhibiting linear dominance hierarchies, we may expect maternal social rank to have profound effects on offspring growth rates (e.g. Altmann and Alberts, 2005).

Because growth rate is so important, the ability of parents to influence the growth rates of their offspring through maternal and other parental effects may have important ramifications for fitness (Mousseau and Fox, 1998, Gluckman et al., 2008). However, ecological and social variables cannot always be manipulated by parents, and may also have important fitness effects (e.g. Altmann and Alberts, 2005, Lindell, 1997). Here we document how ecological, familial, and social variables influence ontogenetic growth rates and adult body size in a large, long-lived mammalian carnivore, the spotted hyena (*Crocuta crocuta*). Specifically we inquire how growth rates and adult size are affected by variation in local prey abundance, maternal social rank, maternal parity, litter size, and intra-litter dominance rank of the offspring. Maternal social rank represents an aspect of the maternal environment that has extensive effects throughout the lifespan of spotted hyenas (Watts et al., 2009, Holekamp and Dloniak, 2009). Maternal parity influences growth rate in a number of mammals (Altmann and Alberts, 2005, Hendriks et al., 2009, Hyatt et al., 2007), and affects survival in spotted hyenas (Watts et al., 2009). Litter size is small in spotted hyenas; usually limited to one or two cubs (Kruuk, 1972, Mills, 1990, Holekamp et al., 1996). Within twin litters, dominance contests among littermates are often fierce, and begin shortly after birth (Frank et al., 1991, Smale et al., 1999, Wachter et al., 2002, Wahaj and Holekamp, 2006). These dominance contests among littermates are exceptionally intense among offspring of low-ranking mothers (Smale et al., 1999, Golla et al., 1999). This relationship between intensity and social rank suggests that intra-litter rank, like maternal social rank, may represent an important source of variation in access to food, and hence potentially a source of variation in growth rates among spotted hyenas (Wahaj and Holekamp, 2006, Hofer and East, 1996). In addition to identifying determinants of growth rates and adult size among hyenas, we also document the effects of growth rates themselves on the timing of life history milestones including age at weaning, first parturition among females, and dispersal among males.

Methods

Study Population

Spotted hyenas live in social groups called clans, which have been observed to contain as many as 120 individuals ordered in a linear dominance hierarchy in which females and their offspring are dominant to immigrant males (Frank, 1986, Smale et al., 1993). Offspring attain ranks immediate below that of their mother, with the youngest offspring outranking its older siblings (Holekamp and Smale, 1990, Engh et al., 2000). The linear dominance hierarchy defining female social rank contains multiple matrilines, each comprised of related females and their young (Engh et al., 2000, Frank, 1986, Kruuk, 1972, Holekamp and Smale, 1990, Holekamp and Smale, 1991, Smale et al., 1993). Rank relationships among matrilines are also linear, such that all females within a matriline have similar social ranks, with no overlap among matrilines (Holekamp and

Smale, 1990). Males usually emigrate to other clans to breed, whereas females are philopatric, and remain in their natal clan for life (Smale et al., 1997, Van Horn et al., 2003, Honer et al., 2007).

Females exhibit a number of sex-role reversed traits; among these, masculinized genitalia in the form of a peniform clitoris, termed the 'pseudopenis', is perhaps the most immediately apparent (Frank and Glickman, 1994, Frank et al., 1990, Glickman et al., 2006, Kruuk, 1972). Both male and female spotted hyenas are physiologically competent to breed at 24 months (Glickman et al., 1992), but both sexes generally delay reproduction for some months or years after puberty. Females generally bear their first litters between 3 and 5 years of age (Hofer and East, 2003, Holekamp et al., 1996), and birth of the first litter is assessed by the tearing of the female pseudopenis (Frank and Glickman, 1994).

Data Collection

Immobilizations were conducted in Kenya using Telazol (Fort Dodge Animal Health, Overland Park, KS; 6.5 mg/kg) administered via a lightweight plastic dart fired from a CO_2-powered rifle (Telinject Inc., Saugus, CA). Ages of most hyenas residing in their natal clans were determined based on known emergence dates from natal dens (+/- 7 days; Holekamp et al., 1996), and ages of other adults were based on patterns of tooth wear (+/- 6 months; Van Horn et al., 2003). During each immobilization several morphological measurements were obtained, including four cranial and nine postcranial measurements. Specifically, we measured scapula length, lower leg length, upper leg length, shoulder height, body length, girth at the chest, neck circumference, hind foot length, front foot length, skull length, head circumference, the distance between the zygomatic arch and the top of the sagittal crest, and the distance between the zygomatic arch and the posterior edge of the sagittal crest. A detailed description of each of these measures is given by Van Horn et al. (2003). We measured prey availability by counting all ungulates within a 100 meter range of two four-kilometer transects in the Masai Mara Game Reserve once every two weeks (three transects after 2001).

Socio-Ecological Predictors of Growth Rates

Because we performed a large number of analyses, using all 13 morphological measurements proved unwieldy, so we used a composite measure of body size. We performed a principal component analysis (PCA) on the 13 linear morphological measures, and calculated size as the scores from the first principal component axis. PCA was performed using the 'prcomp()' function in the 'stats' package in R v 2.14.1 (R Development Core Team, 2012). All further analyses were also performed in R v2.14.1. We fit our multivariate size measure to a mechanistic 'monomolecular' growth model using maximum likelihood estimation (MLE) in the 'bbmle' package (Bolker and R Development Core Team, 2012). Monomolecular growth describes a curve in which growth is most rapid initially, and growth rate continually decreases as it approaches an asymptotic value representing adult size (Monomolecular equation: $y = a^* (1- e^{(-b-(t0-t))})$ where t represents age). The 'a' parameter describes the asymptotic 'adult size', 'b' describes 'relative growth rate' representing the rate of approach to the asymptote, and 't0' describes the age at onset of growth. We used the monomolecular growth model because previous work has shown that the monomolecular growth model generally fit spotted hyena growth data better than other models, such as the logistic, Von Bertanlaffy, and Gompertz curves, as well a second-order polynomial curve (Tanner et al., 2010, Swanson et al., submitted). This is probably due to a combination of the extremely rapid early postnatal growth that young spotted hyenas experience, combined with the absence of prenatal individuals from our data set.

To test for the contribution of socio-ecological factors to growth rates in spotted hyena cubs, we took two approaches. First, we fitted a series of models using MLE in the 'bbmle' package in which we directly examined the contribution of matriline rank, litter size, and intra-litter rank to adult size and growth rate. Second, we fitted a series of multiple regressions with 'size-at-age' residuals for each individual taken from the basic 'null' growth model (see Figure 3.1) as a proxy for growth rate. The predictors of growth rate we tested in the multiple regressions include maternal rank, litter size and intra-litter rank characterized as a single 'litter type' variable with three possible categories, yearly average prey availability, and maternal parity. Although fitting parameters directly to the growth model is more biologically meaningful, and allows simultaneous estimation of the effect on growth rate and adult size, we experienced some model fitting problems when using all available predictors. As such, the multiple regressions are meant as an extension of our growth model analyses, allowing us to expand our range of questions.

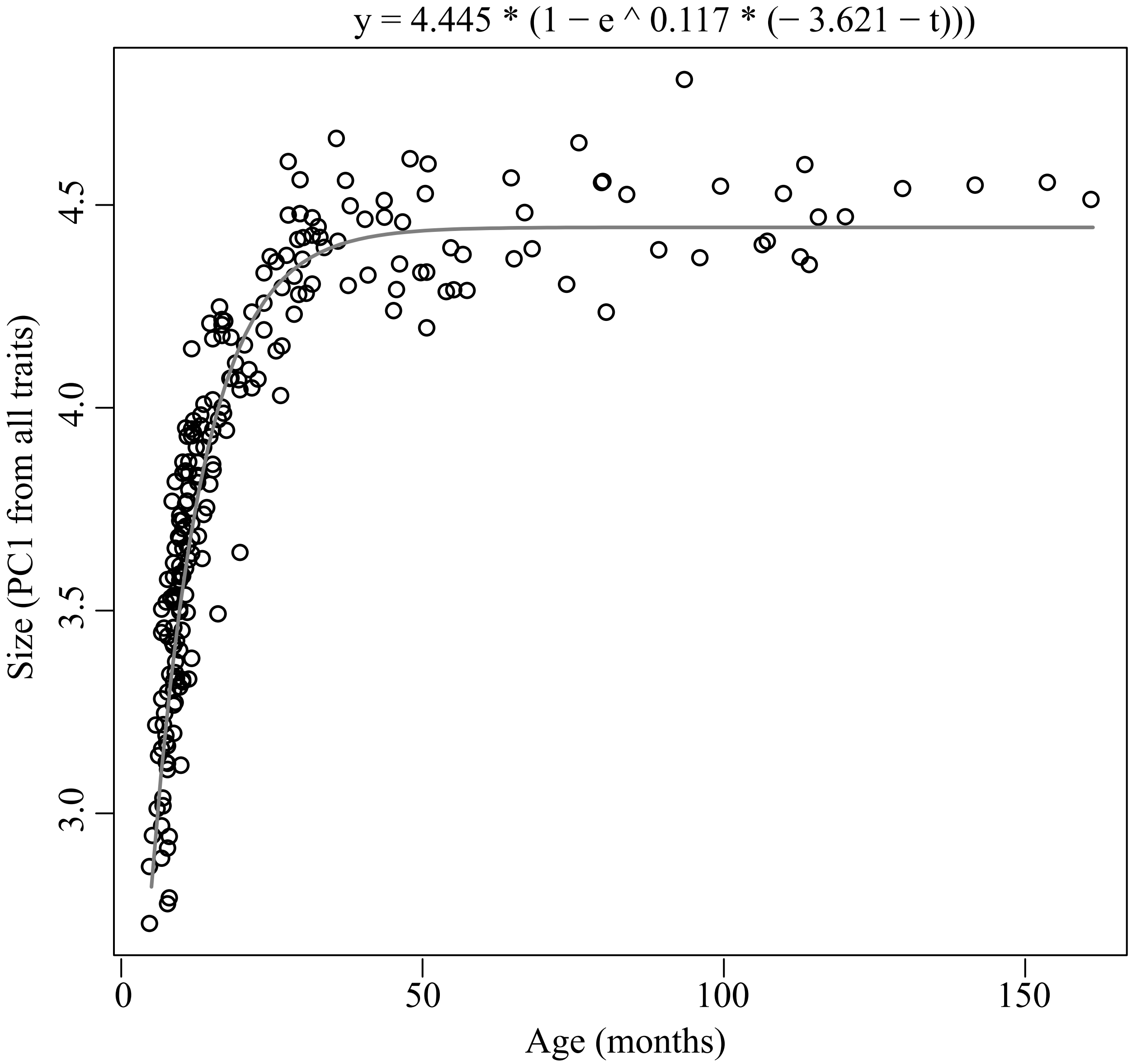


Figure 3.1. Age in months plotted against PC1 calculated from all linear morphological traits (our size measure), with fitted monomolecular model shown as the gray curve through the data. The null model is shown with no predictors fitted to relative growth rate or to adult size. The equation shown above the figure is parameterized with the MLE estimates from the null model. The model is fitted to data from 523 individuals darted a total of 621 times.

For the first, 'growth modeling', approach, we fit predictors using MLE directly to the parameters describing growth rate and adult size in the monomolecular growth models. Maternal rank was coded as a binary variable indicating whether or not the individual in question was a member of the highest ranking, or alpha, matriline. We chose to use a binary matriline rank measure rather than a continuous measure of social rank in this case because the method we used to fit the growth model was meant to fit categorical rather than continuous predictors. This binary matriline rank variable is biologically meaningful because the highest ranking matriline often comprises a large portion of the clan (Holekamp and Smale, 1990, Van Horn et al., 2004), and social rank determines priority of access to feeding sites (Tilson and Hamilton, 1984, Kruuk, 1972, Frank, 1986, Mills, 1990). Because of the linear nature of the social hierarchy in spotted hyenas, females in the highest ranking matriline are always the highest ranking females in a clan (Van Horn et al., 2004), reproducing more rapidly and successfully than other females (Holekamp et al., 1996, Hofer and East, 2003). Litter size for the first approach simply indicated whether the offspring was born in a singleton or twin litter, as seen upon first emergence from the natal den (Holekamp et al., 1996). Intra-litter rank, assigned based on outcomes of early fights between siblings, indicated whether the offspring was the dominant or subordinate cub in its litter. When coding values for intra-litter rank, we treated singleton cubs as dominant. We set the variables up this way because of model-fitting problems when trying to accurately estimate parameters for both adult size and growth rate when all three possible litter characteristics, including singleton, dominant twin, and subordinate twin, were fit separately. These model fitting problems were probably due to small sample sizes relative to the data-intensive nature of these analyses. However, fitting both litter size and intra-litter rank allowed for reasonable interpretation of our results.

To assess the relative importance of the different predictors of adult size and growth rate, we then estimated the second order, sample size corrected Akaike's Information Criterion (AICc; (Burnham and Anderson, 2002)) for each model. We subtracted the AICc of the best model from the AICc of all other models to calculate the AICc difference (dAICc) for each model. The best model is always the model with the lowest AICc, such that all calculated dAICc values are positive, except the dAICc for the best model, which is always 0. We also estimated the Akaike weights for each model, representing the likelihood of the model given the data, normalized relative to the other candidate models as a probability (Akaike, 1978, Burnham and Anderson, 2002). We then took the subset of models in which the dAICc was ≤ 2, renormalized the AICc weights, and performed model averaging (Burnham and Anderson, 2002).

We averaged parameter estimates across models in two ways. First, we estimated the model averaged effect of each variable, only including models in which the variable appeared. We used these model averages and their associated standard errors to perform z-tests, and calculated the weighted proportion of models in which the variables appeared using the AICc weights. These weighted proportions were termed 'variable importance'. The second model averaging technique we used was to calculate the 'shrinkage' estimator for each parameter by calculating a weighted average of the parameter estimates across all models with a dAICc ≤ 2, this time including those models in which the variable was not found, and weighting the parameter estimates by the AICc weights. Variables not found in a model were assigned an effect size of '0' for this approach. Thus, if a variable is only found in one candidate model, its estimate will be biased toward 0, or 'shrunk' when using this method, encouraging relative comparisons among parameter estimates. The two methods are simply different ways of approaching multi-model inference.

Although fitting predictors to the parameters of the growth models themselves is more directly biologically meaningful, we also addressed the relative contributions of socio-ecological and maternal predictors to growth rate using individual residuals from the 'null' growth model as a response variable. These individual residuals represent an individual's size relative to the predicted size of an average individual at that age ('size-at-age') and represent a useful proxy for growth rate. The null growth model is the model fit with no predictors (see Figure 3.1). We fitted general linear models with a normal error distribution, with this measure of growth rate as the response variable, and as predictors we included maternal social rank as a continuous variable, maternal parity, 'litter type', and average yearly prey availability. We used maternal social rank as a continuous variable here rather than matriline rank both to help compare to previous work (e.g. Holekamp et al., 1996), and to establish whether the effect of the matriline rank is similar to that of maternal social rank. The major benefit of this general linear modeling approach is that it is much less data-intensive, allowing us to fit more predictor variables without optimization problems. Maternal parity was defined as either primiparous, meaning that the cub in question represented the mother's first offspring, or multiparous, suggesting that the cub was not the first offspring born to the mother. Litter type was parameterized with three categories: singleton, dominant twin, and subordinate twin, testing the combined contributions of litter size and intra-litter rank more directly. Finally, average yearly prey availability was calculated as the mean prey availability during the year in which the cub was born. All predictor variables were mean centered and standardized by dividing by the standard deviation of the sample before analysis. We used the same approaches for model selection and multimodel inference as described above, but used the 'MuMIn' package in R (Barton, 2012).

Life History Consequences of Growth Rate Variation

To test for the long-term consequences of growth rates during ontogenetic development of spotted hyenas, we took individual residuals from the null model for growth (Figure 3.1; the model with no predictors of growth rate or adult size) for individuals younger than 24 months. We fitted these size-at-age measures, representing a useful proxy for 'growth rate', as a predictor of the timing of three important life history events: weaning age, age at first parturition for females, and age at dispersal for natal males. We also included other predictors that we expected to be important in these models, including sex (for age at weaning only), maternal social rank, offspring age at the time of measurement ('Age'), and the age of the mother upon birth of the offspring ('Mom age at birth'). Maternal social rank was included because it has previously been shown to play a role in determining both weaning age and age at first parturition (Holekamp et al., 1996). Age was included so that we could test for an interaction between age and growth rate, which might suggest that the size-at-age (our growth rate measure) for an individual is more important at some ages than others.

For analyses in which the response variables were age at dispersal and age at first parturition we did not fit age independently of the interaction between age and growth rate. We decided not to consider such models containing age as a predictor without the interaction between age and growth rate on an *a priori* basis because age at dispersal and age at first parturition are both life history events that take place after 24 months. Because all morphological data we used for these analyses were from individuals under 24 months of age, it was unlikely that the age at which an individual was measured as a juvenile would influence life history milestones long after 24 months of age. Instead our goal in fitting age and an interaction between age and growth rate was to allow for variation in the importance of growth rate at different stages of development. We decided *a priori* to allow age to be fit independent of the interaction between age and growth rate for the models with weaning age as a response variable due to the possibility that the event of weaning itself influences growth rate. We also included an interaction effect between rank and growth rate, to test for the possibility that growth rate plays a more important role in the timing of life history events for individuals of lower rather than higher social rank. We used the same model averaging procedures for these models as we did for predictors of growth rate and adult size. However, we automated the process as we did when using the general linear modeling approach to test predictors of growth rate, using the 'MuMIn' package for model averaging and multi-model inference (Barton, 2012). Sample size for these models was 123 natal cubs for age at weaning, 37 natal females measured as cubs for age at first parturition, and 36 natal males measured as cubs for age at dispersal.

Results.

When explicitly fitting predictors to growth rate and adult size as part of a monomolecular growth model, we found strong support for both matriline rank and intra-litter rank as contributors to offspring growth rate (Table 3.1; Table 3.2). Specifically, when fitting predictors to growth rate and adult size, all models with a dAICc of two or less contained both matriline rank and intra-litter rank as predictors of growth rate. Our results thus suggested that subordinate cubs grow slower than dominant and singleton cubs (Table 3.2), and that members of the highest-ranking (alpha) matriline grow more rapidly than members of other matrilines. Litter size was not present as a predictor of growth rate in any model with a dAICc of two or less, therefore parameter estimates for the effect of litter size on growth rate were not included in model averaging. Litter size, intra-litter rank, and matriline rank as predictors of adult size were each present in one model with a dAICc of two or less, but their variable importance was low (the largest was 0.055; see Table 3.2), and the model averaged estimates were not significant for any of the three variables as predictors of adult size. Thus, both singleton cubs and dominant members of twin litters appear to grow more rapidly than subordinate members of twin litters, as do individuals from the highest ranking (alpha) matriline. However, none of these variables appear to influence adult size, suggesting that individuals from low-ranking matrilines and subordinate cubs from twin litters both experience 'catch-up' growth, continuing to grow until they reach the same adult size as dominant cubs and members of the alpha matriline.

For our second approach to testing familial, social, and ecological predictors of growth rate, we used size-at-age residuals from the growth model as our response variable. We found that maternal social rank is positively related to growth rate

Table 3.1. AICc table from model selection with *n* = 225. Models shown in table are those with a dAICc of two or less. Variables included in a model are indicated by an X in the respective column. In regard to our response variables, 'GR' stands for growth rate, and 'AS' stands for adult size. Lit. Size represents litter size, Lit. Rank represents intra-litter rank, and Mat. Rank represents matriline rank category.

