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COOPERATION, COMPETITION AND KINSHIP IN THE SOCIAL RELATIONSHIPS AMONG SPOTTED HYENAS, CROCUTA CROCUTA

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COOPERATION, COMPETITION AND KINSHIP IN THE SOCIAL RELATIONSHIPS AMONG SPOTTED HYENAS, *CROCUTA CROCUTA*

By

Sofia Anne Wahaj

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Zoology Program in Ecology, Evolutionary Biology and Behavior

ABSTRACT

COOPERATION, COMPETITION AND KINSHIP IN THE SOCIAL RELATIONSHIPS AMONG SPOTTED HYENAS, CROCUTA CROCUTA

Bу

Sofia Anne Wahaj

The focus of this dissertation is on social bonds, competition, and cooperation among related and unrelated spotted hyenas (*Crocuta crocuta*). Gregarious animals that live in permanent social groups experience intra-group competition and are expected to repair social bonds damaged by within-group conflict. Indeed, reconciliation in many primates takes the form of affiliative behavior occurring shortly after a conflict. Here I inquired whether reconciliation also occurs among spotted hyenas (*Crocuta crocuta*), gregarious carnivores whose social lives share much in common with those of cercopithecine primates. An affiliative behavior was only identified as having a conciliatory function in hyenas if it occurred more frequently after than before fights, and if it was also associated with reduced rates of aggression between former opponents after fights. Greeting behavior and friendly approach satisfied both these criteria. Mean conciliatory tendency (CT) for individual hyenas was 11.3%, which fell at the low end of the CT range observed among primates.

Kin selection theory predicts that social interactions should vary with relatedness. I examined patterns of affiliation, association and aggression to inquire whether spotted hyenas can distinguish among various groups of maternal and paternal siblings. I also considered familiarity-based recognition and phenotype matching as mechanisms hyenas might use to recognize kin. My data clearly indicate that hyenas can discriminate among various types of siblings, that their social behavior conforms to predictions of kin selection theory, and that they recognize kin using mechanisms of both familiarity and phenotype matching.

Spotted hyena cubs exhibit intense intra-litter aggression immediately after they are born. Indirect evidence suggests that intense early sibling aggression might function in nature to kill siblings, resulting in either obligate or facultative siblicide. One of my aims was to determine what factors influence the occurrence and frequency of aggression among *Crocuta* siblings, and another was to use ultrasonography of pregnant females to test predictions of obligate and facultative models of siblicide. I compared litter sizes in utero to litter sizes after birth to determine frequency of litter reductions in both captive and field settings. Although litters born to multiparous captive females were larger at parturition than were litters of wild multiparous females when cubs were first seen above ground, litter composition did not differ. Litter reductions were common and observed at the same rate in both captive and wild populations. Not only did cubs from twin litters have higher survivorship than cubs from singleton or triplet litters, but cubs from twin litters whose siblings survived for at least three months had higher survivorship than those whose siblings died before three months of age. These data directly contradict predictions of the obligate siblicide hypothesis, but are consistent with those of an hypothesis suggesting that siblicide occurs facultatively in this species.

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CHAPTER ONE

GENERAL INTRODUCTION

Many animals live in groups that contain both kin and non-kin, and they generally cooperate and compete with individuals of both types on a daily basis. Kin selection theory (Hamilton 1964) predicts that relatives should be more valuable and more reliable social partners than non-kin under a wide variety of circumstances, that relatives should compete less intensively than non-kin, and that relatives should cooperate more frequently than non-kin. The purpose of my dissertation is to test these predictions in one species of gregarious carnivore, the spotted hyena (*Crocuta crocuta*). My dissertation compares social bonds, competition and cooperation between related and unrelated hyenas, and the factors that shape these phenomena. Chapters comprising my dissertation focus on conflict resolution, kin discrimination, and sibling rivalry in the spotted hyena.

Living in groups inevitably involves costs to individuals, but group-living persists because the fitness of individual group members exceeds that of solitary conspecifics. Whereas resource competition is perhaps the most common cost of life in groups, cooperation with group-mates to acquire or defend resources and fend off predators are common benefits (Alexander 1974). Competitive and cooperative behavior is often best understood, not in terms of single interactions, but in terms of patterns of interactions between two individuals that occur over extended time periods. Such patterns of interaction define an animal's social relationships (Hinde 1976). Animals that live in groups have opportunities to form long-term cooperative relationships, and these are particularly common

among close kin (Fletcher et al. 1987). However, formation of enduring cooperative relationships with non-kin, through mutually advantageous exchange of benefits, often represents another effective way to increase individual reproductive success (Walters 1987).