	Lit. Size		**Lit. rank**		**Mat. Rank**					
	AS	**GR**	**AS**	**GR**	**AS**	**GR**	**AICc**	**df**	**dAICc**	**weight**
Model 1				X		X	-172.4	6	0	0.19553
Model 2	X			X		X	-171.1	7	1.4	0.09799
Model 3				X	X	X	-170.6	7	1.9	0.0768
Model 4			X	X		X	-170.5	7	2	0.07295

Table 3.2. Results from model averaging for the effect of litter size, intra-litter rank, and matriline rank on adult size, and intra-litter rank and matriline rank on relative growth rate with *n* = 225. Shrinkage estimator represents the slope estimate reached by calculating weighted averages across all models for each variable, and treating the parameter estimate for a variable for a model in which the variable is not found as 0. Non-shrunk estimators are the effect of the parameter averaged across models with a dAICc of 2 or less in which the model is present. Variable importance indicates the weighted proportion of these models in which the variable is actually present. 'Unconditional SE' represents the standard error for the weighted non-shrunk parameter estimate. P-values statistically significant at an alpha = 0.05 are bolded, and noted with an asterisk.

Response variable	**Adult Size**			**Relative Growth Rate**	
Predictor variable	**Lit. Size**	**Lit. Rank**	**Mat. Rank**	**Lit. Rank**	**Mat. Rank**
Shrinkage estimators	-0.0080	-0.0027	-0.0037	-0.0073	0.0082
Non-shrunk Estimator	-0.0362	-0.0164	-0.0214	-0.0073	0.0082
Unconditional SE	0.0418	0.0409	0.0417	0.0027	0.0028
z	-0.8663	-0.4018	-0.5130	-2.7209	2.8737
p	0.3863	0.6878	0.6080	**0.0065***	**0.0041***
Variable Importance	0.055	0.041	0.043	1	1

(Table 3.3; Table 3.4; Figure 3.2). In addition, subordinate twin cubs suffered a reduction in growth rate relative to that of their dominant twins and cubs from singleton litters (Table 3.4; Figure 3.3). Finally, mothers who were caring for their first litter had lower offspring growth rates than experienced mothers (Table 3.4; Figure 3.4). Surprisingly, however, and in contrast to some similar studies (Altmann and Alberts, 2005), we found no effect of prey availability on growth rate (Table 3.3; Table 3.4). We used the same criteria to determine variable importance and statistical significance for these analyses as we did for those in which the variables were fit as predictors of growth rate or adult size of the Monomolecular growth model. All tests in common between the two approaches gave the same results.

We found that offspring with the highest-ranking mothers were weaned at the youngest ages, and that even accounting for variation in maternal rank, individuals that grew most rapidly during their first 24 months of life also weaned earliest. Both variables were included in all four models with a dAICc ≤ 2 (Table 3.5), thus each variable had a relative importance of one (Table 3.6). Also, model averaged estimates and standard errors indicated that these estimates for both maternal rank and growth rate were statistically significant at an $\alpha = 0.05$ (Table 3.7). In addition, the magnitude of parameter estimates was quite large: increasing maternal rank by one standard deviation (SD) led to a predicted reduction of almost nine months for weaning age, and a one SD increase in offspring size predicted a reduction in weaning age of approximately one month. Given that the average weaning age in our sample was just

Table 3.3. Model selection table for predictors of growth rate using a linear modeling approach with 'size-at-age' residuals as the response variable. Models given are those with a dAICc <= 2. Models estimated are given in the first column, with model degrees of freedom, log likelihoods, second-order AIC values, dAICc estimates, and Akaike weights, and *n* = 138.

	df	***logLik***	***AICc***	***Delta***	***Weight***
y~LitterType+Parity+Rank	6	35.704	-58.795	0.000	0.575
y~PreyAvail+LitRank+Parity+Rank	7	36.507	-58.190	0.605	0.425

Table 3.4. a) Model averaged effects of maternal and ecological variables. a) Estimates include litter type coded as singleton, dominant twin, or subordinate twin; maternal parity (primiparous or multiparous), maternal social rank, and total prey counts averaged by the year. Maternal social rank is coded here such that higher values indicate increasing rank. Model averaged standard errors are also given, along with shrinkage estimators, z-tests, and variable importances. Sample size here is 138. b) Remaining contrasts for litter type, specifically including the comparison of the subordinate twin with the dominant twin missing from part a. P-values statistically significant at an alpha = 0.05 are bolded, and noted with an asterisk.

a) Model with Singleton cubs set as intercept

	β	***SE***	***SE (adj.)***	***z***	***p***	***β (w/ shrinkage)***	***Importance***
Intercept	0.052	0.055	0.056	0.932	0.351	0.052	-
Parity (primiparous)	-0.113	0.050	0.050	2.236	**0.025***	-0.113	1
Rank	0.052	0.016	0.016	3.195	**0.001***	0.052	1
Game	-0.020	0.016	0.016	1.233	0.218	-0.008	0.424
Dominant Twin	-0.029	0.060	0.061	0.480	0.631	-0.029	1
Subordinate Twin	-0.126	0.061	0.062	2.043	**0.041***	-0.126	1

b) Same model with dominant twin set as intercept (all other estimates are identical)

	β	***SE***	***SE (adj.)***	***z***	***P***	***β (w/ shrinkage)***	***Importance***
Intercept	0.023	0.024	0.024	0.962	0.336	0.023	1
Singleton	0.030	0.060	0.061	0.480	0.631	0.029	1
Subordinate Twin	-0.097	0.034	0.034	2.823	**0.005***	-0.097	1

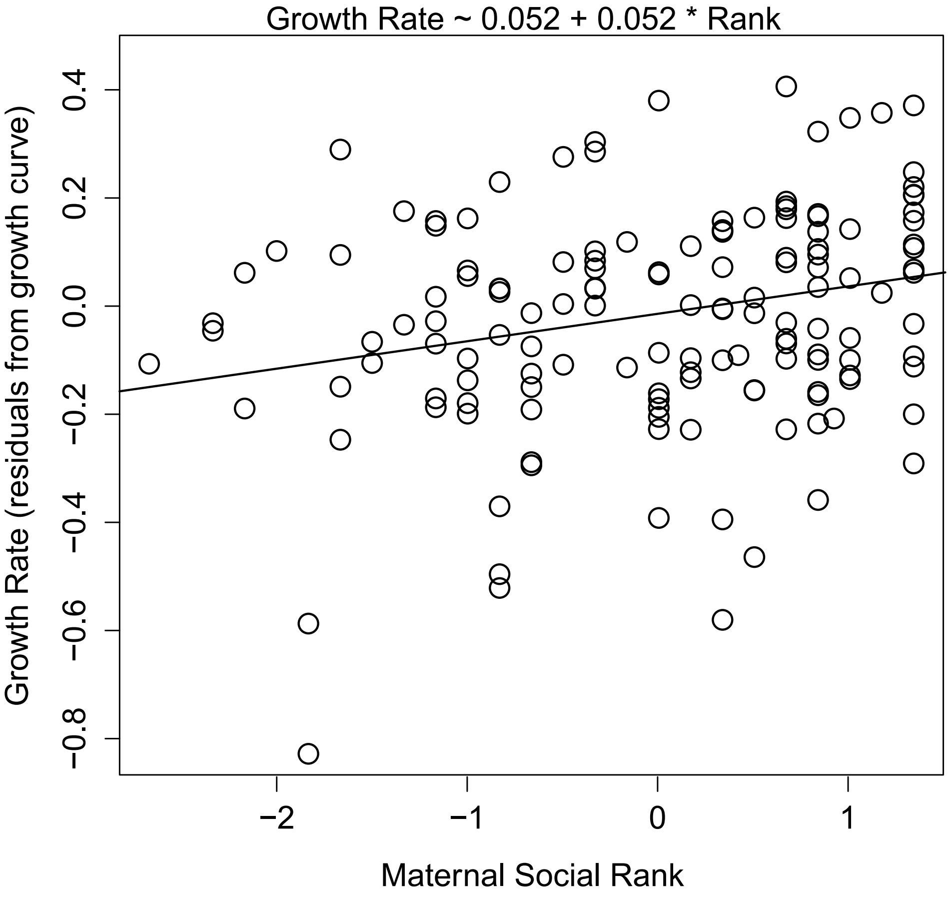


Figure 3.2. Regression of growth rate on maternal social rank. Maternal social rank is coded here such that the highest-ranked females and natal males exhibit the greatest rank. The linear equation describing the relationship between maternal social rank and growth rate is given immediately superior to the plot. Sample size here is 138.

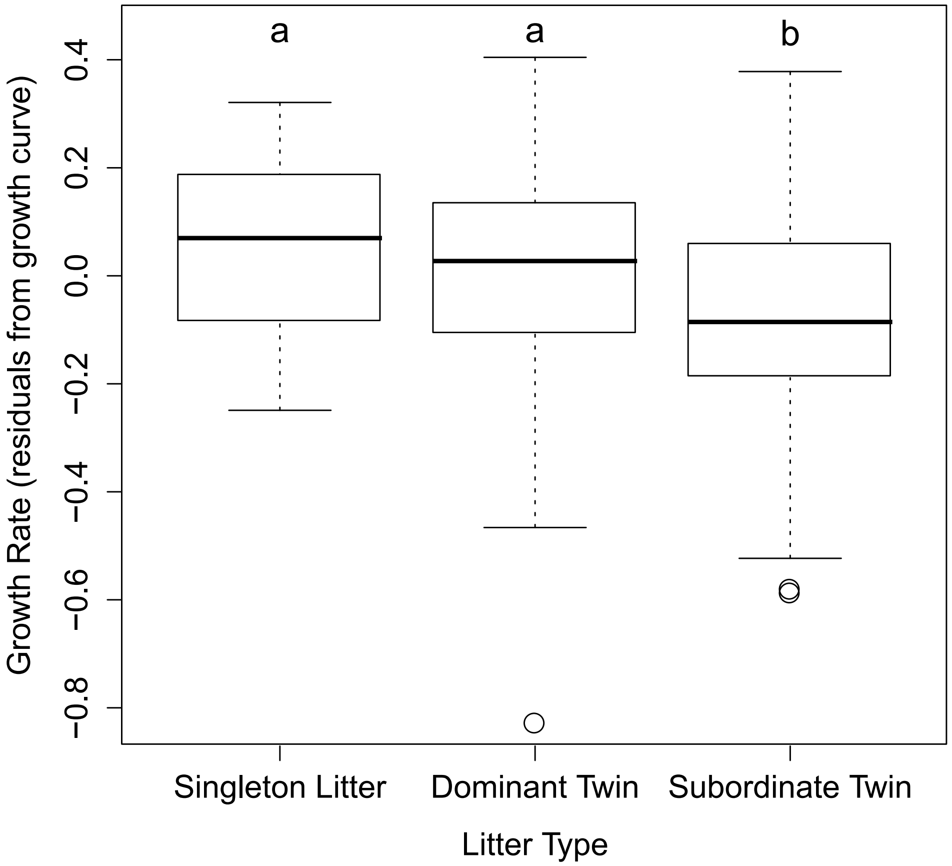


Figure 3.3. Box-and-whisker plot showing the relationship between litter type and growth rate for cubs from singleton litters, dominant twins, and subordinate twins. Growth rates for subordinate twins (group 'b') are significantly reduced relative to those of cubs from singleton litters or dominant twins (group 'a') at an α = 0.05. Growth rates for dominant twins and cubs from singleton litters are not significantly different at an α = 0.05. Sample size in plot is 138.

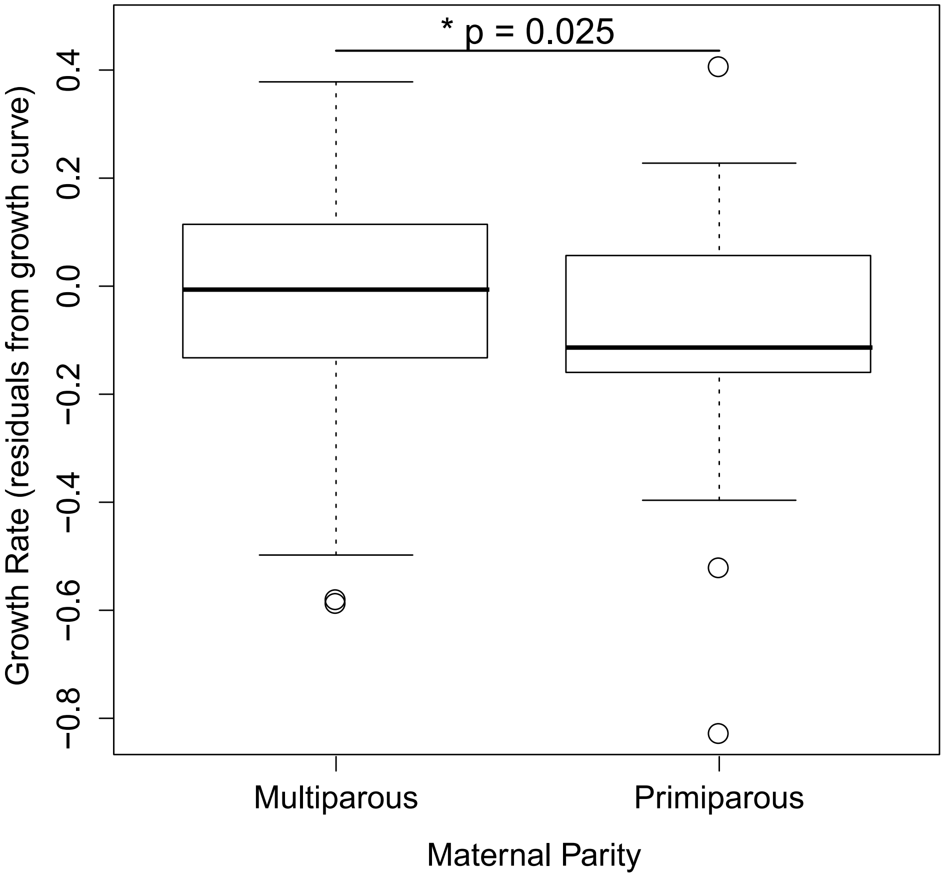


Figure 3.4. Box-and-whisker plot showing the relationship between maternal parity and growth rate. Primiparous mothers caring for their first litter have offspring that exhibit significantly lower growth rates than the offspring of multiparous mothers at an $\alpha = 0.05$.

Table 3.5. Best models for weaning age. All models predicting age at weaning with a dAICc ≤ 2 and *n* = 123. 'df' are the model degrees of freedom (number of parameters + 1), logLik is the log likelihood of the model, AICc represents the second order AIC, corrected for sample size, that is generally preferred (Burnham and Anderson, 2002), dAICc is the difference between the model in question and the best model, with higher values indicated a poorer model fit. Finally, the model weight represents the relative support for the model used in model averaging.

	df	***logLik***	***AICc***	***δAICc***	***Weight***
y~Rank+GR	4	-306.238	620.810	0.000	0.423
y~Rank+GR+MomAgeAtBirth	5	-305.773	622.050	1.240	0.228
y~Age+Rank+GR	5	-305.971	622.446	1.637	0.187
y~Rank+GR+Sex	5	-306.114	622.733	1.923	0.162

Table 3.6. Variable Importance. Variable importance for each variable in each model with growth rate as a predictor. Variable importance is calculated as a weighted proportion of the number of candidate models in which the variable is present. For example, variable importance of one indicates that the variable is present in every model with a dAICc ≤ 2, with *n* = 123. Values with high variable importance are bolded.

	a) Weaning Age	b) Age at 1st Parturition	c) Age at Dispersal
Age	0.187	-	-
Mom Age At Birth	0.228	0.000	**1.000**
Rank	**1.000**	**1.000**	0.000
Growth Rate	**1.000**	0.000	**0.697**
Sex	0.162	-	-
Age:Growth Rate	0.000	0.000	0.000
Rank:Growth Rate	0.000	0.000	0.000

Table 3.7. Model averages for models with weaning age as a response variable. *β* represents the non-shrunk parameter estimate calculated using model averaging. *β* (w/ shrinkage) represents the shrinkage estimator for the parameter. *SE* represents the standard error from the parameter estimate, with *n* = 123.

	β	***SE***	***Adj. SE***	***z***	***p***	***β (w/ shrinkage)***
Intercept	12.698	0.281	0.284	44.759	<0.001	12.698
Maternal Rank	-0.705	0.266	0.268	2.627	0.009	-0.705
Growth Rate	-0.958	0.260	0.262	3.651	<0.001	-0.958
Mom Age At Birth	0.264	0.277	0.280	0.941	0.346	0.060
Age	0.190	0.264	0.267	0.713	0.476	0.036
Sex (male)	0.252	0.515	0.520	0.485	0.628	0.041

under 13 months (Table 3.7; estimate in previous work was 13.5 months (Watts et al., 2009)), these are fairly substantial effects. The mother's age at the birth of the cub, individual age at measurement, and individual sex each appeared in one model with a dAICc of two or less, but all had a weighted variables importance of ≤ 0.228 (Table 3.6), and none were found to be statistically significant after model averaging (Table 3.7) Finally, the effect sizes for each when estimated with shrinkage were very small (Table 3.7); changes of 1 SD for each results in less than a 2 day predicted change in weaning age. The small magnitude of the shrinkage estimates are simply another indication that the variables are unimportant as predictors of weaning age. Finally, neither the interaction between age at measurement and growth rate, or that between rank and growth rate appeared, in any candidate model with a dAICc ≤ 2.

Increasing maternal social rank is associated in females with younger age at first parturition (Table 3.6; Table 3.8; Table 3.9): the variable importance for maternal rank is one, meaning that it is in every model with a dAICc ≤ 2, the magnitude of the estimate is large, and it is statistically significant. There is in fact only one model with a dAICc ≤ 2, and social rank is the only variable included in that model. Thus, we found no support for growth rate, maternal age at birth of the litter, an interaction between age measured and size, or an interaction between rank and size having an effect on age at first parturition (Table 3.6; Table 3.8). However, our data supported previous work with our population demonstrating an effect of social rank on age at first parturition (Holekamp et al., 1996).

Table 3.8. Best models for age at first parturition. All models predicting age at first parturition with a dAICc ≤ 2, with *n* = 37. Column names are the same as in Table 3.5.

	df	***logLik***	***AICc***	***δAICc***	***Weight***
y~Rank	3	-134.853	276.432	0.000	1.000

Table 3.9. Model averaging for age at first parturition. Model averages for models with age at first parturition as a response variable. *β* represents the non-shrunk parameter estimate calculated using model averaging. *β* (w/ shrinkage) represents the shrinkage estimator for the parameter. *SE* represents the standard error from the parameter estimate, with *n* = 37.

	β	***SE***	***Adj. SE***	***z***	***p***	***β (w/ shrinkage)***
Intercept	43.009	1.536	1.592	27.019	<0.001	43.009
Rank	-5.220	1.587	1.644	3.176	0.001	-5.360

Table 3.10. Best models for age at dispersal. All models predicting age at dispersal with a dAICc ≤ 2, with *n* = 36. Column names are the same as in Table 3.5.

	df	**logLik**	**AICc**	**δAICc**	**Weight**
y~MomAgeAtBirth + GR	4	-133.591	276.432	0.000	0.697
y~MomAgeAtBirth	3	-135.687	278.102	1.670	0.303

Table 3.11. Model averaging for age at dispersal. Model averages for models with age at dispersal as a response variable. *β* represents the non-shrunk parameter estimate calculated using model averaging. *β* (w/ shrinkage) represents the shrinkage estimator for the parameter. *SE* represents the standard error from the parameter estimate, with *n* = 36.