Social relationships may have long-term adaptive consequences for the individuals involved, particularly for long-lived animals, such as elephants, cetaceans, carnivores and primates, who often interact with the same group members throughout their lives. The social system of spotted hyenas resembles that of many Old World primates (Mills 1985; Frank 1986; Henschel et al. 1987), in which females remain in their natal group, or clan, throughout their lives while males disperse to join other clans as adults. Spotted hyenas regularly cooperate during group hunts, territorial advertisement and defense, and defense of individual carcasses from both inter- and intra-specific competitors (Kruuk 1972). Hyena social groups, called clans, are structured by linear dominance hierarchies, much like the hierarchies that structure troops of cercopithecine primates. Among females, dominance status is determined by maternal rank, and rank remains quite stable throughout a female's lifetime. Among adult immigrant male hyenas, dominance rank appears to be determined primarily by length of tenure in the group (Henschel et al. 1987). In stable groups of mammals, where individuals interact repeatedly over extended time periods, the presence of a dominance hierarchy speeds settlement of disputes over access to particular resources (Pusey et al. 1997). In hyena society, an individual's position in the hierarchy determines its priority of resource access, and high-

ranking females and their offspring have the best resource access in the entire group. This superior resource access allows high-ranking females to enjoy the greatest reproductive success (Holekamp et al. 1996). One important form of cooperative behavior among free-living *Crocuta* is coalition formation (Zabel et al. 1992), which often enhances the competitive effectiveness of individuals within the clan. Coalitionary attacks among spotted hyenas tend to reinforce the existing dominance hierarchy (Zabel et al. 1992).

As is also true in cercopithecine primates, spotted hyenas prefer to associate with and direct affiliative behavior toward individuals who are higherranking than themselves (East et al. 1993). In fact, high-ranking female Crocuta are more popular social companions than are lower-ranking females (Holekamp et al. 1997). Thus, it appears that hyenas, like many primates, recognize that some group members are more valuable social partners than others. The value of a relationship reflects the magnitude of social or ecological benefits likely to accrue from it, with valuable relationships most worthy of maintenance and protection (Kummer 1978; Cords 1988; Cords et al. 2000). When individuals interact repeatedly over long periods of time, the security and reliability of relationships also become important factors shaping sociality in long-lived gregarious animals. Greeting ceremonies among hyenas, an expression of affiliative behavior in which individuals inspect each other's anogenital region, are often preferentially directed at dominant animals. These greeting ceremonies may represent one means of strengthening social bonds within the clan (Kruuk 1972; East et al. 1993).

Aggression is often a manifestation of competition within groups and it represents an integral part of most social relationships. Aggressive behavior in the spotted hyena occurs in a variety of contexts, and it varies in intensity from subtle intention movements to killing bites. With their powerful musculature and sharp teeth, spotted hyenas are well-equipped to cause serious injury to attacked individuals. Aggressive interactions among spotted hyenas are typically characterized by approach or attack by one or more dominant animals directed at a lower-ranking individual, who responds with unambiguous submissive behavior and withdrawal (Smale et al. 1993). Female *Crocuta* are generally more aggressive than males, and male aggression toward other males is relatively low in frequency and intensity (Szykman 2001).

Because the genetic interests of individuals are not identical, conflicts of interest perpetually endanger the survival of cooperative relationships (Silk 1987). Even the most intimate kin, such as mothers and offspring, interact in both affiliative and competitive ways (Trivers 1972; Nicolson 1987). Siblings are often the closest relatives within a population, and they are often one another's most direct competitors as well (Alexander 1974). Thus, relationships between siblings represent a mix of cooperative and competitive interactions. Female spotted hyenas give birth to one or two cubs (rarely three) in an isolated natal den (East et al. 1989). When cubs reach 2-4 weeks of age, they transfer their cubs to the clan's communal den, where up to 20 offspring from several different females may reside concurrently (Kruuk 1972). Siblings live in close proximity during early life and interact in a variety of ways, such as huddling together,

playing together, nursing together, grooming one another, fighting, establishing dominance relationships within the litter, and sharing food. Siblings may also suppress one another's growth and reproduction (Golla et al. 1999). Sibling relationships in spotted hyenas may be shaped by a number of different variables, and part of this dissertation focuses on elucidating factors that determine when hyena siblings should cooperate and when they should act selfishly.