	β	***SE***	***Adj. SE***	***z***	***p***	***β (w/ shrinkage)***
Intercept	44.000	1.555	1.612	27.295	<0.001	44.000
Mom Age At Birth	-4.038	1.837	1.894	2.132	0.033	-4.038
Resids	-3.493	1.729	1.793	1.948	0.051	-2.436

We found strong evidence that males with older mothers disperse at younger ages: the variable importance was one, the estimate was statistically significant with model averaging, and the averaged estimate suggested a predicted decrease in dispersal age of almost 10% associated with a one SD increase in maternal age at birth (Table 3.6; Table 3.10; Table 3.11). Our data also suggest that more rapidly growing males disperse earlier; variable importance was 0.697, and the pattern was nearly statistically significant at an $\alpha = 0.05$ ($p = 0.051$, Table 3.6; Table 3.11). The magnitude of effect was nearly the same as maternal age at birth with a one SD increase in growth rate resulting in about a 7.5% decrease in dispersal age (a little over 5% using the shrinkage estimate).

Discussion

Our results suggest that the rank positions occupied by an individual spotted hyena in its social group and in its family have important fitness consequences that can persist throughout the individual's life. Our results contribute to a number of previous studies demonstrating the profound effects of social and familial rank in spotted hyenas (e.g. Kruuk, 1972, Holekamp et al., 1996, Watts et al., 2009, Holekamp et al., 1997). The critical role of dominance rank seems especially pervasive in the data presented here. Both an individual's rank in society and the nature of an individual's relationship with its own littermate strongly influence growth rate, and thus subsequently the timing of successive life history events such as age at weaning age and age at dispersal. Furthermore, we demonstrate that maternal social rank affects both weaning age and age at first parturition, independent of growth rate. We do not, however, find an effect of prey availability on growth rates. Considering the importance of food availability in other mammals that also exhibit strong maternal effects (Altmann and Alberts, 2005), it is particularly interesting that prey availability does not seem important to growth rates in spotted hyenas.

Instead, social and familial factors are crucial determinants of growth rate in spotted hyenas. For social factors, matriline rank and maternal social rank both affect growth rate similarly. Specifically, members of the alpha matriline, as well as females with higher linear social rank, both exhibit more rapid growth than their lower-ranking counterparts. For familial factors, our results suggest that subordinate members of twin litters suffer a reduction in growth rate relative to that of either their dominant twins or cubs from singleton litters. The fact that litter size and intra-litter rank only matter for subordinate twins suggests that mothers are unable to fully provision two cubs, and in twin litters allow the dominant twin priority of access for nursing. The lower growth rate of subordinate cubs may be a first-order result of poorer access to nutrients, which can directly reduce growth hormone levels (Clemmons and Underwood, 1991, McGuire et al., 1992, Straus, 1994). Alternatively, lower growth rates among subordinate twins might represent an adaptive modification of offspring phenotype by mothers to reduce growth rate under low food conditions, in order to reduce starvation risk (Gotthard et al., 1994). Interestingly, the lower growth rate experienced by a young hyena associated with its status as the subordinate member of a twin litter is similar in magnitude to the difference in growth rate between members of the alpha matriline and those of lower-ranking matrilines (Table 3.2). Given the well-documented importance of maternal social rank, intra-litter rank thus represents a critical source of growth-related variation in the early life of a spotted hyena, worth the potential costs of intra-litter dominance contests (Frank et al., 1991, Smale et al., 1999, Wachter et al., 2002, Wahaj and Holekamp, 2006, Golla et al., 1999).

'Catch-up growth' is a process in which some individuals grow for a longer period of time than they would under standard conditions as a mechanism to make up for unfavorable environmental conditions (Metcalfe and Monaghan, 2001, Hector and Nakagawa, 2012, Wilson and Osbourn, 1960). Catch-up growth engenders less physiological or immediate fitness costs relative to the related, but more rapid, 'compensatory growth' (Roff, 1992, Stearns, 1992, Arendt, 1997, Dmitriew, 2011). Documented costs of compensatory growth include oxidative stress (Metcalfe and Monaghan, 2003, Mangel and Munch, 2005, De Block and Stoks, 2008), and reduced survival due to increased starvation risk (Arendt, 1997, Blanckenhorn, 2000). Catch-up growth still likely carries fitness costs in terms of a protracted pre-reproductive period, but should theoretically not have severe physiological costs, and might in fact be beneficial in some environments (Hector and Nakagawa, 2012, Oli et al., 2002, Wilbur and Rudolf, 2006).

The slow growth associated with low priority of access to food both in the form of low maternal social rank and the subordinate rank for twin litters, appears to be overcome during a prolonged period of 'catch-up' growth in spotted hyenas. In both cases, this 'catch-up' growth eventually results in attainment of the same adult size as their higher ranking peers. Instances of 'catch-up' growth are often seen when the fitness benefits of reaching a critical adult size outweigh the potential costs of prolonged growth. Swanson et al. (2011) demonstrated higher fitness in female spotted hyenas with larger adult size for a number of morphological traits, and in Chapter 5 we show that larger individuals of both sexes have higher fitness for some morphological traits. These results suggest possible benefits of catch-up growth despite the delayed achievement of adult size with which such growth is associated. Costs for catch-up growth are generally thought to be expressed in the form of life history tradeoffs, because this requires that recruitment into the breeding population be delayed. Interestingly, although we found evidence for potential second order influences of growth rate on weaning age and age at dispersal, we did not find such influences on age at first parturition among females. It remains to be seen if more subtle, but equally pervasive life history consequences, such as the relatively poor survival within a female's first litter, can be explained in part by variation in growth rate.

Such pervasive fitness consequences of growth rate variation are common, but generally vary greatly among species because they are strongly dependent upon the ecological context of the species in question. For example, rapid growth is generally good because it allows juveniles to outgrow some predators (Urban, 2007, Mittelbach, 1984, Reznick, 1983, Spitze, 1991, Werner and Gilliam, 1984, Day et al., 2002), acquire food or territories (McAdam and Boutin, 2003b, McDonald et al., 2005), overcome seasonal constraints (Sibly et al., 1985, Dmitriew, 2011) and breed at a younger age (Blanckenhorn, 2000, McDonald et al., 2005). At the same time, excessively rapid growth can have physiological costs (Arendt, 1997, Dmitriew, 2011, Dantzer and Swanson, 2012), as well as survival costs due to nutrition limitation or starvation (Arendt, 1997, Gotthard et al., 1994, Blanckenhorn, 2000, Urban, 2007, Dmitriew, 2011). Furthermore, such growth can lead to increased predation on larger individuals due to greater visibility or the increased foraging effort required to sustain the growth (Dmitriew, 2011, Blanckenhorn, 2000). Rapid growth can also delay reproduction in species that are not able to immediately switch from growth to reproduction with full efficiency (Arendt, 1997).

We document two primary effects of growth rate on subsequent life history events in spotted hyenas that may be important to variation in fitness. First, increased growth rate results in earlier weaning, suggesting that one cue mothers might use to determine cessation of lactation is in some way related to body size of the offspring independent of the mother's rank. Although it is not fully clear how early weaning might benefit offspring, variation in the timing of this life history event probably represents an important contribution to maternal fitness. That is, early weaning reduces the length of the inter-litter interval, which is an important fitness component influenced both by maternal size (Swanson et al., 2011) and maternal social rank (Swanson et al., 2011, Holekamp et al., 1996). Age at first parturition in females is similarly related to rank, but interestingly, is unrelated to early-life growth rate. Age at dispersal, unlike weaning age and age at first parturition, does not appear to be under strict control by maternal rank independent of growth rate. However, more rapidly growing males do appear to disperse early, as do males with older mothers. Males that reach a larger size at an earlier age should be able to hunt and escape predators better at an earlier age, making early dispersal a viable possibility. There are clear benefits to early dispersal because males queue to gain social rank in the clan into which they emigrate, and because their social rank in the new clan is strictly determined by their tenure there (East and Hofer, 2001, Holekamp et al., 1997, Holekamp and Smale, 1998). Furthermore, previous research suggests that males generally do not breed for a year or two after arriving in the new clan (Szykman et al., 2001, Curren, 2012). Therefore, an earlier start in a new clan might have important fitness consequences for males by resulting in an earlier onset of reproduction, and all else being equal, greater lifetime fitness. The mechanism by which maternal age might influence age at dispersal for males is not fully clear, but we can suggest several possible explanations. First, because cumulative mortality probabilities increase with maternal age, older mothers will be more likely to die sooner, and thus dispersal may be triggered in some males upon their mother's death. Second, more experienced mothers may be better at resource acquisition, which may contribute to male preparedness for dispersal in ways independent of size. Hence, variation in dispersal age resulting from growth rate and maternal age are two important ways in which maternal effects can persist throughout life even for males that emigrate to other social groups.

Our results suggest that maternal parity and social rank, as well as priority of access to milk, contribute to variation in spotted hyena growth rate. Previous work has shown that both social rank and intra-litter rank affect survival in cubs. Because rank and intra-litter rank influence growth rate, and increased growth rates contribute both to more rapid weaning for cubs, and to earlier dispersal for males, our results elucidate new direct and indirect fitness consequences that persist throughout the lives of offspring. Interestingly, it appears that the very mechanisms used by offspring to mitigate some potential deleterious fitness consequences of unfavorable conditions early in life, such as prolonging the growth period to reach the same critical adult size, may have fitness consequences of their own, at least for males, in the form of a later start at breeding. A number of interesting questions remain, however. For example, there is some evidence that mechanisms exist for female spotted hyenas to selectively alter offspring sex ratios (Holekamp and Smale, 1995). If litter size can be similarly modified in response to environmental conditions, in some cases females may be able to effectively 'plan' for the future environment of their offspring by selectively retaining the sex and number of offspring that will maximize inclusive fitness. Finally, the full range of potential life history consequences faced due to prolonging the growth period is yet to be determined. Similarly, the more rapid growth rate of high ranking littermates and matrilines may carry their own life history and physiological consequences, such as the oxidative damage and reduced lifespan associated with rapid growth rate (Dantzer and Swanson, 2012, Bartke, 2005, Berryman et al., 2008, Holzenberger et al., 2003, Kappeler et al., 2008).

CHAPTER 4

Swanson, E.M., I. Dworkin, and K.E. Holekamp. 2011. Lifetime selection on a hypoallometric size trait in the spotted hyena. *Proceedings of the Royal Society B: Biological Sciences* **278** (1722) 3277-3285. doi: 10.1098/rspb.2010.2512

Introduction

Both body size and morphological scaling relationships are critical factors in ecology and evolution, as they are central to energetics, niche partitioning, life history strategies, reproduction (e.g. (Stearns, 1992, Peters, 1983, Schmidt-Nielsen, 1984, Calder, 1984)) and ultimately fitness (Blanckenhorn, 2000, Bonner, 2006). However, selection on size can be complex. Consistent positive sexual selection is common among male mammals for access to females, or for control of resources required by females, and is hypothesized to result in the observed pattern where males are generally larger than female conspecifics. [7, 8]. In many classes of animals, females are often larger than males, likely because fecundity increases with increasing body size [9]. By contrast, female mammals are predicted to be smaller than males due to a tradeoff between somatic growth and reproduction [7]. Deviations from these common patterns likely result either from relaxation of widespread selection, the imposition of novel selection, or both.

Selection analyses are most reliable when performed on lifetime fitness data (Clutton-Brock, 1991, Arnold and Wade, 1984, Endler, 1986, Lande and Arnold, 1983). Lifetime reproductive success (LRS) is often considered a gold standard for measuring fitness (Clutton-Brock, 1991, Endler, 1986), performing well even against rate-sensitive measures of lifetime fitness such as lambda (Brommer et al., 2004). However, estimates of lifetime selection remain rare due to the difficulty of conducting long-term evolutionary studies, particularly those involving large carnivorous mammals (see (Kingsolver et al., 2001) and references therein). We use LRS data from a long-term field study to evaluate selection on size-related traits among female spotted hyenas (*Crocuta crocuta*); spotted hyenas exhibit female-biased sexual size dimorphism (SSD), a condition that is arguably unique among terrestrial carnivores (see (Ralls, 1976)). We apply the results of our selection analysis to test a number of the most commonly invoked hypotheses explaining reversed SSD in spotted hyenas.

One of the first hypotheses forwarded to explain female-biased SSD in spotted hyenas, the 'infant defense' hypothesis, suggests that larger mothers might be better at preventing infanticide by conspecifics (Kruuk, 1972, Ralls, 1976). Another possibility is suggested by the observation that increased size decreases the relative metabolic cost of nursing in a variety of species (Peters, 1983), permitting larger mothers to invest more heavily in offspring (e.g. (Crocker et al., 2001)). The metabolic costs of lactation are extremely high among spotted hyenas (Hofer and East, 2003) and this 'inexpensive lactation' hypothesis provides an explanation for why female size might be under positive selection. The third hypothesis we test is the 'hunting success' hypothesis, suggesting that hunting success increases with size. Finally, the 'feeding competition' hypothesis suggests that larger females fare better during intense competition to obtain food at kills dominated by individuals of high social rank. All hypotheses predict a positive relationship between female size and fitness. Thus we would fail to support any of these hypotheses without evidence for positive selection on morphological size traits in female spotted hyenas. However, the 'infant defense' and 'inexpensive lactation' hypotheses both further predict that one fitness component in particular, cub survival, should increase with maternal size, whereas the ‘feeding competition’ and ‘hunting success' hypotheses make no specific prediction regarding which fitness components might be affected by maternal size.

Despite its clear importance in ecology and evolution, there is no well-accepted method for measuring animal body size, nor is there a consensus on what body size truly represents; this may result in widely divergent interpretations of co-variation of fitness with size. Body mass can be problematic as a size measure, because it may fluctuate temporally due to feeding, reproduction, and other factors (Fairbairn, 2007). Mammalian carnivores such as spotted hyenas represent extreme examples, as they consume large amounts of tissue in a single meal (e.g. (Kruuk, 1972)). A common approach to estimating body size is use of taxon-specific univariate proxies, such as snout-vent length in reptiles or body mass in birds and mammals. However this approach assumes both a strong correlation and an isometric relationship, or 1:1 log ratio, between the trait and overall size (Fairbairn, 2007). We define overall size here as a measure of all morphological traits where all traits increase isometrically. Any allometric variation, or deviation from isometry, represents a change in shape rather than size. A common alternative to univariate size measures is use of the first axis (PC1) from a principal components analysis (PCA) on the covariance matrix of a set of log-transformed morphological measures (e.g. (Massemin et al., 2000, Schulte-Hostedde et al., 2002)). However, this approach also assumes an isometric relationship between overall size and each trait, indicated by the loading of the trait with PC1. Failure to meet this assumption suggests that traits do not contribute equally to the size measure, and thus that PC1 represents both size and shape, obscuring interpretation. Interestingly however, this assumption is likely seldom met, as allometric variation is more the rule than the exception (Lindenfors et al., 2007).

The current approach in selection analysis is to use all available size-related morphological traits in a selection gradient analysis to assess direct and indirect components of selection (Lande and Arnold, 1983). However there are potential conceptual and practical drawbacks to this approach. Conceptually, if all of the size-related traits in fact reflect an underlying, but unmeasured, body size factor, then the contribution of the size factor to fitness will be spread among the traits. The high degree of multicollinearity in the model will inflate standard errors for the partial regression coefficients. This can lead to an inability to statistically detect selection, even when it is operating. In practice, unrealistically large sample sizes may be required to obtain reasonable estimates with many traits and few a priori expectations concerning those under selection.

Here we use a novel approach for estimating body size that represents a compromise between using PC1 as the sole size proxy and a selection gradient analysis that includes all traits. Our approach integrates multivariate allometric techniques (Klingenberg, 1996, Tzeng and Yeh, 2002), identifies unequal contribution of traits to PC1 and, if necessary, allows groups of traits to be chosen for inclusion in multivariate measures of size based on allometric relationships. We then test for selection among female hyenas on three composite size traits grouped by their multivariate allometric coefficients, using LRS as a measure of fitness. We demonstrate an explicit link between fitness and a composite size trait that scales hypoallometrically in adult female spotted hyenas, and use path analysis to identify fitness components influenced by size. Interestingly, we do not observe a significant relationship between fitness and either mass or PC1 from a PCA performed on all traits. We discuss these results within the context of measuring size as a target of selection, and with respect to the evolution of the rare form of SSD reversal observed in spotted hyenas.

Materials and Methods

Study Organisms

Spotted hyenas are characterized by small litter sizes, slow life histories and unusual genital monomorphism (Holekamp et al., 1996, Kruuk, 1972, Watts et al., 2009, Hamilton et al., 1986). Spotted hyenas live in social groups called clans, consisting of up to 90 individuals including multiple females born in the clan and their young, as well as several adult immigrant males. Each clan is structured by a strict linear dominance hierarchy (Holekamp and Smale, 1990, Frank, 1986, Smale et al., 1993), and an individual's position in this hierarchy has profound effects on both survival and reproduction by mediating differential access to food at kills (Kruuk, 1972, Watts et al., 2009, Hofer and East, 2003). Female spotted hyenas are physiologically competent to breed after 24 months of age, but first parturition usually occurs in the third or fourth year of life; the timing of first parturition varies greatly with rank (Holekamp et al., 1996, Hofer and East, 2003). Female hyenas are philopatric, whereas nearly all males emigrate and join neighboring clans after puberty (Smale et al., 1997, Van Horn et al., 2003). Spotted hyenas live up to 19 years in the wild (Drea and Frank, 2003).

Study site, population and field methods

We used data from a total of 170 immobilizations: 68 immobilizations of 46 females in the Talek clan, 22 immobilizations of 14 females in the Mara River clan and 80 immobilizations of 80 females in other Mara clans; the latter 80 females were only included in the allometric and correlation analyses, as we had no reproductive data for them. For individual hyenas immobilized more than once as adults, we used their mean values. Males could often not be monitored or immobilized after dispersal from their birth clan, so their lifetime fitness could not be accurately assessed here. Therefore our analyses were performed only on adult females. From each immobilized hyena we obtained the 4 cranial and 9 post-cranial linear morphological measurements shown in Figure 4.1. We only included measurements taken after 36 months of age or after first parturition, whichever came first; 36 months represents a conservative estimate of the age at which reproductive and morphological maturity is achieved among females (Watts et al., 2009, Holekamp et al., 1996). Females were included if they met these criteria even if they died without giving birth. All morphological data were natural log transformed prior to analysis.

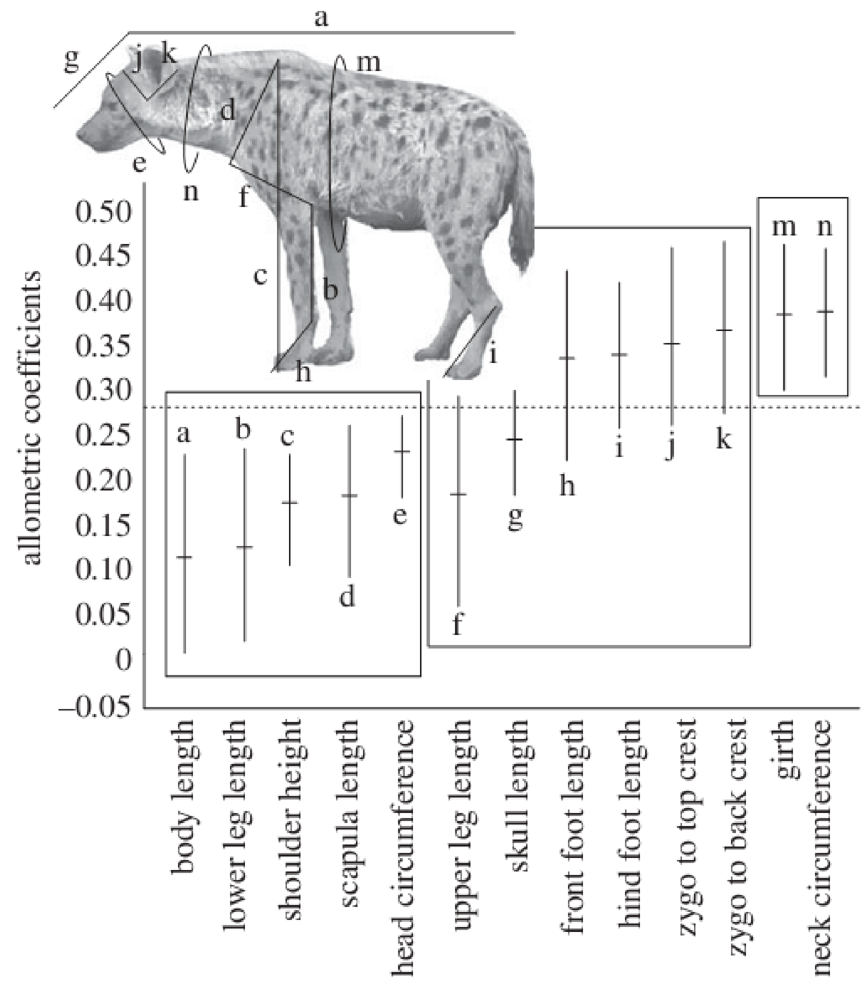


Figure 4.1. Morphological measurements taken from 140 adult female spotted hyenas, displayed with allometric intervals and labeled by corresponding letters; each letter refers to only one trait. The isometric hypothesis for overall body size is designated by the horizontal dotted line. Values on the y-axis represent allometric coefficients, or the loadings on PC1, for each trait.