OVERVIEW OF CHAPTERS

The highly structured group life characteristic of most gregarious species did not evolve through a decline in aggressive and competitive tendencies, but rather through the development of mechanisms to deal with such tendencies (de Waal 1987). Reconciliation is one form of conflict resolution animals may use to repair social relationships damaged by aggression. Reconciliation is an important behavioral mechanism regulating social relationships and reducing social tension in hierarchical primate societies (Aureli et al. 2000). Given that hyenas and many cercopithecine primates live in similarly complex societies, in Chapter Two I sought to determine whether spotted hyenas possess reconciliation tendencies comparable to those found in primates. Here I demonstrate that greeting and non-aggressive approach behavior serve a conciliatory function in spotted hyena society since these behaviors occur more frequently after than before fights, and they also tend to reduce the probability of subsequent attack. Conciliatory tendency, a measure of reconciliation behavior,

in spotted hyenas is relatively low when compared to that documented in primates, and may reflect the fission-fusion nature of hyena society. That is, whereas cercopithecine primates live in highly cohesive societies in which individuals are always together, spotted hyenas have the option of using dispersive mechanisms of conflict resolution by separating after fights rather than reconciling. Although kin selection theory predicts social relationships with kin should be more valuable than those with non-kin, I found that kin exhibited lower conciliatory tendencies than did non-kin. These results suggest relationship security, which is higher within kin than non-kin dyads, has a stronger effect on conciliatory tendency in *Crocuta* than does relationship value. The work presented in Chapter Two has been published in *Ethology* (Wahaj et al. 2001).

Because kinship can have profound effects on social relationships, kindifferentiated behavior in gregarious mammals requires significant social discrimination abilities (Sherman et al. 1997). Although earlier studies (e.g. Holekamp et al. 1999) suggested that *Crocuta* could discriminate maternal kin from non-kin, genetic data were not available until now to permit analyses of kin recognition abilities among other kin classes, including paternal kin. Full siblings, half-siblings, and non-kin each share different proportions of their genes, yet all of these kin classes overlap in time and space within spotted hyena clans. Chapter Three demonstrates that hyenas can discriminate among these relatedness classes, and that they cooperate most with their most closely related kin. I further illustrate that hyenas use mechanisms of both familiarity and phenotype matching to differentiate among various categories of siblings.

Results of Chapter Three have recently appeared in *Behavioral Ecology and Sociobiology* (Wahaj et al. 2004).

Sibling rivalry in the form of sibling aggression is unusually intense and frequent in young spotted hyena cubs (Frank et al. 1991). Chapter Four attempts to determine what factors influence the occurrence and frequency of aggression between *Crocuta* siblings. Independent variables considered here include cub age, litter composition, identity of the target of aggression and resource availability. This study illustrates intense sibling aggression during the first two months of life, when neonates are establishing intra-litter rank relationships, with rates of aggression decreasing thereafter. Although maternal rank did not have a significant influence on aggression between *Crocuta* litter-mates in this study, levels of local prey abundance were negatively correlated with rates of fighting between siblings. Results of Chapter Four suggest that competitive relationships among spotted hyena siblings are not invariant, but instead fluctuate with shifting environmental conditions.

Although spotted hyena cubs clearly display intense aggression during early neonatal life, the adaptive significance of this early fighting is not clear. It has been suggested in the literature that this early aggression routinely results in sibling death (Frank et al. 1991). Obligate siblicide, as is observed in some avian species (reviewed in Mock et al. 1997), occurs when aggression between siblings is almost always fatal to the subordinate individual. However, in facultative siblicide, siblings adjust fighting intensity as costs and benefits of intra-

litter aggression vary with environmental conditions. Chapter Five tests predictions of obligate and facultative models of siblicide in wild spotted hyenas using ultrasonography techniques to assess litter reduction in captive and wild hyenas. Litter sizes and patterns of litter reduction in captive and wild hyenas were similar, and survivorship data suggest individuals survive better with a sibling than without one. Thus, results from both Chapters Four and Five provide support for the facultative model, but contradict predictions of the obligate model of siblicide in the spotted hyena.

CHAPTER TWO

Wahaj, S. A., K. R. Guse, and K. E. Holekamp (2001). Reconciliation in the spotted hyena (*Crocuta crocuta*). *Ethology* 107: 1057 – 1074.

CHAPTER TWO

RECONCILIATION IN THE SPOTTED HYENA (CROCUTA CROCUTA)

Gregarious animals that live in permanent social groups experience intragroup competition, and are therefore expected to exhibit non-dispersive forms of conflict resolution (de Waal 1986; de Waal 1989). Aggression often has negative consequences aside from injury and resource loss in that it may also damage social relationships. An attacked animal may be uncertain about the aggressor=s future intent regarding either tolerance or further aggression (Aureli 1992). Thus, behavioral mechanisms are often needed after fights to repair social relationships and reduce the uncertainty experienced by victims of aggression regarding their future social interactions with aggressors. De Waal & Van Roosmalen (1979) suggested reconciliation behavior as one mechanism that might modulate aggressive conflicts among primates. Defining reconciliation as "any friendly reunion between former opponents occurring soon after an agonistic conflict", they documented its occurrence in chimpanzees. Since 1979, conciliatory behavior has also been described in many other primate species (reviewed in Aureli et al. 2000).

Whereas affiliative post-conflict behavior has now been widely studied in primates and appears to occur throughout this entire order (de Waal 1986; de Waal 1986; Kappeler et al. 1992; de Waal 1993; Silk 1997; Aureli et al. 2000; de Waal 2000), few data exist on post-conflict behavior in non-primate mammals (reviewed by Schino 2000). Data on this from a wide array of taxa, particularly

from gregarious animals other than primates, are needed to develop a more comprehensive understanding of the social conditions under which it occurs, the cognitive capacities required for it (Kappeler et al. 1992; Kappeler 1993), and the selective forces that have shaped its evolution (Silk 2000).

We focus here on reconciliation behavior observed among free-living spotted hyenas (*Crocuta crocuta*). These are gregarious carnivores whose complex social lives share much in common with those of cercopithecine primates (Holekamp et al. 1991; East et al. 1993; Holekamp et al. 1999). Both spotted hyenas and old-world primates are long-lived mammals that live in permanent social groups, the members of which cooperate to acquire and defend resources (Henschel et al. 1991; Harcourt 1992; Boydston et al. 2001). Spotted hyenas also often depend on help from other group members during hunts of large-bodied prey, defense of ungulate carcasses against inter- and intraspecific competitors, and coalition formation important in both the acquisition and maintenance of social rank (Kruuk 1972; Zabel et al. 1992; Holekamp et al. 1997; Engh et al. 2000). Thus, as also occurs in primates, the enduring social relationships found among these long-lived carnivores affect survival and reproduction of individual group members.

Like those of many primates, social groups of spotted hyenas usually contain multiple adult males and several matrilines of adult female kin with offspring, including individuals from several overlapping generations. Males in both hyena and cercopithecine primate societies disperse from their natal groups whereas females are usually philopatric (Cheney et al. 1983; Henschel et al.

1987; Pusey et al. 1987; Mills 1990; Smale et al. 1997). Spotted hyenas appear to recognize the other members of their social group as individuals using visual, olfactory (Kruuk 1972), and acoustic cues (East et al. 1991; Holekamp et al. 1999). In social groups of *Crocuta*, macaques, baboons, and vervet monkeys, adults can be ranked in a linear dominance hierarchy, members of the same matriline occupy adjacent rank positions, and female dominance relations are stable for extended periods and across a variety of contexts (Tilson et al. 1984; Andelman 1985; Frank 1986). In hyenas as in many primates, an individual's position in the group's hierarchy strongly determines its priority of access to food (Kruuk 1972; Frank 1986). The one respect in which hyena societies differ from those of cercopithecine primates is that, among adults, female hyenas are socially dominant to males. Female dominance is very rare in primates other than lemurs (Kappeler 1993).

Reconciliation is an important behavioral mechanism regulating social relationships and reducing social tension in hierarchical primate societies (Aureli et al. 2000). Given that primates and hyenas live in similarly complex societies, we sought to determine whether spotted hyenas possess reconciliation tendencies comparable to those found in primates. First, we inquired whether spotted hyenas reconcile by comparing rates at which various affiliative behaviors occur before and after fights, and ensuring that emission of particular affiliative behaviors is subsequently associated with reduced aggression between former opponents. Second, we attempted to identify variables affecting conciliatory tendencies (CTs) among hyenas (de Waal et al. 1988; Kappeler et al.

1992; Chaffin et al. 1995). Specifically, we examined post-conflict behavior in *Crocuta* in light of theoretical expectations based on potential costs and benefits to the individuals involved (Schino 2000).

Levels of direct feeding competition, both within and between social groups, are extremely high in *Crocuta*, and this should theoretically favor high rates of conciliatory behavior in this species compared to others in which feeding competition is less intense (van Schaik 1989; Sterck et al. 1997). On the other hand, Crocuta live in fission-fusion societies in which dispersive conflict resolution is always a viable option for individuals that are attacked by conspecifics. Thus Crocuta might be expected to reconcile their conflicts less frequently than do members of more cohesive groups, and instead avoid shortterm costs of conflict by separating from former opponents (Schino 2000). Spotted hyenas are well armed, so risk of injury to recipients of aggression from continued or escalated fighting is high in this species. Since repair of social bonds should therefore be more valuable to recipients of aggression than aggressors, we anticipated that recipients would be more likely to reconcile than aggressors. Close relatives in *Crocuta* associate most closely, are most likely to hunt together, and join most frequently as allies during coalition formation (Smale et al. 1995; Holekamp et al. 1997; Holekamp et al. 1997). Therefore, if social behavior of Crocuta conforms to predictions of the 'valuable relationship' hypothesis (Kummer 1978; Cords 1988), then kin should exhibit greater CTs than non-kin. Alternatively, if relationship security (Kummer 1978; Cords 1988) affects probability of reconciliation, then kin should exhibit lower CTs than non-kin, since

interactions among non-kin are considerably less predictable and more aggressive in this species than are interactions among kin.