Fitness measures

As our measure of fitness we used lifetime reproductive success (LRS), defined as the total number of offspring produced that survived to weaning. As fitness components we included cub survival to weaning, average annual reproductive success (ARS) and reproductive lifespan. Measuring fitness of mothers and their offspring accurately can be difficult, and it may not be clear whether to assign a particular component of fitness to the mother or to her offspring. Assigning the fitness component of cub survival to weaning as a maternal fitness component is common in mammalian studies. This practice has been criticized because selection theory emphasizes that the fitness of individuals in one generation (e.g. offspring) should not be assigned to individuals in another generation (e.g. parents), as this can result in incorrect predictions regarding evolutionary dynamics (Lande and Arnold, 1983). However, when the effect of the parental phenotype on a component of offspring fitness greatly overshadows the effect of the offspring phenotype and there is no genetic correlation between the parental trait of interest and the component of offspring fitness, then it may be beneficial to assign this component of fitness to the parent (Wolf and Wade, 2001). Explicit consideration of a genetic correlation between the parental trait and the component of offspring fitness of interest has specifically been suggested, based on researchers' knowledge of the species' biology and the traits in question (Wolf and Wade, 2001). In the case of spotted hyenas, there is no reason to suspect a direct genetic correlation between maternal size and offspring survival. In fact, offspring survival to weaning is dominated by the mother's ability to provide milk and protect her cubs (Hofer and East, 2003, Watts, 2007), and is strongly influenced by maternal social rank (Watts et al., 2009), which is learned and is not under genetic control (East et al., 2009, Engh et al., 2000).

Data Analysis

Allometric Methods

For log-transformed morphological data the multivariate allometric coefficient for each trait is indicated by the trait’s loading in the first eigenvector of the variance-covariance matrix (PC1), called the allometric vector. In order for PC1 to represent an isometric size measure, the allometric coefficients should equal $1/(p^{1/2})$, called the isometric hypothesis where p is the number of traits included in the PCA (Jolicoeur, 1963). To compare coefficients among traits we used a bootstrap approach to estimate 99% confidence intervals (allometric CIs) on the loadings, resampling with replacement 10,000 times (Tzeng and Yeh, 1999, Klingenberg, 1996). Because the allometric coefficients are estimated from loadings, they are dependent on the covariance matrix of included traits, and thus on the traits included in the analysis. Thus, the specific allometric coefficient of a trait is dependent upon the relative scaling relationship of the trait with other traits included in the PCA. If a trait's allometric CI overlapped the expected value for isometry, representing the null hypothesis, the trait was considered isometric to overall body size (Jolicoeur, 1963, Tzeng and Yeh, 1999). If the CI fell below the isometric value, the trait was considered to be hypoallometric to body size, or scale at less than a 1:1 log ratio with overall body size, whereas a CI wholly above the isometric value indicated a hyperallometric trait scaling at greater than a 1:1 log ratio with body size. If all included traits did not scale isometrically, PC1 would not be a good measure of overall body size because each trait would not contribute equally, and thus increases in PC1 would result in disproportionate changes in the trait in question. Disproportionate increases in some traits suggest that the size measure is conflated with shape. Because PC1 with all traits included failed to represent a good isometric measure of body size here, we split the measured traits into three groups: traits that scaled hypoallometrically, isometrically and hyperallometrically with body size, respectively. We then performed a separate PCA on each group and used each resulting PC1 as a new composite measure of size. This of course altered the resulting covariance matrix, and thus the relative scaling relationships of the traits. We therefore estimated the allometric coefficients of each group, comparing them to the predicted isometric value for each group. Any traits that demonstrated mild departures from isometry were left in their respective groups, as perfect isometry is unlikely. As an additional precaution however, we also temporarily moved traits that exhibited mild departures from isometry to new groups, and repeated all further analyses to determine whether their placement influenced our results. Currently we do not know whether the measured traits are functionally, developmentally or evolutionarily integrated, but merely present them as appropriate and practical proxies for size. Although we formed trait groups based on their allometric CIs, we also wanted to determine whether these or similar groups would also be generated if we used other grouping methods. If our trait groups were robust to use of alternative methods, this would support the idea that trait groups were natural groups produced by similar evolutionary and developmental processes, and not merely artifacts of any particular covariance matrix. Therefore we used the 'pvclust' package in R (Suzuki and Shimodaira, 2009) to perform a hierarchical cluster analysis, with bootstrap support values for nodes, to further investigate relationships among the univariate morphological traits of interest. We used 10,000 bootstrap replicates with uncentered correlations subtracted from one as a measure of distance between two traits. We used a variety of agglomeration methods including Ward’s, single, average, median and complete. Using a bootstrap resampling method allowed us to estimate confidences in the various topologies of each dendrogram. The combination of dimensional reduction and the preservation of some allometric information makes this technique very useful when a large number of size traits are measured, especially when there are no clear a priori hypotheses regarding the importance of specific traits.

Selection Gradients and Path Analysis

We used Conner's (1996) approach to understanding natural selection operating on one or more traits using selection gradient analysis (Lande and Arnold, 1983), multiplicative fitness components (Arnold and Wade, 1984) and path analysis (Li, 1975). LRS was converted to relative fitness by dividing it by mean absolute fitness for selection gradient analysis (Arnold and Wade, 1984, Lande and Arnold, 1983). LRS and all fitness components were standardized for path analysis (Conner, 1996). We calculated standardized selection gradients to estimate the strength of selection by regressing relative LRS on the standardized traits of interest (Conner, 1996, Lande and Arnold, 1983). Although we had full morphological trait data for 140 females, we had reproductive data on 50 females, seven from the Mara River clan and 43 from the Talek clan, and full LRS data for a reduced set of 31 individuals, all from the Talek clan. For the selection gradient analysis we only used the 31 individuals from the Talek clan with full LRS. The females used in each analysis represent all females for which we had all necessary data. Although bias could possibly be introduced into the path analysis by inclusion of data from two clans, the ecological conditions experienced by both clans are very similar (Kolowski and Holekamp, 2009), and the results of the selection gradient analysis match the path analysis closely, so we believe it unlikely. The three composite size measures were included in a multiple regression along with the standardized social rank of each animal. Social rank was included to remove any correlated effects of rank, as rank affects most aspects of life history, behavior and ecology in the spotted hyena (Holekamp and Smale, 1993, Holekamp and Smale, 1990, Smale et al., 1993, Hofer and East, 2003, Holekamp et al., 1996, Watts et al., 2009). However, social rank among adult female spotted hyenas is not correlated with mass or other univariate measures (Holekamp et al., 1996). We also assessed the relationship between rank and morphology in our current data set, described in the section on path analysis. We did not include mass in the current analysis because any effect of mass would conflate the effects of size and condition, either of which might influence fitness (Melis et al., 2010, Fairbairn et al., 2007). We did, however, perform a selection gradient analysis with only social rank and mass to assess the value of this measure in spotted hyenas, as well as a similar model with PC1 from a PCA of all 13 traits replacing mass.

We performed path analysis using ordinary least-squares (OLS) regression to determine which fitness components are influenced by size-related traits and social rank, and to elucidate the importance of different fitness components in determining total fitness (Conner, 1996), as well as the potential influence of social rank on size in the current data set. Offspring achieve ranks directly subordinate to their mothers in this species with no evidence that rank acquisition is influenced by adult size (Engh et al., 2000). We expected any correlation between rank and size observed to be due to effects of rank on size rather than vice versa. We thus fit the relationship between rank and size as a causal path. The multiplicative fitness components we included were reproductive lifespan, average number of offspring born each year, or ARS, and proportion of cubs born that survived to weaning. We confirmed this approach by using structural equation models (SEMs), which allow simultaneous estimation of all paths using maximum likelihood, utilizing bootstrapping to construct confidence intervals. However, we only utilized SEM in a confirmatory role to OLS path analysis due to complications engendered by the estimation of all paths simultaneously (see Appendix B). Because sample size limited the number of variables that could reliably be included in the path analysis, only traits found to be under significant ($p \leq 0.05$) lifetime selection in the selection gradient analysis were included in the path analysis. We confirmed that this was appropriate using AICc (corrected Akaike's Information Criterion) to compare the fit of the model containing only the significant traits with other possible models.

To maximize sample size in our path analysis we used all 50 females for which we had at least three years of fitness measures after reproductive maturity. Nineteen of 50 females included in the path analysis had left- or right-censored data; left-censored individuals started breeding before our study began, and right-censored individuals were alive at the end of the study. To test whether the effect of size differed between censored and uncensored individuals, we performed model selection on ANCOVAs using likelihood ratio tests and AICc (detailed in Appendix B). No method indicated a difference regarding effects of hypoallometric size on reproductive longevity between individuals with full lifetime data and either left- or right-censored individuals (Tables B.2 and B.3), so we subsequently pooled the data for these three groups. All statistical analyses were performed in R version 2.9.2 (R Development Core Team, 2009).

Results

Allometric Analyses

As has been observed in studies with other organisms, the loadings on PC1 from the principal components analysis from morphological traits were unequal among female hyenas (e.g. (Tzeng, 2004, Tzeng and Yeh, 2002, Klingenberg, 1996)), with allometric confidence intervals (CIs) only overlapping the expected value for isometry for 6 of 13 traits (Figure 4.1). Using the observed multivariate allometric patterns, we grouped hyperallometric, isometric and hypoallometric traits separately, performed a PCA on each group and used the new PC1s as multivariate proxies for size. We refer to each resulting multivariate size measure by its original allometric relationship to isometric size (e.g. "the hypoallometric size trait"). The bootstrapped correlation analysis indicated that groups identified by allometric relationships were also generally robust to other clustering methods (Figure 4.2). The meaning of multivariate traits calculated from such groupings is easier to interpret than that of a PC1 calculated from all measured traits because they can be interpreted more easily as a set of proxies for overall size, not conflated with allometric effects. For the purposes of this paper we explicitly limit ourselves to utilizing these measures as size proxies, and we make no inferences about possible genetic, functional or evolutionary explanations for the observed groupings. After reanalyzing the allometric CIs of the new multivariate traits, skull length and upper leg length appear to be slightly hypoallometric to the new predicted value for the multivariate isometric trait. Thus, both traits appear to straddle the isometric and the hypoallometric groups. However, moving these traits into the hypoallometric group and repeating all analyses does not change any of the remaining results (see Appendix B).

Correlation Analyses

The groups chosen using the allometric CIs were robust to alternative grouping methods. Specifically, hierarchical cluster analyses using a variety of agglomeration methods, including Ward's, average, single, complete and Mcquitty's, returned topologies similar to the groupings chosen using the allometric CIs (Figure 4.1; Figure 4.2). Although complete congruence between the correlation and allometric analyses was not universal across agglomeration methods, especially at higher dimension topologies, all agglomeration methods generally corresponded with the allometric CIs at lower levels. Specifically, small clusters of traits commonly found using one method were usually seen using others, and were also recovered by partitioning traits using allometric coefficients. Furthermore, two out of five agglomeration methods recovered nearly the same partitions identified by allometric coefficients (e.g. Figure 4.2). The general congruence observed here suggests that the allometric CIs broadly reflect the action of the evolutionary and developmental processes that generate bivariate correlations between morphological traits. Therefore, it seems reasonable to consider

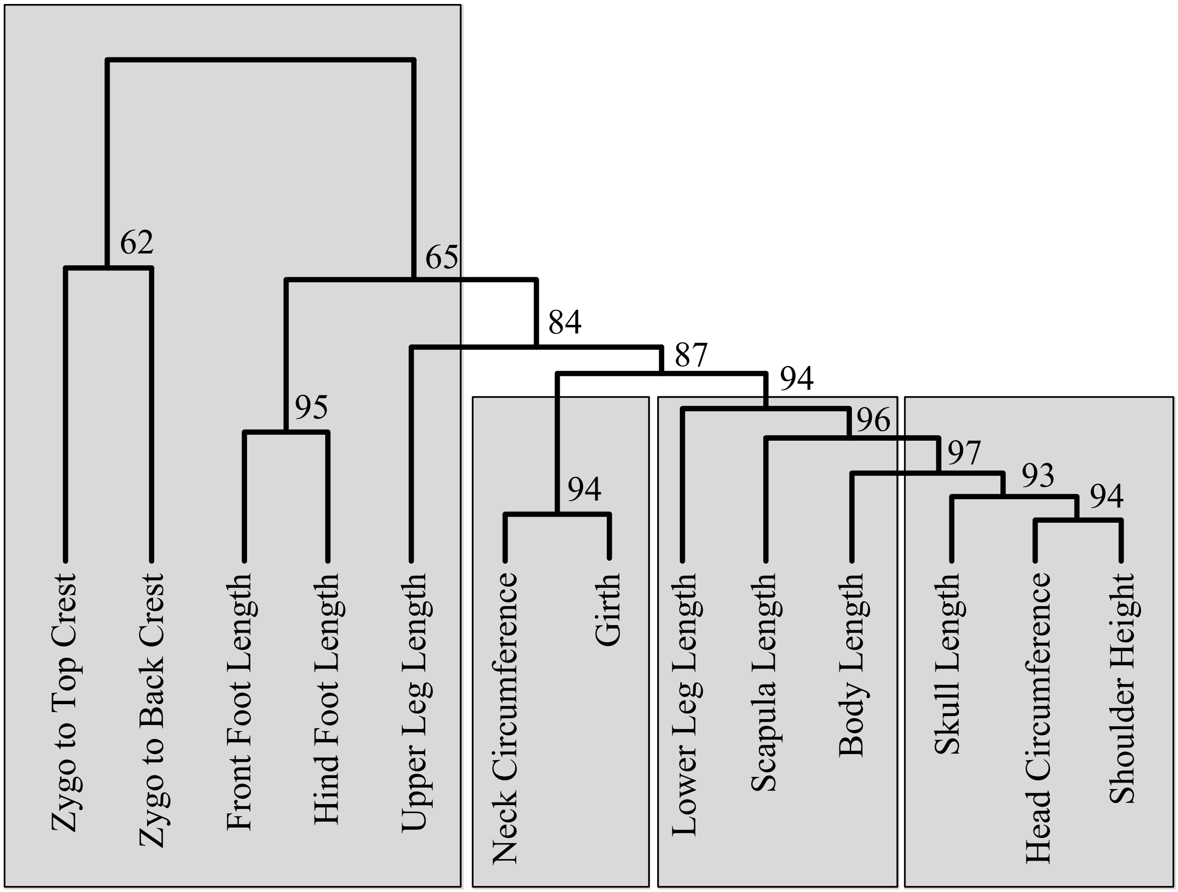


Figure 4.2. Cluster diagram from hierarchical cluster analysis on univariate measures. Cluster analyses were performed using five different agglomeration meth ods, and distance measures calculated from uncentered correlations. Shaded regions designate groups of traits that are returned by four out of five agglomeration methods. The exact topology shown was returned by two out of five agglomeration methods.

Table 4.1. Parameters from multiple regression selection analysis performed using data from 31 adult female spotted hyenas. β values are standardized selection gradients. Significant effects at α ≤ 0.05 are indicated by asterisks.

	β	SE	t	p-value
Rank	-0.276	0.108	-2.545	0.0172*
Hypoallometric Size	0.313	0.112	2.788	0.0098*
Isometric size	-0.105	0.145	-0.729	0.4726
Hyperallometric Size	0.139	0.143	0.977	0.3378

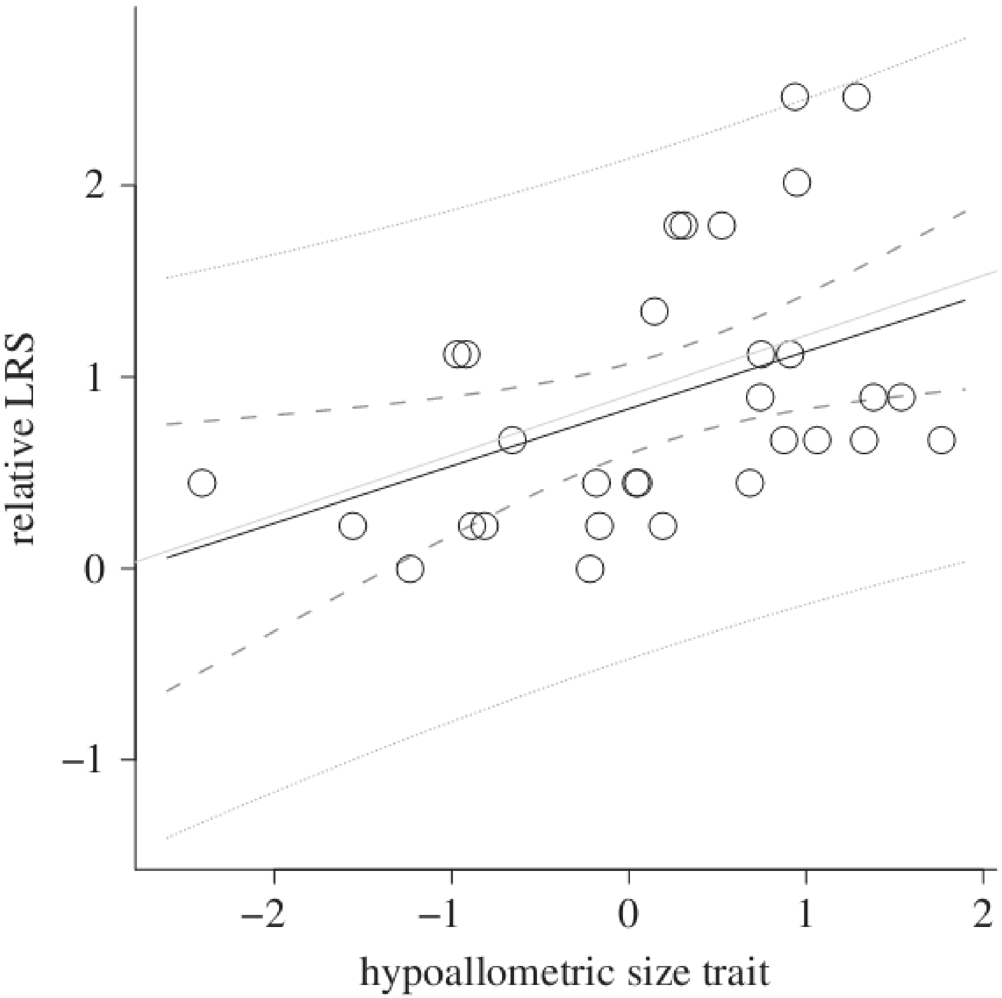


Figure 4.3. Relationship between relative LRS and the standardized hypoallometric size trait observed among 31 female spotted hyenas. The black line indicates the selection differential, whereas the grey line indicates the selection gradient. The dashed lines indicate the 95% confidence intervals while the dotted lines are the 95% predictive intervals.

the groups we identified using allometric CIs appropriate, both in terms of multivariate statistical methods and interpretation of our results, thus reinforcing the utility of our approach for partitioning morphological traits.