METHODS

Subjects and Study Site

This study took place study between June 1988 and April 1998, in the Talek region of the Masai Mara National Reserve, Kenya, an area of open rolling grassland. Subjects were members of one large *Crocuta* clan that occupied an area approximately 65 km5. The borders of the home range of the Talek clan have been stable since at least 1979 (Frank 1986).

All members of the Talek clan were identified by their unique spot patterns and other conspicuous characteristics, such as ear notches. We determined the sex of each clan member using methods described by Frank et al. (1990). We estimated their birth dates (to _ 7 days) by using their pelage, size, and other aspects of their appearance and behavior when cubs were first observed above ground (Holekamp et al. 1996). Here we considered individuals up to the age of two years to be juveniles, and older animals were classified as adults. On average during each year of the study period (N=10 yrs), the Talek clan contained 25 ± 1 adult females, 18 ± 2 adult males, 16 ± 1 juvenile females, and 15 ± 2 juvenile males.

Mother-offspring relationships were established based on regular nursing associations, and other genealogical relationships within matrilines were as described by Holekamp et al. (1993). Although information about paternity was

unavailable for many hyenas used as subjects in this study, we were able to compare CTs between kin and non-kin by calculating coefficients of relatedness (r, Hamilton 1964) from matrilineal pedigrees for members of each hyena dyad, based on the assumption that there is no multiple paternity within twin litters. Because we found previously that vocal recognition of maternal kin occurs among animals with *r*-values as small as 0.125 (Holekamp et al. 1999), we defined kin here as members of dyads with *r*-values of 0.125 or greater.

Social ranks were determined based on wins and losses in dvadic agonistic interactions, as described previously (Holekamp et al. 1990). Briefly, we identified the subordinate member of a dyad engaged in an agonistic interaction as the animal responding with retreat and appeasement behavior to aggression directed at it by its social partner. Aggressive behaviors monitored throughout this study included head wave, lunge, aggressive posture (ears cocked forward while the tail was bristled and raised), chase, displace, stand over, bite, and push. Appeasement behaviors included head-bob, grin, openmouth appeasement, carpal crawl, skitter away, and submissive posture (body crouched with tail between legs and ears flattened back against the head). These behavior patterns have been described in detail elsewhere (Kruuk 1972; Frank 1983; Holekamp et al. 1990). Outcomes of agonistic interactions were organized into a hierarchical matrix from which each individual hyena could be assigned an 'absolute' social rank in the clan's dominance hierarchy (Martin et al. 1988). Rank distance was then calculated between members of each hyena

dyad by subtracting the absolute rank of the subordinate individual from that of the dominant animal.

Data Collection

Throughout the study period, observers were present in the study area at least 23 days/month except for April 1991, when observers were present for 14 days. We observed members of the Talek clan daily from our vehicles, which we used as mobile blinds. An observation session started when we found two or more hyenas separated from other hyenas by at least 200m. Observation sessions occurred at natal and communal dens, at ungulate kills, and away from both dens and kills as, for example, when animals were traveling or resting. Throughout every observation session, we recorded all occurrences (Altmann 1974) of aggression and appeasement behavior by all hyenas present. Agonistic behavior recorded during every dyadic aggressive interaction included both the aggressive behavior emitted by the initiating individual, and the response to that aggression emitted by its victim. We assigned each aggressive act to a category describing its intensity as low (head wave, aggressive posture), medium (lunge, displace, stand over), or high (push, chase, bite). Responses to aggression emitted by victims included retreat, appeasement behavior, failure to respond, and counter-attack.

During observation sessions we conducted 30 minute focal animal observations on particular individuals, including adults and juveniles of both sexes. During each focal animal observation period, the following eight

candidate affiliative behaviors were recorded if either the focal animal directed them toward a recent opponent or that opponent directed them toward the focal individual: sniff, groan (a friendly 'come-hither' vocalization), lick, lie or rub against, initiate play, present, greet, and friendly approach to within one meter of the former opponent. During a greeting one hyena lifted its hind leg and offered its genitals for inspection to the other animal, who often reciprocated and sniffed them while raising its own hind leg. Presenting involved one hyena standing perpendicular to its social partner and stretching both its hind leas out posteriorly to give the social partner opportunity to sniff its belly or genitals. During a friendly approach, the focal animal expressed no signals of aggressive intent with either body postures or facial expressions, although the approaching animal often showed unambiguous expressions of fear (e.g. grinning with ears back) as it came near a former opponent (Kruuk 1972). When the recipient of an attack responded with retreat accompanied by these signs of fearfulness, this was not considered to be affiliative behavior. Instead this was recorded as a response to conspecific aggression, and was therefore considered part of the original fight. However, when an individual subsequently initiated a new interaction with its former opponent by approaching with no aggressive intent, yet while emitting signs of nervousness or fear, we considered this to be affiliative behavior. Given the impressive weapons carried by these animals, and the abundant opportunities for dispersive conflict resolution in the fission-fusion society characteristic of this species, approach without aggression toward a recent opponent appeared to represent an unambiguous, even risky, affiliative gesture.

When reviewing printed field notes, we selected focal animal observations in which a dyadic agonistic interaction occurred, and in which victim and aggressor were present within 200 m of each other for at least 5 minutes before, and 5 minutes after, the fight. A total of 220 focal animal observations were used that involved 73 hyenas and their 87 social partners, with an average of 3.01 + 0.32 focal animal observations per individual. We recorded the occurrences of all affiliative behaviors during both pre- and post-fight intervals, calculated the rates at which each occurred during the post-conflict interval, and used the preconflict interval as a matched control (de Waal et al. 1983). We elected to use the pre-conflict interval as a matched control period rather than use a comparable control interval on a different day, because the same set of individual hyenas are rarely found together at the same time and under the same circumstances on different days in the fission-fusion society characteristic of this species (Hofer et al. 2000). We were able to use a small subset of samples (N=14 dyads) to compare behavior within dyads on days they fought with that observed within 30 days of the original conflict, on days when they did not fight. However, we were generally unable to use these superior control intervals preferred by primatologists in our field study, as has also been the case in some earlier field studies of reconciliation in primates (e.g. Cheney et al. 1989) and hyenas (e.g. Hofer et al. 2000).

Data Analysis

We required that any affiliative behavior satisfy two criteria to be considered as a functional conciliatory gesture. First, we required that a particular affiliative behavior occur at a higher rate during the 5 min post-fight interval than during the 5 min pre-fight interval. Our rate data were not normally distributed so we used Wilcoxon matched-pairs signed-rank tests to compare mean rates at which particular affiliative behaviors were emitted by individuals during the pre-fight interval with mean rates of behavior exhibited by those same individuals during the post-fight interval. After eliminating those affiliative behaviors that did not occur at significantly higher rates after than before fights, we used Wilcoxon matched-pairs signed rank tests to compare mean rates of attack on individuals during post-conflict periods when one of the remaining affiliative behaviors occurred with mean rates of attack on those same individuals during post-conflict periods when no affiliative behaviors were emitted. Functional conciliatory behaviors were identified as those for which attack rates in the former post-conflict intervals were lower than those observed during the latter post-conflict intervals. Mann Whitney tests were used to compare rates at which different affiliative behaviors were emitted within each time interval.

After specific affiliative behaviors were identified as having a conciliatory function, we examined the temporal patterning of occurrences of these behaviors, and used Chi-square tests to compare the probability of initial occurrence of each identified affiliative behavior during each minute of both preand post-conflict periods. Using fights as the units of analysis, we constructed a 2

by 2 contingency table for each minute, in which columns represented pre- or post-conflict intervals and rows whether or not initial affiliative contact was observed during that minute. In comparing pre- and post-conflict intervals here, we numbered minutes moving forward in time during the post-conflict period, and moving backward in time from the conflict during the pre-fight interval. Thus for example, the Chi-square analysis performed for Minute 1 compared events occurring during the first minute after fights with those occurring during the last minute before fights.

We next calculated CTs within hyena dyads (Veenema et al. 1994), and inquired whether these were affected by kinship, age-sex classes of opponents, and rank distance between opponents. In these calculations, a hyena pair was called 'attracted' (A) if conciliatory behavior between opponents occurred earlier in the post- than in the pre-conflict interval, or if it occurred only during the post conflict period. A pair was called 'dispersed' (D) if conciliatory behavior between opponents occurred earlier in the pre- than the post-conflict interval, or if it occurred only before the fight. A pair was considered 'neutral' (N) if conciliatory behavior between opponents occurred at the same time in respective intervals, or if no conciliatory behavior occurred in either interval. We then calculated the CT for all hyena pairs using the formula, [CT=(A-D)/(A+D+N) * 100], suggested by Veenema et al. (1994). A Wilcoxon matched-pairs signed rank test was used to compare the number of attracted to the number of dispersed pairs per individual. To compare our results with those from studies of reconciliation in
other species, the Wilcoxon matched-pairs signed rank test was used to compare the number of attracted pairs to the number of dispersed pairs per individual.