Selection Analysis

We found no selection on isometric or hyperallometric size measures (Table 4.1). The model did, however, reveal significant positive selection on the hypoallometric trait (Figure 4.3; Table 4.1), as well as a negative effect of rank on LRS (Table 4.1). By convention, the highest-ranked animal is assigned a rank of one, so a negative effect of rank on LRS indicates that higher-ranked individuals have higher LRS. The observed selection on the hypoallometric trait is fairly strong (β=0.313), as Kingsolver et al. (2001) demonstrated that absolute magnitudes of selection estimates ($|\beta|$) roughly follow an exponential distribution, with a mean of 0.22 and a median of 0.16. In addition, the magnitude of selection on the hypoallometric trait is more than twice that of selection on the other traits. In the two separate selection gradient models, one with mass and rank, and the other with rank and PC1 from all size-related traits, neither commonly-used size proxy contributed significantly to fitness (Mass: β=0.223, SE=0.111, t=2.000, p=0.055; PC1: β=0.200, SE=0.120, t=0.168, p=0.105). Additional descriptive statistics and information on the opportunity for selection (I) appear in the supplementary material and Table B.1.

Fitness components

To understand how the hypoallometric trait contributes to LRS, we used a path analytic approach, which demonstrated that the hypoallometric size trait contributes to LRS through its impact on both reproductive lifespan and ARS (Figure 4.4). Also, rank had a positive effect on ARS, a marginally significant positive effect on cub survival and no effect on reproductive lifespan (Figure 4.4). Rank also had a non-significant positive effect on the hypoallometric trait, suggesting that low-ranking females may be larger as adults than high-ranking females (Figure 4.4). All fitness components had strong effects on LRS, though the effect of reproductive lifespan was much stronger than ARS or cub survival (Figure 4.4). Finally, the negative correlation between ARS and cub survival (Figure 4.4) suggests a possible reproductive tradeoff. The negative correlation between annual reproductive success (ARS) and cub survival to weaning in Figure 4.3 was taken from the structural equation model (SEM) because the correlated errors did not meet the assumptions of a recursive model, but other path coefficients were unaffected. Using OLS path analysis, this negative correlation was present, but not significant at $\alpha \leq 0.05$. There were no other discrepancies between the traditional path analysis and the SEM (Figure 4.4; Table B.4).

Discussion

The intensive sampling and individual identification necessary to obtain long term fitness data are difficult in free-living populations, particularly for animals that are cryptic, nocturnal, dangerous or long-lived. We present, to our knowledge, the first phenotypic selection analysis on a large carnivore using lifetime reproductive success as a

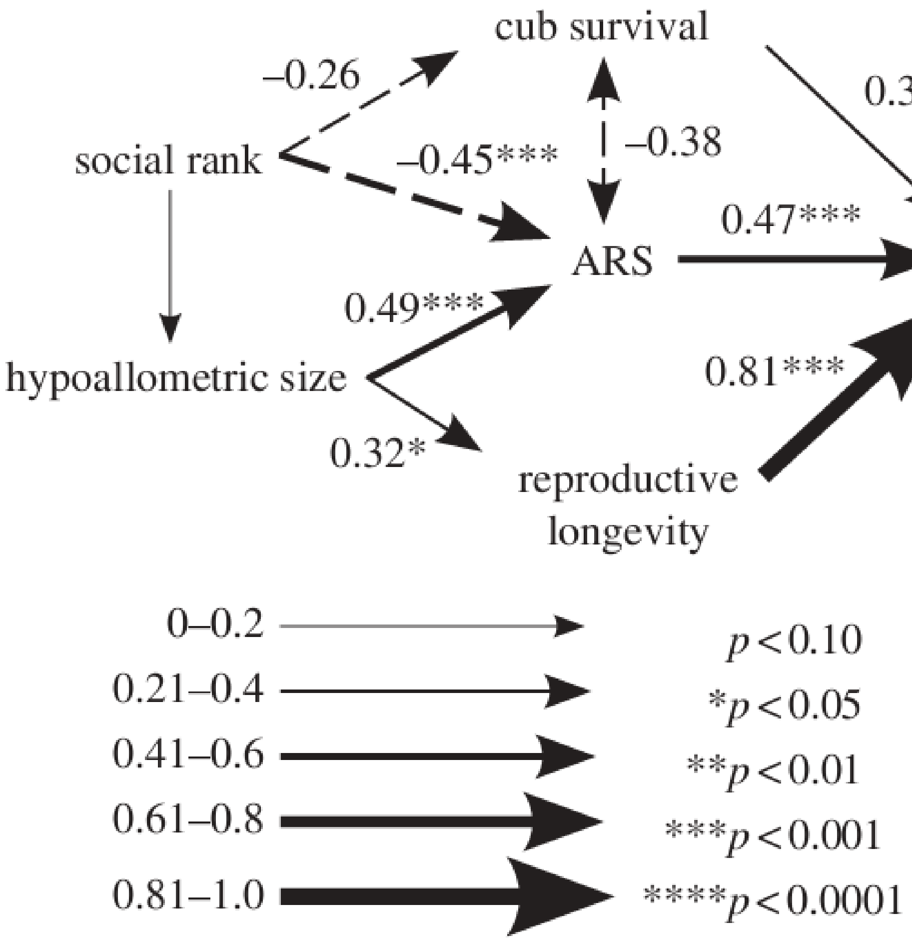


Figure 4.4. Results of path analysis showing relationships between social rank, the hypoallometric size trait, and fitness components among 50 adult female spotted hyenas. All paths are shown with relationships significant at p≤0.20. Path coefficients are given above paths that are significant at p≤0.10. Arrow width corresponds to magnitude of the path coefficient, with positive coefficients indicated by solid lines and negative coefficients indicated by dashed lines. ‘One’ is the highest social rank an individual can achieve, so negative path coefficients indicate that rank is positively related to the response variable. Path coefficients are almost identical to MLE estimates; 95% bootstrap CIs are given in Table B.5, and agree in all cases with significances of path coefficients. The correlation shown between ARS and cub survival is the MLE estimate, for reasons discussed in results.

measure of fitness. We also showed that LRS among female spotted hyenas is affected by a multivariate morphological trait that scales hypoallometrically with body size (Figure 4.3; Table 4.1). However, we failed to find evidence for selection on either an isometric size trait, which is often thought of as 'idealized size,' or on a hyperallometric size trait (Table 4.1). Although the results of the selection gradient analysis support all four adaptive hypotheses considered here, the path analysis results (Figure 4.4) fail to support either the 'infant defense' hypothesis or the 'inexpensive lactation' hypothesis, which predict an effect of size-related morphological traits on cub survival. Instead, the results of our selection and path analysis (Figure 4.3; Figure 4.4) are consistent with both the 'feeding competition' hypothesis and the 'hunting success' hypothesis.

(Clutton-Brock, 2009, Ralls, 1976). Under this hypothesis, food is more critical to the reproductive success of females than males, and this appears to be the case among spotted hyenas (Holekamp and Smale, 2000). Reproductive opportunities among female spotted hyenas are strongly limited by food; feeding competition among females is very intense, and priority of access to food has profoundly important effects on most measures of female reproductive success (Hofer and East, 2003, Holekamp and Smale, 2000, Holekamp et al., 1996). Although rank is the most important determinant of access to food, size may influence food access through a greater ability to steal or process food more quickly, increasing intake rates without affecting priority of access. In addition, size may be beneficial in situations where intrasexual rank plays a lesser role, such as during interspecific interactions at kills. The 'hunting success' hypothesis, which is also supported by our data, suggests that hunting success increases with size. In contrast to other extant hyenas, spotted hyenas are proficient predators, and even solitary hunters can bring down prey up to four times their own body mass (Holekamp et al., 1997). If selection is acting on hunting ability, then females with larger values of the hypoallometric trait should exhibit higher success rates during hunts. Furthermore, if the 'hunting success' hypothesis is correct, we should see the same relationship between hunting success and hypoallometric size traits in males and females, but a greater effect of hunting success on fitness in females. Although we view these hypotheses as the most likely candidates, size data from adult males is necessary to provide conclusive evidence in support of any particular explanation for SSD in this species. Other hypotheses proposed to explain SSD in spotted hyenas, such as a pleiotropic effect of female masculinization or selection for smaller males, cannot be directly addressed with our current data.

The importance of ARS as a route through which body size influences LRS among female spotted hyenas will allow us to compare selection on size-related traits in males and females directly, shedding further light on the maintenance of female-biased SSD, which represents a derived trait in this species (Holekamp and Kolowski, 2009). Yet one remaining question concerns the source of variation in ARS. Namely, does size influence ARS via inter-litter interval, litter size or both? In a multiple regression, increases in the hypoallometric trait resulted in larger litters ($\beta=0.431$, $SE=0.135$, $t=3.192$, $p=0.003$), but rank had no effect ($\beta=-0.229$, $SE=0.135$, $t=-1.696$, $p=0.097$). In a separate multiple regression, females with larger values of the hypoallometric trait had more litters per year ($\beta=0.377$, $SE=0.132$, $t=2.859$, $p=0.006$), as did higher ranking females (β=-0.396, SE=0.132, t=-3.007, p=0.004). In a third multiple regression, females with more litters per year had increased ARS (β=0.715, SE=0.053, t=13.623, p<0.001), as did females with a greater average litter size (β=0.388, SE=0.053, t=7.384, p<0.001). All variables in these analyses were mean centered and standardized. Sample size was 49 because we did not have enough information on the exact frequency of one female's litters.

There is little explicit support for any current hypothesis explaining female-biased SSD in spotted hyenas, but it is commonly assumed in the literature that larger body size in female hyenas evolved as part of an integrated suite of "sex-role reversed" traits, including enhanced aggressiveness, social dominance and male-like genitalia in females. However, our data suggest instead that SSD in the spotted hyena may result from direct positive selection on size-related morphological traits in females. Positive selection may also play a role in the generation of patterns of static allometry among females. It is not currently known whether the hypoallometric trait affects fitness among male spotted hyenas as it does among females. Direct evidence regarding how female-biased SSD is maintained will come from comparing effects of size on ARS between the two sexes. Selection on females that is absent or negative in males, combined with positive heritability, would provide strong support for positive selection on size-related morphological traits as a mechanism maintaining female-biased SSD in spotted hyenas. Another result relevant to future analyses comparing males and females is that our path analysis identified a negative correlation between ARS and cub survival (Figure 4.4), possibly representing a life history tradeoff in which females that reproduce at younger ages experience lower cub survival simply due to inexperience at first parturition. However, a more likely possibility is that litter loss before weaning brings females rapidly back into estrus (Holekamp et al., 1996), allowing them to produce more litters, albeit unsuccessful ones, per unit time than females whose cubs survive to weaning.

In addition to comparing selection on size in males and females in future work, it may be informative to incorporate our results concerning the specific morphological traits under selection in attempts to address the remaining hypotheses explaining female-biased SSD in spotted hyenas. Specifically, we note that we failed to find evidence for selection on mass or PC1 from a PCA on all morphological traits. We interpret this to mean that selection is likely acting on the subset of morphological traits contained within the hypoallometric measure rather than overall size itself. Notably, body length, mass and PC1 are all commonly used as proxies for overall size, yet in our study they vary greatly in their relationships to fitness. PC1 calculated from all traits is generally not as condition-dependent as mass, and is widely considered a standard comprehensive measure of size. However, the effect of PC1 on fitness here is even less clear than the effect of mass. Thus our results suggest that, despite the importance of size in biology (e.g. (Roff, 1986, Stearns, 1992, Bonner, 2006)), overall size is not always the trait of interest. Our data thus underscore the value of determining whether size itself, or specific size-related morphological traits, are under selection. In situations where size appears vital in mediating an ecological process, it may be that the proxy used for size was the relevant trait, not overall size.

Although we currently have little understanding in hyenas of how post-cranial morphological traits contribute to running speed, feeding performance or hunting ability, identifying morphological traits that are targets of selection is an important first step. Interestingly, in post-hoc analyses, we found that all hypoallometric traits except scapula length significantly influenced LRS when each univariate trait was included in a multiple regression alone with social rank. However, no traits that scaled isometrically or hyperallometrically exhibited evidence for even indirect selection (Table B.5). The robustness of this pattern supports the notion that our allometric grouping technique has identified a set of functionally integrated traits, and suggests that selection is acting either on the hypoallometric trait as an integrated unit or on individual hypoallometric traits.

Research on the evolutionary forces shaping allometric patterns in animals has mainly focused on sexually selected male traits, including ornaments, weaponry and genitalia (e.g. (Eberhard, 2009, Emlen, 2008)). Although these traits are of great interest, when investigating the generation of allometric scaling relationships, such strict focus may limit our thinking, and a broader base of empirical work would most likely benefit the entire field. We do not yet know whether the hypoallometric size trait documented here among female spotted hyenas is shaped by natural or sexual selection; in keeping with Darwin’s (1871) original definition of sexual selection, the latter possibility would most likely not have been considered at all even a decade ago. However, Clutton-Brock (2007, 2009) has recently argued that competition for reproductive opportunities among female animals can generate strong selection favoring competitive ability, and that in extreme cases, selection may reverse the usual direction of sex differences in behavior and morphology. If the definition of sexual selection is broadened to encompass the consequences of reproductive competition and mate choice in both sexes (Clutton-Brock, 2009), then the spotted hyena probably represents one such extreme case. If the hypoallometric size trait affects fitness in female but not male spotted hyenas, this would suggest it is indeed a sexually selected trait, and that selection on the hypoallometric size trait drives the rare pattern of female-biased SSD in spotted hyenas.

APPENDIX

Published as Supplementary Material for Swanson et al. (2011)

Supplementary methods

Morphological data collection and inclusion

Data were collected between 1988 and 2009 from spotted hyenas inhabiting the Talek and Mara River regions of the Masai Mara National Reserve in southwest Kenya (1 degree 40’ S, 35 degrees 50’ E). Individuals in the Talek and Mara River clans were recognized by their unique spots, and their sexes were determined based on phallic morphology (Frank et al., 1990). Ages of natal individuals were known to +/- 7 days based on their appearance when first observed above ground (Holekamp and Smale, 1998). Mother-offspring pairs were established on the basis of regular nursing associations and genotyping (Holekamp et al., 1996, Engh et al., 2002), and rank was determined based on outcomes of dyadic agonistic interactions (Frank, 1986). For immobilizations and collection of morphological data, female hyenas were anesthetized with Telazol (Fort Dodge Animal Health, Overland Park, KS; 6.5 mg/kg) administered in a lightweight plastic dart via a CO_2-powered rifle (Telinject Inc., Saugus, CA). All statistical analyses were performed in R version 2.9.2 (R Development Core Team, 2009).

Multicollinearity of selection gradient analysis

Multicollinearity is a common issue with multiple regression techniques when predictor variables are highly correlated with one another. To determine the strength of multicollinearity in our multiple regression, we calculated variance inflation factors (VIFs) and condition indices for our selection gradient analysis. VIFs are a common measure of multicollinearity that indicate the degree to which standard errors are inflated due to correlation among predictor variables. Condition indices are another common method of assessing multicollinearity that are best used complementarily with VIFs. We calculated VIFs using the 'vif()' function in the 'car' package in R (Fox et al., 2009a). There is no theoretical cut-off point for VIFs where multicollinearity is considered severe, but ten is a commonly-used value, and values as low as four have been considered significant (O'brien, 2007). Condition indices are calculated by dividing the square root of the first eigenvalue of the design matrix of the multiple regression by the square root of each successive eigenvalue. The most important condition index is the condition number, which is the condition index with the greatest magnitude, calculated on the last eigenvalue. Condition index values below 10 indicate low multicollinearity, and values above 30, severe multicollinearity (Belsley, 1991).

Path analysis and structural equation modeling

Our sample size for the path analyses was 50 females, such that we included 8.3 individuals per variable in the analysis. Ten samples per variable included has been suggested as a rule of thumb for path analysis (Mitchell, 1993), therefore our sample size is slightly lower than suggested. However, the main problems with small sample size are a lack of power and potential convergence problems for the MLE estimates. We thus limited the number of predictor variables and estimated 95% bootstrap CIs on the SEMs to confirm all path coefficients, which should have offset all problems resulting from low sample size except low statistical power.

Differences may arise between path models estimated using OLS regression and SEM if models are not fully recursive, such that they have correlated error terms or the causal effects are not all 'unidirectional' (Berry, 1984). Standardized path coefficients were estimated here because it is only useful to measure selection in units of evolutionary change for total fitness, not for fitness components (Conner, 1996). We confirmed the results of our traditional path analysis using structural equation modeling (SEM), a technique that estimates all paths simultaneously using maximum likelihood estimation (MLE). All SEMs were fitted using the 'sem' package in R (Fox et al., 2009b). For our study, a major advantage of this technique is that the correlated error between fitness components is estimated after accounting for the effects of maternal rank and the hypoallometric size trait. The correlated error thus indicates the correlation between the fitness components after accounting for such predictors, representing a closer estimate of a true life history tradeoff than does a simple correlation. However, two complications arose from the use of SEM here because all paths are estimated simultaneously with this method. First, because the fitness components together completely determine total fitness, simple regressions must be used between each fitness component and total fitness (Conner, 1996). We dealt with this problem by creating 4 path models. The first path model was a full model estimating all paths of interest (chosen a priori). Each of the other three models estimated the effect of only one fitness component on LRS, so each model provided the equivalent of a simple regression of LRS on the respective fitness component. The slope of the regression of LRS on each of the fitness components and their respective strengths did not differ between the full model and the respective reduced models. All parameter estimates and 95% bootstrap CIs, except those leading from a fitness component to total fitness, were taken from the full model. For SEMs, bootstrapping was done using the 'boot' package in R (Canty and Ripley, 2009). Parameter estimates and bootstrap CIs were taken from each of the reduced models for the path from the appropriate fitness component to total fitness. We observed no qualitative differences between results obtained using this approach and those obtained using the full path model. In addition, though the absolute magnitudes of the paths between the fitness components and total fitness differed somewhat, the relative magnitudes did not change. Therefore, both approaches indicated that reproductive lifespan contributed most strongly to fitness, followed by ARS and finally cub survival.

The second problem engendered by simultaneous estimation of all paths was that complete data are required for SEMs. In our dataset two out of 50 individuals lacked values for cub survival because although each lived over six years, they never gave birth to a single cub. We can determine this unequivocally because the tearing in the posterior surface of the female's phallus at first parturition allows us to determine when she has borne her first litter, even when no cubs survive to emerge above ground. Neither of these two females ever exhibited a torn phallus. To solve the problem posed by these two females while also retaining the maximum amount of valid information in our analysis, we used pairwise deletion, also known as available case analysis (Allison, 2001). To carry out pairwise deletion, we calculated three covariance matrices (Fig. B.1), the third being a combination of the first two. The first covariance matrix (C1) only included females for which we had data on cub survival, and thus contained only 48 cases. The second covariance matrix (C2) included all females, and thus contained 50 cases, but incomplete data on cub survival. We combined these to form the third covariance matrix by taking from C1 every covariance or variance pertaining to cub survival, and values not associated with cub survival from C2 (illustrated in Fig. B.1). For example, C3 had a value estimated from all females for the variance in ARS or the covariance between maternal rank and reproductive lifespan. However, C3 had a value estimated only from females for which we had full data on cub survival for the variance in cub survival or the covariance between maternal rank and cub survival. C3 was re-estimated for each random bootstrap replicate. We assigned degrees of freedom conservatively, using 48 rather than 50 when estimating p-values because we had data on cub survival for only 48 individuals.

Model comparison for selection on reproductive lifespan

For both the path analysis and the SEM we needed to test whether the effect of the hypoallometric size trait on reproductive lifespan differed between individuals for which we had full lifetime data and individuals that were either left- or right-censored. Therefore, we created four possible models, three of which contained covariates indicating different selection pressures on the group with full lifetime data compared to those without. Model 1 was a multiple regression containing only hypoallometric size and female social rank. Models 2, 3 and 4 were ANCOVAs. Model 2 contained the variables in Model 1, a dummy term representing whether or not the individual was present at the beginning of the study and an interaction term between the dummy variable and size. Model 3 contained Model 1, and also a dummy term representing whether or not the individual was alive at the end of the study, as well as an interaction term between this dummy variable and size. Model 4 was a full model, containing all variables present in Models 1, 2, and 3; this final model was created simply to assess the importance of the dummy variables themselves. We then used likelihood ratio tests (LRTs) and corrected Akaike's Information Criterion (AICc) to assess whether the added terms significantly improved upon Model 1. However, the larger models showed no significant improvement over Model 1, indicating that there are no appreciable differences in the effect of size on reproductive lifespan between females with full lifetime data and censored females. Therefore, for the path analysis and the SEM we pooled data from females with full lifetime data and censored females.

Supplementary results

Descriptive statistics

The opportunity for selection (I), calculated as the variance in relative fitness, was 0.50 among adult female spotted hyenas (Table B.1). Extreme values of I from a nonexhaustive survey of the literature range from 0.04 (Houck et al., 1985) to 32.9 (McAdam and Boutin, 2003b), but the distribution of these values is decidedly right-skewed, with males generally experiencing greater opportunity for selection.

Allometric and correlation analyses

Table B.1. Fitness measures for female spotted hyenas. Relative LRS was calculated by dividing all LRS measures by the mean value for the sample. Variance in relative LRS is equal to the opportunity for selection (**I**) on lifetime fitness.

	Fitness Measures				
	Relative LRS	**LRS**	**ARS**	**Reproductive lifespan**	**Cub survival**
Mean	1	4.32	1.08	7.13	0.56
Variance	0.50	9.89	0.14	11.18	0.04
Std. Dev.	0.70	3.14	0.38	3.34	0.21

After comparing the 99% allometric CIs to the predicted isometric value for each of the new multivariate traits, we found that two traits originally included in the isometric trait, skull length and upper leg length, now scaled hypoallometrically to the predicted isometric value. When we moved these to the hypoallometric trait and repeated all analyses, all multivariate traits were now internally isometric, and there were no qualitative differences (differences in statistical significance or direction of effect) in the results of the selection analyses. Because there were no qualitative differences in the results, we present results in the main text from the original analysis, where skull length and upper leg length were included in the isometric trait. Skull length did not correspond well between the correlation analysis and the allometric analysis using confidence intervals. In fact, skull length was the only trait to violate the congruence between groups identified by allometric coefficients and those identified by correlations. Skull length was originally included in the isometric trait, but when we moved this measure from the isometric to the hypoallometric trait and repeated all analyses, we found no qualitative differences in the resulting conclusions. In fact, most results differed very little quantitatively between the two analyses.

VIFs calculated for each predictor variable were all less than two. While values above four have been suggested in some cases as indicating moderate multicollinearity, ten is the most common rule of thumb for a cutoff point, with higher values indicating severe multicollinearity (O’brien, 2007). The condition number here, calculated as the largest condition index, was 2.6. Values lower than ten are considered to indicate only weak multicollinearity (Belsley, 1991). Thus, multicollinearity does not appear to be a problem in our selection gradient analysis.

Slopes of the regression of reproductive lifespan on the hypoallometric trait did not differ between females with full lifetime data and right-censored data (β=0.095, SE=0.333, t=0.284, p=0.778), or females with full lifetime data and those with left-censored data (β=0.438, SE=0.573, t=0.764, p=0.449). LRTs and AICc indicated no improvement in model fit for models in which the effect of size on reproductive longevity differed among females with full lifetime data and either type of censored females (Table B.2; Table B.3).

Table B.2: Likelihood ratio tests (LRTs) demonstrating that introducing a dummy term indicating censored individuals as an interaction term with the effect of body size on reproductive lifespan does not improve the model.

Model 1 vs.	X2	df	p
Model 2	0.931	2	0.628
Model 3	1.31	2	0.518
Model 4	2.28	4	0.685

Table B.3: Corrected AIC (AICc) values demonstrating, in corroboration with Table B.2, that model fit is not improved by including a term specifying a difference between individuals with full lifetime data and either right- (Model 2) or left-censored (Model 3) individuals, or both (Model 4). dAICc values of >4 indicate moderately strong evidence that the model fit is not improved. Thus, there is no appreciable difference in the effect of the hypoallometric trait on reproductive lifespan between individuals with full lifetime data and those for which we were not able to observe either the beginning or the end of their reproductive careers.

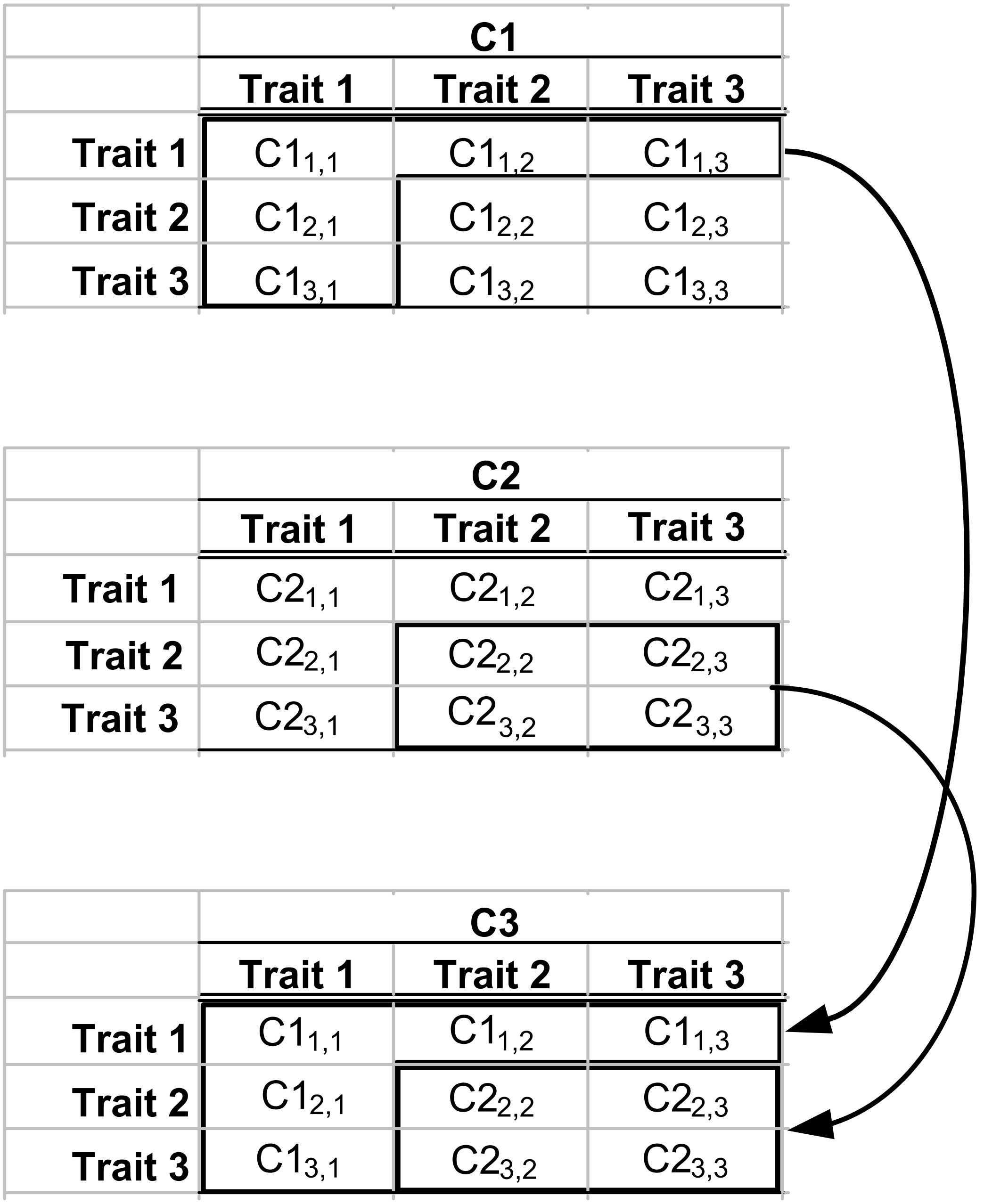
	AICc	df	dAICc	weights
Model 1	144.2	4	0	0.772
Model 2	148.0	6	3.8	0.118
Model 3	148.4	6	4.1	0.098
Model 4	152.6	8	8.3	0.012

Table B.4: Maximum likelihood estimates (MLE) for path coefficients and 95% CIs from 10,000 random bootstraps of an SEM. Double headed arrows in the path designation indicate correlations, whereas single-headed arrows indicate hypothesized causal paths. Asterisks next to the MLE indicate results significant at α≤0.05 for the basic SEM.

	MLE	**Low 95%CI**	**Hi 95% CI**
Rank->Surv	-0.126	-0.386	0.145
Rank->ARS	-0.448*	-0.634	-0.230
Rank->Cub	-0.262	-0.510	0.047
Rank->Hypoallometric trait	0.189	-0.099	0.435
Hypoallometric trait->ARS	0.490*	0.291	0.670
Hypoallometric trait ->Cub	0.028	-0.217	0.271
Hypoallometric trait ->Surv	0.323*	0.035	0.560
ARS<->Cub	-0.375*	-0.514	-0.168
Surv<->ARS	0.087	-0.111	0.266
Cub<->Surv	0.032	-0.266	0.330
Surv->LRS	0.812*	0.698	0.885
ARS->LRS	0.471*	0.225	0.645
Cub->LRS	0.395*	0.088	0.642

Table B.5: Results from separate post-hoc multiple regressions on univariate traits. Each variable had LRS regressed upon it with social rank as a second predictor. Rank was significant at α≤0.05 in all cases with β of approximately 0.5 and a similar SE in each case. Asterisks indicate significance of the morphological trait at α≤0.05. All statistically significant results included measurements contained in the hypoallometric trait, whereas only one non-significant result was from the hypoallometric trait. Rows with values in bold are significant at α≤0.05. The trait group that each trait was part of is given next to the name of the trait.

	β	**SE**	**t**	**p**
Lower Leg Length (Hypo-)	**0.214**	**0.099**	**2.161**	**0.039**
Head Circumference (Hypo-)	**0.285**	**0.124**	**2.296**	**0.029**
Shoulder Height (Hypo-)	**0.243**	**0.115**	**2.111**	**0.043**
Body Length (Hypo-)	**0.243**	**0.112**	**2.172**	**0.039**
Scapula Length (Hypo-)	0.136	0.110	1.232	0.228
Girth (Hyper-)	0.187	0.112	1.671	0.106
Neck Circumference (Hyper-)	0.115	0.124	0.929	0.361
Front Foot Length (Iso-)	0.118	0.107	1.101	0.281
Upper Leg Length (Iso-)	0.112	0.117	0.951	0.350
Hind Food Length (Iso-)	0.108	0.113	0.957	0.347
Zygo To Back Crest (Iso-)	0.053	0.104	0.511	0.613
Zygo To Top Crest (Iso-)	-0.060	0.135	-0.444	0.660
Skull Length (Iso-)	0.009	0.116	0.078	0.938



	C1		
	Trait 1	**Trait 2**	**Trait 3**
Trait 1	$C1_{1,1}$	$C1_{1,2}$	$C1_{1,3}$
Trait 2	$C1_{2,1}$	$C1_{2,2}$	$C1_{2,3}$
Trait 3	$C1_{3,1}$	$C1_{3,2}$	$C1_{3,3}$

	C2		
	Trait 1	**Trait 2**	**Trait 3**
Trait 1	$C2_{1,1}$	$C2_{1,2}$	$C2_{1,3}$
Trait 2	$C2_{2,1}$	$C2_{2,2}$	$C2_{2,3}$
Trait 3	$C2_{3,1}$	$C2_{3,2}$	$C2_{3,3}$

	C3		
	Trait 1	**Trait 2**	**Trait 3**
Trait 1	$C1_{1,1}$	$C1_{1,2}$	$C1_{1,3}$
Trait 2	$C1_{2,1}$	$C2_{2,2}$	$C2_{2,3}$
Trait 3	$C1_{3,1}$	$C2_{3,2}$	$C2_{3,3}$

Figure B.1: Illustration of pairwise deletion process to incorporate all values for ARS and reproductive longevity despite two missing values for cub survival. Each table represents a covariance matrix estimated on the same data set, but on different segments. The cells in each covariance matrix are designated $CX_{i,j}$, where X designates which covariance matrix the variance or covariance was estimated in, i represents the row and j the column. C1 is a covariance matrix estimated for individuals with no missing data. C2 is a covariance matrix estimated for all individuals, but not for trait 1 (ie. cub survival in our model). C3 is an agglomerate covariance matrix, with row 1 and column 1 made up of covariances and variances from C1, but the other cells made up of values from C2. Each SEM was estimated on the equivalent of C3. Note that there were more traits in our model than are shown here.

CHAPTER 5

Selection and heritability in spotted hyenas: an evolutionary explanation for female-biased sexual size dimorphism

Introduction

Intersexual variation in body size-related morphological traits is a common phenomenon, known as sexual size dimorphism (SSD). SSD is generally explained by invoking different fitness optima for size or size-related traits in males and females (Badyaev, 2002, Fairbairn, 2007, Blanckenhorn, 2000, Blanckenhorn, 2005). SSD is of special interest because males and females share most of their genes, so intersexual variation in size requires sex-specific expression of the same growth-related genes (Badyaev, 2002, Fairbairn, 2007, Blanckenhorn, 2000). In most animals, females are larger than males, a pattern often explained by 'fecundity selection' on females, in which larger females produce a greater quantity or quality of offspring than smaller females (Andersson, 1994, Fairbairn, 2007). In mammals, however, males are usually the larger sex, a pattern often attributed to physical competition among males for access to females or mating opportunities (Alexander et al., 1979, Darwin, 1871, Rhen, 2007, Lindenfors et al., 2007, Ralls, 1976, Weckerly, 1998, Isaac). As one of the rare mammals, and arguably the only mammalian carnivore in which females are the larger sex (Ralls, 1976), spotted hyenas (*Crocuta crocuta*) represent an interesting exception to this pattern.

Spotted hyenas in fact exhibit a variety of uncommon, and often unique, sex-role reversed traits for their taxonomic position. For example, female spotted hyenas exhibit more intense aggression than males (Goymann et al., 2001, Van Meter, 2009), are socially dominant to males (Engh et al., 2000, Frank, 1986, Kruuk, 1972), and bear uniquely masculinized genitalia (Matthews, 1939, Kruuk, 1972, Hamilton et al., 1986). The evolution and ontogeny of some of these sex-role reversed traits, especially of masculinized genitalia, have been the subject of intensive research. However, their other sex-role reversed traits, notably female-biased SSD, have received little attention beyond documentation of the pattern; see Chapter 2 and (e.g. Matthews, 1939, Kruuk, 1972, Whateley, 1980, Sillero-Zubiri and Gottelli, 1992, van Jaarsveld et al., 1988, McElhinny, 2009).

Although the most parsimonious explanation may be that this entire suite of sex-role reversed traits may be driven evolutionarily or developmentally by a single set of factors, there are other interpretations of this unusual concentration of sex-role reversed traits in one species. For example, the evolutionary origin of some of these traits may alter the usual fitness landscape for morphological traits such that females that grow to be larger than males experience a fitness benefit. Alternatively, the evolution of some of these traits may be completely independent of one another. Here we set out to test predictions consistent with a specific evolutionary explanation for female-biased SSD independent of other sex-role reversed traits. Our previous research has shown that female spotted hyenas characterized by large size in some morphological traits have greater lifetime reproductive success than females exhibiting smaller size in those same traits (Swanson et al., 2011). Specifically, larger females live longer and have more cubs per year on average than smaller females (Swanson et al., 2011). Here we compare differences in selection between females and males to determine whether the presence and strength of selection on multiple morphological traits are consistent with the hypothesis that female selection documented by Swanson et al. (2011) contributes to the patterns of SSD documented by Swanson et al. (submitted). We also test three hypotheses forwarded concerning the importance of selection among males to female-biased SSD in the spotted hyena. First, the 'small-male advantage hypothesis' predicts that smaller males are favored by selection (Ralls, 1976, Blanckenhorn et al., 1995, Isaac, 2005). The second hypothesis, 'no selection on males', simply suggests that size traits are not under directional selection in male hyenas, and predicts that there should be no relationship between size traits and fitness in males for traits exhibiting female-biased SSD. Third, the 'weaker positive selection in males' hypothesis predicts that traits exhibiting SSD are under positive selection in both sexes, but also that selection is weaker in males than in females. If the observed selection contributes to the maintenance of SSD in this species, then both the 'small-male advantage' and the 'weaker positive selection in males' hypotheses predict that the difference between the selection coefficients for males and females with respect to particular morphological size traits should correlate positively with the degree of dimorphism separating the sexes in those traits. The 'no selection on males' hypothesis predicts that selection in males is irrelevant; thus selection coefficients for size traits expressed in females should correlate significantly with the degree of dimorphism observed in different morphological traits.

To test these hypotheses, we used general linear mixed modeling to estimate selection coefficients for 14 univariate morphological traits in both sexes. Swanson et al. (2011) demonstrated that selection operates in females through annual reproductive success (ARS). Because this fitness component has roughly the same implications in both sexes, we used ARS as our measure of fitness here. We also estimated the heritability of each morphological trait. Heritability is equal to the additive genetic variance underlying a trait, divided by the phenotypic variance (V_A/V_P). This measure is important because the ability of a phenotypic trait to respond to selection in a population is limited by the heritability of that trait. Finally, to determine whether our data are consistent with the explanation that selection contributes to observed patterns of female-biased SSD in the spotted hyena, we inquired whether the calculated difference between selection gradients in males and females is positively correlated with estimates of the degree of dimorphism expressed in particular traits.

Methods

Spotted hyenas live in social groups called clans, which can contain over 100 individuals, and consist of females born in the clan, their young, and several adult immigrant males. Clans are structured by strict linear dominance hierarchies (Holekamp and Smale, 1990, Frank, 1986, Smale et al., 1993), and an individual’s position in this hierarchy has profound effects on both survival and reproduction by mediating differential access to food at kills (Kruuk, 1972, Watts et al., 2009, Hofer and East, 2003). Social ranks are based here on the outcomes of dyadic agonistic aggressions (Holekamp and Smale, 1993, Smale et al., 1993). Female spotted hyenas are physiologically competent to breed after 24 months of age, but first parturition usually occurs in the third or fourth year of life, and the timing of first parturition varies greatly with rank (Holekamp et al., 1996, Hofer and East, 2003). Female hyenas are philopatric, whereas nearly all males emigrate and join neighboring clans after puberty (Smale et al., 1997, Van Horn et al., 2003). Here the ages of hyenas born in the study clan were known to within ± 7 days based on the date of emergence from the natal den

(Holekamp et al., 1996). The ages of immigrant males, however, were estimated based on tooth wear data to within 6 months (Van Horn et al., 2003).