In analyses of effects of specific independent variables on CT, we mainly used individual hyenas as the units of analysis, and used individuals as their own controls. For example, to examine the effect of kinship on reconciliation behavior, we calculated a mean CT for each individual in its interactions with kin, and we also calculated a mean CT for that same individual during its interactions with non-kin. We then compared these mean CTs for kin and non-kin across all individuals observed to interact with both groups, using a Wilcoxon signed rank test. Similarly, to ascertain whether victims or aggressors exhibited higher CTs, we calculated a mean CT for each individual in interactions when it was the aggressor, and calculated a second mean CT for that same individual when it was the victim in fights. We then compared these mean CTs for victims and aggressors across all individuals observed to play both roles, using a Wilcoxon signed rank test.

Next we used the dyad as the unit of analysis to inquire whether CT varied with the age-sex classes of the opponents involved in fights. For recipients of aggression of each age-sex class, we calculated mean CTs in interactions with each age-sex class of attacker, and compared these means using Kruskal-Wallis tests. We also compared CTs of males and females using a Mann-Whitney test. Since our study spanned several years, we were able to compare the CTs of individual observed when they were juveniles with the CTs of those same

individuals when they were observed as adults, using a Wilcoxon signed rank test.

Dyads were used as the units of analysis in Kruskal-Wallis tests to determine whether CT varied with context in which the fight occurred (at dens, over food at kills, or away from both dens and kills), or intensity of aggression occurring during the fight (low, medium or high). Finally, to determine whether CT was affected by rank distance, we regressed the CTs calculated for dyads against the rank distance between victim and aggressor within each of those same dyads to obtain a Spearman's rank correlation coefficient. Differences between groups were considered significant when $P \le 0.05$. All statistical tests were two-tailed.

RESULTS

During the study period we observed 160 hyenas participating as either victims or aggressors in 698 fights in which we could monitor the behavior of both victim and aggressor for at least five minutes before and after the fight, and in which the full five minutes preceding the fight elapsed without any aggression between these opponents. On average, each individual hyena was involved in 8.7 ± 0.9 conflicts. In total, 494 different hyena dyads were involved in the 698 fights. Of the eight candidate affiliative behaviors recorded during focal animal surveys, only five satisfied our first criterion for classification as reconciliation behavior. That is, rates at which individuals emitted affiliative behaviors were significantly higher in the post- than the pre-fight interval for the following

behaviors: greet (Z=2.848; P=0.004), present (Z=2.807; P=0.005), friendly approach (Z=-2.475; P=0.013), rub or lie against (Z=-2.023; P=0.043), and lick (Z= -2.805; P=0.005). None of the other three candidate affiliative behaviors satisfied our first criterion so these were not considered further (play: Z=-1.841, NS; groan: Z=-0.631, NS; sniff: Z=0.069, NS). We subsequently treated present and greet together as one category of affiliative behavior, which we called 'greetings,' and the other three behaviors that satisfied our first criterion were treated together as 'approaches.'

Individual hyenas initiated greetings at significantly lower rates than they initiated friendly approaches during both pre- and post-fight intervals (Figure 2.1a: pre-conflict interval Mann Whitney U= 10350, N=137 individuals, P < 0.001; post-conflict interval U= 11224, N=137 individuals, P < 0.001). Both greetings (Wilcoxon Z= 3.028, N=137 individuals; P < 0.001) and approaches (Wilcoxon Z= 3.918; N=137 individuals; P < 0.001) occurred at higher rates after than before fights (Figure 2.1a). Furthermore, hyenas experienced lower rates of attack by their former opponents when they greeted or approached them during the post-conflict interval than when these same individuals neither greeted nor approached their former opponents (greeted: Wilcoxon Z= 3.290; N=19 individuals; P=0.001; approached: Wilcoxon Z= 4.871; N= 44 individuals; P < 0.001; Figure 2.1b). Thus, both greetings and approaches satisfied both our criteria for classification as conciliatory gestures.

In 411 of the 698 fights, both opponents remained together for at least 15 minutes after the conflict. In these 411 cases, which involved 96 hyenas that

participated on average, in 4.27 ± 0.43 fights per individual, we found that initial greetings occurred most commonly during the first two minutes immediately after the fight, but that initial greetings were observed up to 9 minutes after the fight (Figure 2.2). Similarly, initial approaches occurred most commonly during the first three post-conflict minutes, but were observed up to 12 minutes after the fight (Figure 2.2). We found that over 72% of all greetings and 74% of all approaches occurred within the first 5 minutes after the fight. Therefore, in order to maximize sample sizes in subsequent analyses, we used all 698 fights in which opponents could be observed together for 5 min before, and 5 min after, the conflict.

Latency to occurrence of the first affiliative behavior in these 698 fights was greater before than after the fight (Figure 2.3). Thirty two percent of initial affiliative contacts occurred during the minute after fights, whereas only 13% of initial affiliative contacts occurred during the last of five minutes before the fight.