Morphological data were collected in the field from immobilized hyenas. We used data from a total of 420 immobilizations of 301 individuals. For individual hyenas immobilized more than once as adults, we used mean values for morphological traits in selection analyses, but all data in heritability analyses, allowing us to estimate repeatability within traits. From each immobilized hyena we obtained 4 cranial and 9 post-cranial measures. Specifically, we measured skull length, head circumference, the distance between the widest point on the zygomatic arch and the top of the sagittal crest (henceforth 'zygo to top crest'), the distance between the widest point on the zygomatic arch and the posterior end of the sagittal crest (henceforth 'zygo to back crest'), body length, girth, neck circumference, shoulder height, lower leg length, upper leg length, hind foot length, front foot length, and scapula length. We also obtained a body mass measure for each individual at each immobilization. For all selection analyses using females, we only included measurements taken after 36 months of age or after first parturition, whichever came first; 36 months represents a conservative estimate of the age at which reproductive and morphological maturity is achieved among females (Watts et al., 2009, Holekamp et al., 1996, Tanner et al., 2010). Females were included if they met these criteria even if they died before giving birth. For males, we used measurements taken after 36 months of age. For heritability analyses, we included measurements taken after the measure reached maturity in Swanson et al. (submitted). All morphological data were natural log transformed prior to selection analysis, as traditionally suggested (Lande and Arnold, 1983, Arnold and

Wade, 1984) but raw data were used for heritability analyses, to estimate variances on non-transformed data.

The pedigree we used for heritability analyses was based on a combination of known maternity based on nursing associations (Holekamp et al., 1999), and assigned paternity based on genotypic data (see Engh et al., 2002 for details). Briefly, paternity was assigned based on 11 polymorphic autosomal microsatellite loci and one X-linked locus (Engh et al., 2002, Libants et al., 2000) when a 95% confidence criterion was satisfied in the program CERVUS (Marshall et al., 1998). Maternity was assigned based on regular nursing bouts and confirmed using microsatellites (Engh et al., 2002, Holekamp et al., 1999).

We estimated selection in each sex using annual reproductive success (ARS) as our fitness measure, as indicated by maternity and paternity data. To do this, we used the 'lme4' package (Bates et al., 2011) in R v.2.14.1 (R Development Core Team). We used ARS as the response variable in mixed models, including individual identity (ID) as a random effect in each model. This approach allowed us to include individuals only present in the population for one year without bias, and it allowed us to incorporate the social rank of each hyena during each year; ranks were calculated and standardized on a yearly basis, as these may change within individuals over time. Relative rank was calculated within each year. All variables included were mean-centered and standardized. Standardization was accomplished by dividing each value by the standard deviation of the sample for each sex separately. Models were built for males and females separately; selection coefficients were represented by the estimated slope of the regression. To determine whether selection estimates through ARS and reproductive lifespan documented in Swanson et al. (2011) are consistent with the idea that selection through the different fitness components results from the same factors, we estimated selection on individual female traits using reproductive lifespan estimates from Swanson et al. (2011). To do so, we regressed reproductive lifespan on the size of each morphological trait in separate univariate linear regressions. To test for a relationship between the magnitudes of both measures of selection we then regressed the magnitude of selection estimates using female reproductive lifespan as a measure of fitness on those using ARS as a measure of fitness.

To account for the fact that selection estimates on individual traits likely exhibit some degree of autocorrelation due to covariances among the morphological traits measured, we also estimated a second set of models estimating the correlation between selection estimates using reproductive lifespan as a measure of fitness and those using ARS as a measure of fitness. These models estimated and accounted for the relationships among the individual morphological traits. Accounting for these relationships is important because in this case, the selection estimates themselves are not independent due to the covariances among the included morphological traits. Specifically, we calculated the degree of dissimilarity among morphological traits on which selection was estimated by converting the covariance matrix of the log-transformed morphological traits into a matrix of Euclidean distances using the 'dist()' function in R. Then we used the 'hclust()' function in R on the distance on the resulting distance matrix. This approach dendrogram, or tree, from the results of a hierarchical cluster analysis, describing the hierarchical relationships and degree of difference among morphological traits. We then treated this set of relationships as a phylogeny, and performed phylogenetically corrected generalized least squares (PGLS) regression using the resulting tree (Paradis, 2006, Martins and Hansen, 1997). Although this approach is traditionally used on among-species data to account for phylogenetic relationships, it provides a straightforward method of dealing with the similarity that we observe among morphological traits that exhibit non-zero within-population covariances. PGLS regression simultaneously estimates and incorporates a parameter λ, that describes the degree to which the phylogenetic distances among species matches the autocorrelation among the residual errors from the model. When λ equals 0, it suggests no phylogenetic autocorrelation among residual errors, and when λ equals 1, it suggests that trait evolution is approximately modeled by Brownian motion. In this context, λ represents the degree to which residual error in the model reflects autocorrelation among morphological traits. If λ is equal to 0, it suggests that covariances among morphological traits do not explain any of the variation in the selection estimates. If λ is equal to 1, it suggests that there is essentially a 1:1 correspondence between the covariance among morphological traits and autocorrelation among the residuals. We tested three different models and compared the second-order sample-size corrected Akaike's Information Criterion (AICc) between each model to calculate a dAICc, the difference between an individual model and the model with the lowest, or 'best' AICc. The first model tested fixes λ to 0, the second model fixes λ to 1, and the final model estimates λ using maximum likelihood estimation (MLE). We consider the results of the resulting 'best' model alongside those of the 'naive' or basic model lacking a correction for covariances among morphological traits.

We next compared the differences between selection estimates for males and females (selection estimate in females - selection estimate in males), to the degree of dimorphism in each trait between males and females. To make these comparisons we used linear regressions with percent dimorphism calculated as log (Percent dimorphism + 1), estimated by Swanson et al. (submitted) as the response variable and the difference between selection estimates, from the current analyses as the predictor. A positive relationship would mean that the greater difference a trait exhibits in selection strength between females and males, the greater the related degree of female-biased SSD. Thus, a positive relationship would be consistent with the hypothesis that observed selection in males and females contributes to phenotypic patterns of SSD in our study population. To account for the fact that both estimates of dimorphism and selection may be influenced by covariance among individual morphological traits, we also took a PGLS-based approach here to estimate the relationship between selection in females and selection in males using ARS as a measure of fitness. The analyses here were performed identically to those comparing selection in females through the fitness components of lifespan and ARS.

We treated selection estimates (represented by circles in Figure 5.1) as statistically significant if they lay more than 1.96 standard errors (SEs) from 0 (error lines in Figure 5.1 represent 1 SE). This criterion is roughly equivalent to statistical significance at an $\alpha = 0.05$ using a z-test, or a t-test with a small sample size, and also equivalent to a 95% confidence interval. We used this criterion because the degrees of freedom for simultaneously testing fixed effects in mixed models are not well understood statistically, and thus exact p-values are uncertain (Elston, 1998). Therefore for all selection estimates, 'statistical significance' indicates that the 95% confidence interval does not overlap 0. Traits with positive parameter estimates were considered to be under positive selection, and traits with negative parameter estimates were considered to be under negative selection, whether or not the estimates were statistically significant at an $\alpha = 0.05$. The strength of evidence for selection was thus assessed by the magnitude of selection relative to the standard error.

We estimated repeatability and heritability of each morphological trait in a Bayesian mixed modeling framework implemented with Markov chain Monte Carlo (MCMC) methods using the 'MCMCglmm' package (Hadfield, 2010) in R. Repeatability was estimated by fitting a random effect of ID in each model, whereas heritability was calculated by estimating the variance explained by the pedigree. Repeatability represents the upper bound of one's ability to estimate heritability such that heritability can never be higher than repeatability. Repeatability is important to include in any heritability analysis to avoid biasing heritability estimates upward. In other words, estimates of heritability without repeatability risk concluding that some of the variance attributable to environmental factors is in fact genetic (Kruuk et al., 2008, Wilson, 2008). To ensure we were not biasing heritability estimates by failing to include important fixed effect parameters, we estimated a model with age as a predictor for each morphological trait, and a model with both age and sex as predictors in addition to the base model, with only the effect of multiple replications on the same individual and the effect of the pedigree fit. It has been suggested however, that controlling for variation due to some factors may remove variation visible to natural selection (Wilson, 2008). As such, the estimates from the base model may be just as valid as, or more valid than, estimates from the models with additional fixed effects. We fit MCMCglmm models using a weakly informative prior suggesting a conservative, somewhat smaller variance than that obtained by partitioning the variances equally among random terms (variances explained by each random effect set to 1/5 of the total phenotypic variance, nu = 1; see (Hadfield, 2010)). Fitting alternative priors did not alter any conclusions reached from repeatability or heritability estimates, although more informative priors had a quantitative effect on posterior estimates, and less informative priors sometimes resulted in chain mixing problems. The posterior distributions for MCMC models were sampled every 10,000 iterations after a burn-in of 500,000 iterations for a total of 1000 samples. Autocorrelation of Markov chains was less than 0.1, and chain mixing was visually inspected for problems.

Results

A number of morphological traits were under clear selection using ARS as a measure of fitness for both sexes (Figure 5.1). Specifically, for females, shoulder height, body length, head circumference, girth, and mass were all under statistically significant positive selection. Positive selection estimates indicate traits in which larger individuals are favored by selection. Among males, shoulder height and skull length were under statistically significant positive selection. We did not observe any statistically significant negative selection estimates for either males or females (Figure 5.1). Additionally, a number of other traits exhibited non-significant positive trends (e.g. scapula length for

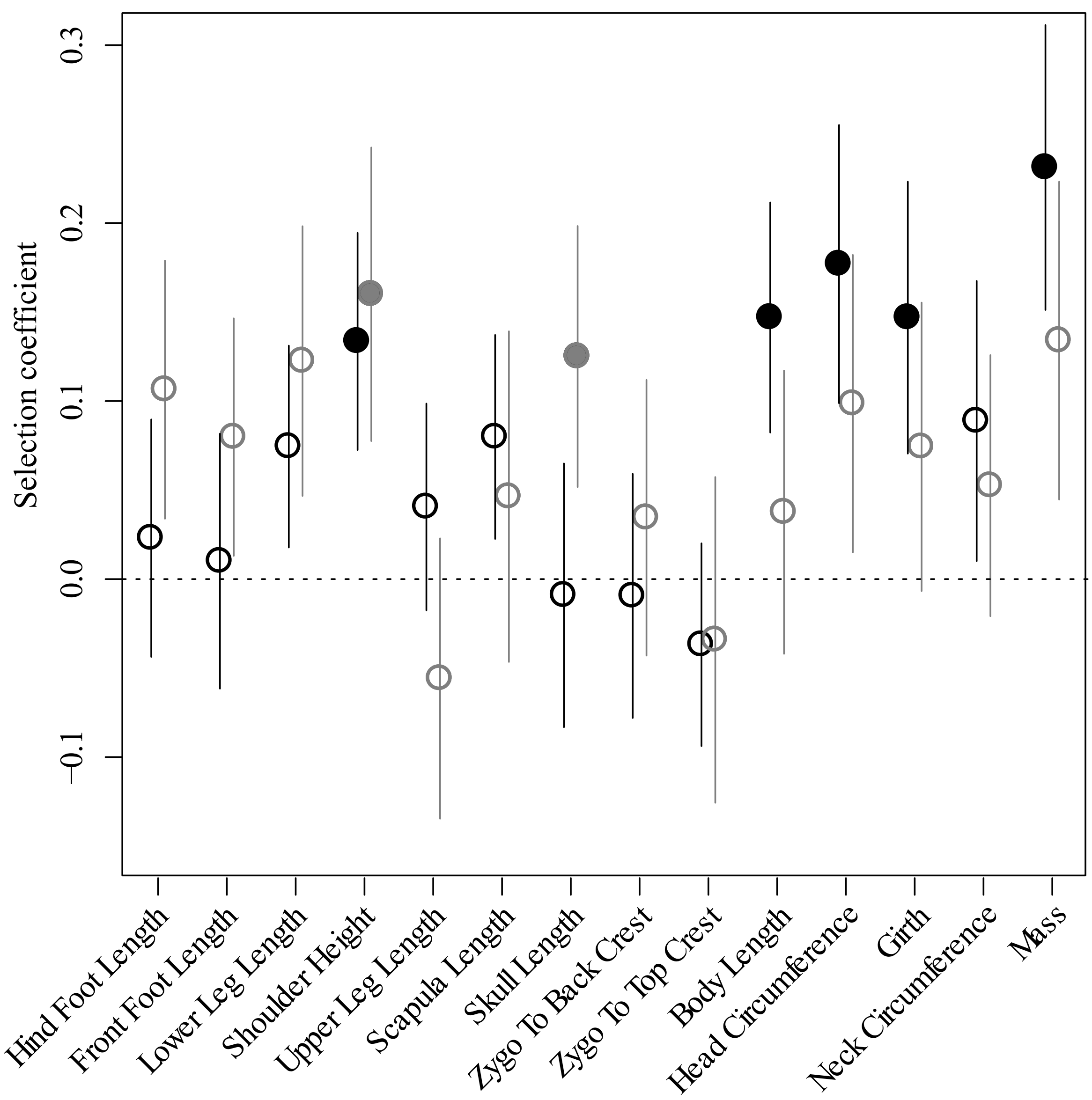


Figure 5.1. Comparison of selection between males and females using annual reproductive success as a measure of fitness. Gray circles and lines represent selection coefficients in males, and circles and lines in black represent selection coefficients in females. Open circles represent selection coefficients that are not statistically significant ($z < 1.96$) and filled circles represent selection coefficients that are statistically significant ($z \geq 1.96$).

females and lower leg length for males), and only 5 of 28 selection estimates (including estimates for both males and females) were negative (Figure 5.1). Furthermore, none of the 5 negative selection estimates were even one standard error from 0, which could be considered a much weaker version of 'statistical significance' at an $\alpha = 0.32$. Social rank significantly influenced female ARS in all models, as in previous work with this species (Holekamp et al., 1996, Swanson et al., 2011). Social rank also influenced ARS in males.

Interestingly, we found that selection estimates in females using ARS as a measure of fitness were strongly correlated with selection estimates in females on the same traits using reproductive lifespan estimates (Swanson et al., 2011) as measures of fitness (Figure 5.2). The model correcting for the covariance among morphological traits and therefore lack of independence among the selection estimates agreed. Specifically, the model with the lowest AICc was the model in which λ was fixed to 0 suggesting that there was no congruence between the degree of covariance among the morphological traits and the model residuals. The dAIC of the model with λ fixed to 1 was 1.4, and the dAIC of the model in which λ was estimated by MLE was 3.7. The results of the 'best' model agreed with the base 'naive' model, suggesting that selection estimates in females for morphological traits were correlated with selection estimates in females on the same traits using reproductive lifespan as a measure of fitness ($\beta = 0.853$, $SE = 0.237$, $t = 3.60$, $p = 0.004$).

Calculated differences between selection estimates in females and males using ARS (female selection coefficient - male selection coefficient) were strongly correlated with percent dimorphism both using a traditional linear regression, and one corrected for

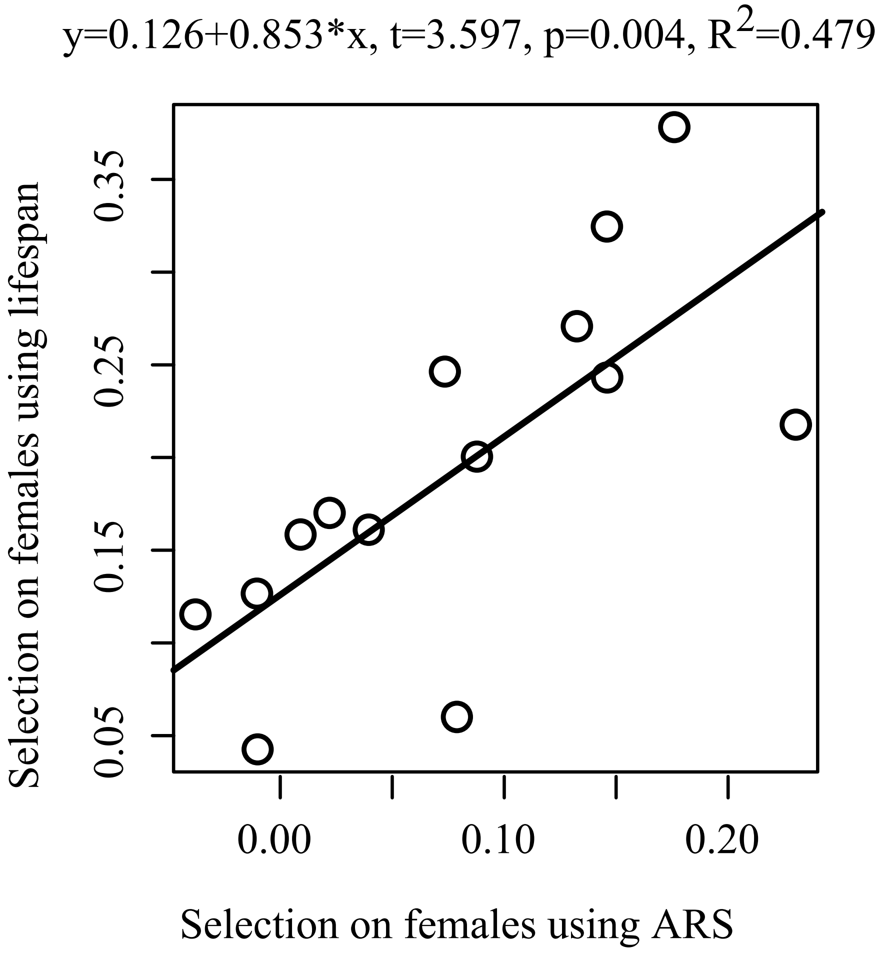


Figure 5.2. Comparison of selection estimates using lifespan and annual reproductive success as measures of fitness for females using reproductive lifespan estimates from (Swanson et al., 2011).

the potential dependence engendered by covariances among included morphological traits (Figure 5.3; Table 5.1). This result was consistent with our hypothesis that differences in selection coefficients between males and females contribute to the evolutionary maintenance of SSD in extant populations of spotted hyenas.

Posterior repeatability estimates for morphological traits ranged between 0.156 and 0.564 (mean = 0.322, SD = 0.111; shown in Figure 5.4 with 95% credible intervals). Including age and sex as fixed effects in Bayesian 'MCMCglmm' models generally reduced repeatability estimates, but only slightly, usually by less than 0.1. In some cases, for example that of shoulder height, the inclusion of these fixed effects increased the repeatability estimates by similar amounts (Figure 5.4). There was considerable variation among repeatability estimates, with mass, shoulder height, lower leg length, body length, and head circumference exhibiting the greatest repeatability, and zygo to back crest, girth, zygo to top crest, and upper leg length all exhibiting the weakest repeatability. Heritability estimates were generally smaller in magnitude than repeatability estimates, varying between 0.058 and 0.342 (mean = 0.150, SD = 0.091). Most suggested fairly low heritability for morphological traits (12 are < 0.5, 6 are < 0.1) but both shoulder height and head circumference estimates were around 0.4 for the base models (Figure 5.5). In six cases, including age as a fixed effect increased the heritability estimate, whereas in eight cases it decreased heritability. The effect of including sex was similar, increasing heritability in eight cases, and decreasing it in six. Most changes due to including a fixed effect were less than 0.1 in magnitude (Figure 5.5). As stated in the methods, the base models may in fact represent better estimates,

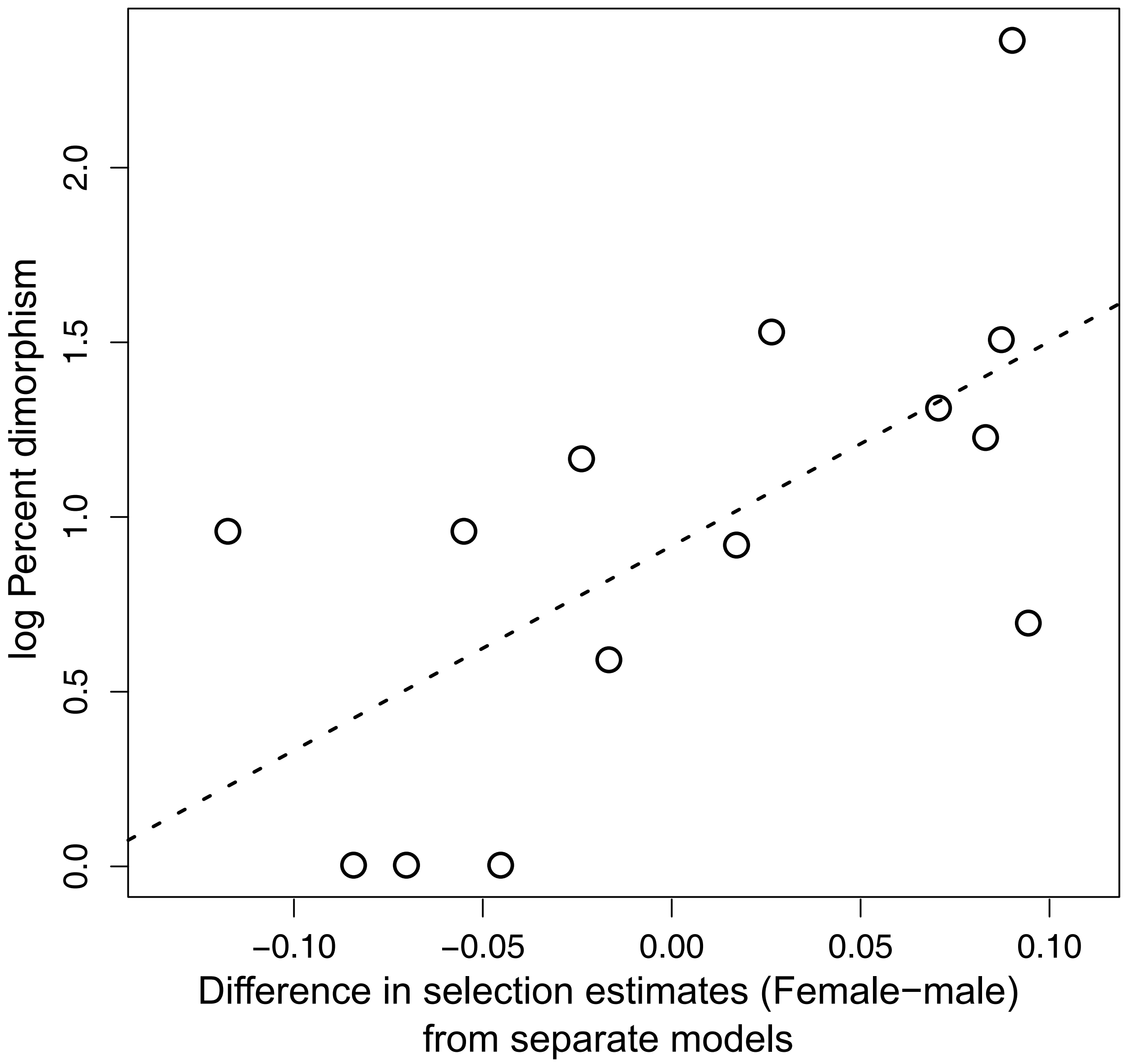


Figure 5.3. Plot of difference in selection estimates using annual reproductive success as a predictor of log transformed percent dimorphism for each trait. Open circles and dashed line represent differences calculated from a separate regression for each sex.

Table 5.1. Relationships between female-male selection estimate differences and degree of dimorphism in morphological traits expressed as [log (percent dimorphism + 1)]. Model **a** describes the relationship between percent dimorphism and the differences in selection coefficients between the sexes calculated from separate regression models, and corresponds to the black circles and lines in Figure 5.3. Model **b** describes the same relationship, but represents the slope and p-value adjusted for the covariances among the morphological traits on which both selection and the degree of sexual dimorphism was estimated.

	β	***SE***	***t***	***p***
a) Sex differences	5.493	1.906	2.881	**0.014***
b) PGLS sex differences	2.933	1.303	2.250	**0.044***

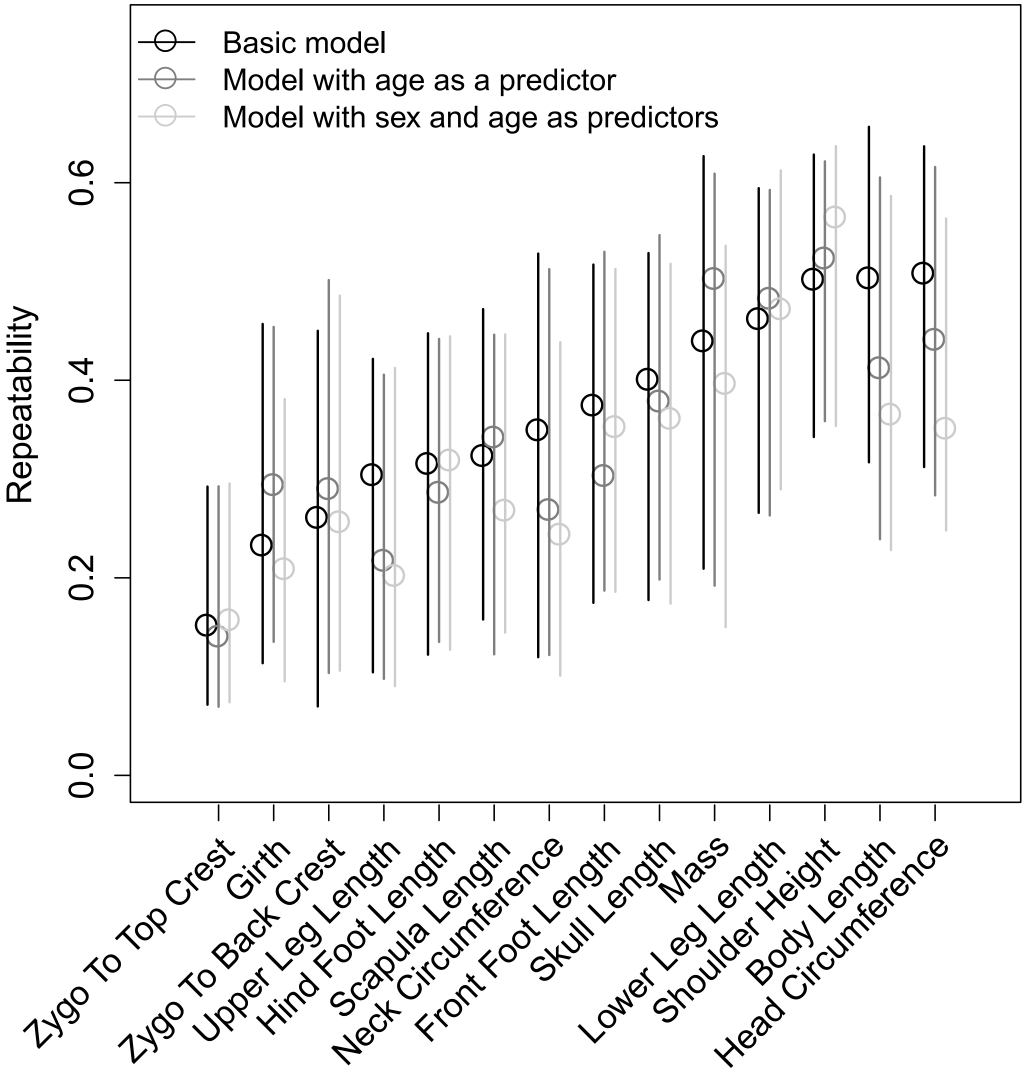


Figure 5.4. Posterior estimates and 95% credible intervals of repeatabilities estimated from 'MCMCglmm' models with different fixed effects. Repeatability has the same conceptual meaning as a coefficient of variation (CV), although the measures won't be exactly the same for a given trait because they are calculated differently. Black circles and lines represent estimate and credible interval for the base model with no fixed effects, dark gray with age, and light gray with age and sex as fixed predictors. Traits are ordered by the repeatability (from lowest to highest), estimated from the base model with no fixed effects.

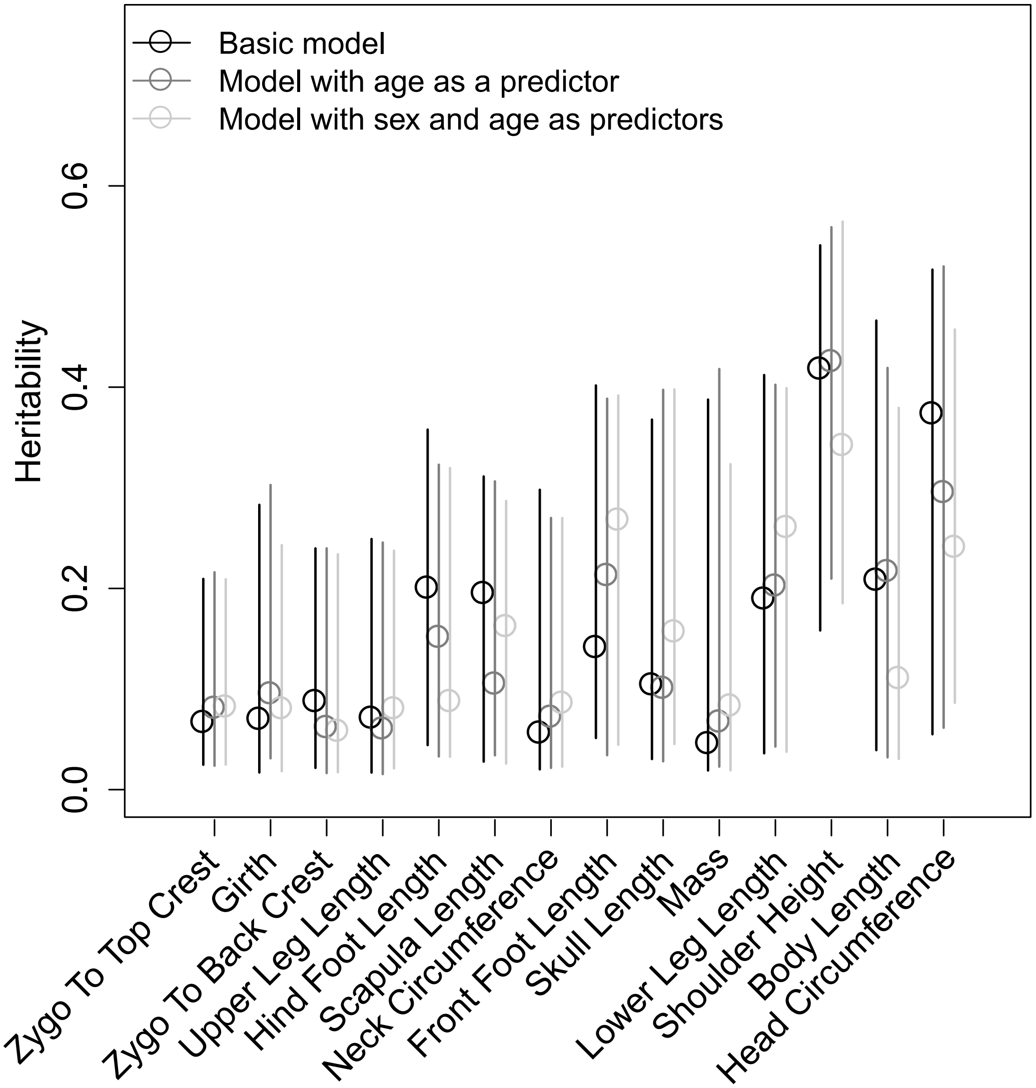


Figure 5.5. Posterior estimates and 95% credible intervals of heritabilities estimated from MCMCglmm models with different fixed effects. Black points and lines represent estimate and credible interval for the base model with no fixed effects, dark gray with age, and light gray with age and sex as fixed predictors. Traits are ordered by the repeatability estimated from the base model with no fixed effects.

because fixed predictors can account for phenotypic variance due to the permanent environment, or variance visible to natural selection (additive genetic variance), obscuring repeatability and heritability estimates, respectively (Wilson, 2008). In this context, accounting for variance refers to the variance explained by the variable in the statistical model.

Discussion

Our results are consistent with the hypothesis that the selection observed and quantified here contributes to the evolutionary maintenance of female-biased sexual size dimorphism (SSD) in the spotted hyena. Specifically, we rejected the 'small-male advantage' hypothesis, finding no evidence for negative selection on size in males. Statistically significant negative selection is not apparent on any trait in either sex, and there is in fact little evidence even for very weak negative selection (see Figure 5.1). We also rejected the 'No selection in males' hypothesis. Two morphological traits are under positive selection in males, and others exhibit non-significant trends in the same direction. Furthermore, it is notable that very few selection estimates are negative, even those estimates for which the standard errors strongly overlap 0 (Figure 5.1).

Our data instead supported the 'Weaker positive selection in males' hypothesis for most traits. Specifically, most selection estimates for males are closer to 0 than the estimates in females for the same traits; this is most apparent for traits under positive selection in females, especially for the most dimorphic traits. Body length, head circumference, neck circumference, girth, and mass represent examples of significant positive selection estimates in females where estimates in males exhibit a similar pattern, but are smaller in magnitude (right-hand side of Figure 5.1). Conceptually, it is not the female or male selection estimates alone that matter in the evolution of SSD, but rather the differences between male and female estimates that are important (Blanckenhorn, 2005). This is empirically apparent in our data because some of the traits that exhibit weak sexual dimorphism are under positive selection in females, but also under positive selection in males (e.g. shoulder height), while others are only under positive selection in males (e.g. skull length). In addition, it appeared that males were under stronger selection than females for the most monomorphic traits (left side of Figure 5.1), although the standard errors overlap among male and female selection estimates for all of these monomorphic traits (Figure 5.1). This suggests the possibility that some traits are monomorphic because they are under positive selection in males as well as females. The significant relationship between percent dimorphism and the differences in selection between males and females (female selection coefficient - male selection coefficient) suggests that selection in males is playing a role in current patterns of SSD in spotted hyenas.

The significant relationship between selection coefficients using ARS and lifespan as fitness components in females suggests that the same factors may be contributing to selection through both of these components of fitness. This is especially interesting given that Swanson et al. (2011) found that ARS and lifespan are not highly correlated in their own right, and our PGLS approach found that incorporating covariances among morphological traits does not improve the model in this case. If the same ecological factors are driving selection through both distinct fitness components, then that would also suggest that selection coefficients using ARS as a measure of fitness should be correlated to selection coefficients using lifespan within the same sex as well. This is indeed the case (Figure 5.2). Therefore, the correlation observed here strongly suggests that the agent of selection is such that it would influence both lifespan and ARS similarly.

Positive heritability estimates represent the final piece of evidence suggesting that our results are consistent with the selection we observe contributing to maintenance of SSD in extant populations of spotted hyenas. Our estimates of repeatability and heritability suggest a fair amount of variation in both of these measures (Figure 5.4; Figure 5.5). This is important because in order for selection to result in an evolutionary response, the trait under selection must have non-zero heritability. Heritabilities for some traits are quite low, especially compared to previous estimates of mean heritabilities on morphological traits. Previous work has estimated the mean heritability for morphological traits at 0.461 with a standard error of 0.004, with a sample size of 570 (Mousseau and Roff, 1987). Therefore our largest heritability estimate is slightly below the mean heritability for morphological traits, and the heritability for a number of our other traits is quite low. It does not appear that the heritability estimates are limited by repeatability for most traits. For example, there are a number of traits, such as mass, with high repeatability, but low heritability. There may be a number of reasons for this. Measurement error and variation in the degree to which temporary environmental conditions affect traits may both influence repeatability estimates, whereas both additive genetic and phenotypic variation can influence heritability. In addition, low heritability estimates can result from consistent stabilizing or directional selection operating on traits over long periods of time, and thereby reducing available additive genetic variance. The low heritability estimates we observe have other potential causes as well. For example, because heritability is calculated as the ratio of additive genetic to phenotypic variance (V_A/V_P), low heritability can result from high phenotypic variance, which sometimes characterized poor environmental conditions (Hoffmann and Merila, 1999, Gebhardt-Henrich and Van Noordwijk, 1991). Increasing phenotypic variance is an important possible contributor to decreasing heritability because heritability is calculated as a ratio such that increases in phenotypic variance decrease heritability similarly to decreases in additive genetic variance (Falconer and Mackay, 1996).

If individual hyenas respond variably in terms of growth rate and adult size to favorable and unfavorable conditions such as high vs. low rank, stress, good vs. bad mothers, etc., the result may be high phenotypic variance, or a large contribution of environmental variance to phenotype, either of which tends to reduce heritability. Maternal effects appear to be generally very strong in spotted hyenas as well (Holekamp and Dloniak, 2009, Dloniak et al., 2006, East et al., 2009, Hofer and East, 1993, Smale et al., 1995, Watts et al., 2009, Hofer and East, 1996), and may contribute to generally low heritabilities among morphological traits.

Although our results here are consistent with the observed selection contributing to variation in degree of SSD among morphological traits in contemporary spotted hyenas, the responsible agents of selection have not yet been identified. For females, Swanson et al. (2011) supported socio-ecological agents such as increased ability to hunt or steal food successfully from lions or other hyenas, while directly rejecting hypotheses that predicted a benefit of increased size on cub survival. Here we reject the 'small-male advantage' and the 'no selection in males' hypotheses, and generally support the 'weaker positive selection in males' hypothesis. In addition, we suggest here that selection in females is operating similarly through the fitness components of ARS and reproductive lifespan. Furthermore, among the most dimorphic traits, males exhibit patterns of selection statistically significant positive selection among females for those same traits (Figure 5.1). Among the least dimorphic traits, we observe a similar pattern, but males exhibit stronger selection than females. These results force us to reject purely sex-specific hypotheses such as fecundity selection, and support hypotheses that posit that size is important in performance of one or more fitness-related tasks for both males and females. We thus suggest that the agent of selection is ecological, influencing both fitness components concurrently. The remaining hypotheses suggested by earlier workers are 1) that hunting success improves with some size measures, 2) that larger individuals are more successful at winning fights, and 3) that individuals larger for some traits are better at accessing foods successfully in other ways such as through feeding competition. This is a non-exhaustive list, but given the importance of access to food for spotted hyenas, we suggest that these are the most likely hypotheses for selective agents explaining SSD in spotted hyenas. Importantly, it seems that the relevant fitness-related task does not influence male and female fitness to the same degree, which is reasonable due to the high costs of reproduction that females, but not males, must face. Essentially, for both sexes, we suggest that increased size for some traits is tied to some measures of critical ecological performance, leading to positive selection on increased size.

Clearly, even if the ecological agent of selection and physiological drivers of dimorphism are found to be the same in males and females, the reasons for increased ARS with increasing size may differ between the sexes, simply because the processes enhancing fecundity among females are different than those improving mating success among males. Female spotted hyenas have essentially complete control over mate choice due to their social dominance, larger size, and masculinized genitalia (East et al., 1993, Frank et al., 1995, Holekamp et al., 2012, Kruuk, 1972). This limits the possible mechanisms thorough which selection might be operating on males using ARS as a measure of fitness. For males specifically, we suggest that the role of size is in some way related to female mate choice or the ability of males to spend time engaging in mate guarding or attendance behaviors. For example, if larger males are better hunters, they may be able to spend more time trying to gain access to females, or time with females trying to gain mating opportunities; such 'shadowing' has been suggested previously in spotted hyenas (East and Hofer, 2001, Goymann et al., 2003), and in cercopithecine primates by (Bercovitch, 1997). Identifying the specific agents of selection operating to maintain SSD in spotted hyenas will require further work. In conclusion, the correlation between strength of selection and degree of sexual dimorphism, as well as the presence of low to moderate heritabilities for morphological traits measured here are consistent with the explanation that the observed selection plays an important role in the evolutionary maintenance of female-biased SSD in the spotted hyena.

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