After identifying approaches and greetings as functional reconciliation behaviors, we found that 14.6% of 698 fights were followed by approaches or greetings during the 5-minute post-conflict interval, whereas only 4.4% of these fights were preceded by these affiliative gestures during the 5-minute pre-conflict interval. Of the 494 dyads involved in these fights, 17.6% had positive conciliatory tendencies compared to 5.1% with negative conciliatory tendencies. Spotted hyenas increased their affiliative contacts with former opponents after conflicts compared with control periods in 698 fights (96 attracted pairs; 29 dispersed pairs; mean CT per individual = 11.3%). Thus individual hyenas participated in significantly greater numbers of attracted than dispersed pairs

(Wilcoxon Z= -4.687; N=140 individuals; P<0.001). For 14 dyads, we were able to calculate CTs using a matched control period on a different day within 30 days of the observed fight, when no fight occurred between these two individuals. Here we found that CTs calculated as [(Attracted-dispersed)/ total PC-MC pairs] did not differ from CTs calculated for the same dyads as [(Attracted-dispersed)/ total PC-BC pairs], where BC=the preconflict period, PC=the post-conflict period, and MC= a matched control period on a different day when no fight occurred. Both calculations yielded identical mean CTs of 16.6% (Wilcoxon Z=0.00; NS; N=14 dyads).

In 89% of 698 fights, victims were of lower social rank than aggressors. Eighty-three hyenas were observed to be both victims in some fights and aggressors in others. When individuals were victims, their mean CTs were significantly higher than when they were aggressors (Figure 2.4a: Wilcoxon Z= -2.846; N=83 individuals; P=0.004). The vast majority of 698 fights (88.4%) occurred within non-kin dyads. Twenty-eight hyenas were observed to be recipients of aggression in fights with both kin and non-kin. The mean CT among kin calculated for these 28 animals was negative, indicating that kin do not reconcile their fights, and was significantly smaller than the mean CT calculated for non-kin (Figure 2.4b: Wilcoxon Z=2.367; N=28 individuals; P=0.018).

We found no significant differences in CTs among dyads containing various combinations of age-sex classes. Specifically, we compared CTs of adult female victims when their aggressors were adult females, adult males, juvenile females, or juvenile males, and found that CT did not vary with age-sex

class of the aggressor (Kruskal-Wallis T= 2.059, NS, N=97 adult female aggressors, 8 adult male aggressors, 18 juvenile female aggressors, and 13 juvenile male aggressors, NS). We also examined the CTs of adult male victims in various dyad types, but found no significant differences based on age-sex class of aggressor (Kruskal-Wallis T= 2.788, NS, N=89 adult female aggressors, 19 adult male aggressors, 9 juvenile female aggressors, and 10 juvenile male aggressors, NS). Although adult males never attacked juveniles, we found that CTs did not vary significantly when juveniles of either sex were attacked by peers or by adult females (juvenile female victims: Kruskal-Wallis T= 1.635, NS, N=67 adult female aggressors, 18 juvenile female aggressors, and 20 juvenile male aggressors; juvenile male victims: Kruskal-Wallis T= 1.635, NS; N=67 adult female aggressors, 35 juvenile female aggressors, and 22 juvenile male aggressors). After determining that there were no significant differences among age-sex classes, we compared CTs among female-female dyads, female-male dyads, and male-male dyads, but we found no differences (Kruskal-Wallis T= 1.15, N=189 female-female, 246 female-male, and 50 male-male dyads, NS). CT also did not vary among dyads containing opponents of different ages (Kruskal-Wallis T= 0.825, N= 215 adult-adult, 186 adult-juvenile, and 96 juvenilejuvenile dyads, NS). We found no difference in mean CTs between male and female victims (Mann-Whitney U= 2551.5, N=82 males and 58 females, NS), nor did CTs calculated for juveniles differ significantly from CTs calculated for those same individuals when they were adults (Wilcoxon Z= -0.197; N=15 individuals; NS).

CT did not vary with intensity of aggression recorded during a fight (Kruskal-Wallis T= 4.65, N= 101 victims at aggression level 1, 97 victims at aggression level 2, and 62 victims at aggression level 3; NS), nor did CT vary with the context in which the aggression occurred (Kruskal-Wallis T= 1.40, N= 60 victims in context of dens, 55 victims in context of kills, and 39 victims away from dens and kills; NS). Finally, when we regressed conciliatory tendencies of individual dyad pairs against rank distance between victim and aggressor, we observed no relationship between CT and rank distance (R_s = 0.016, N= 477 dyads, NS).

DISCUSSION

The present study documents the occurrence of post-conflict conciliatory behavior among spotted hyenas in their natural habitat. During the moments following a dyadic aggressive interaction, opponents tend to engage in friendly approach or greeting behavior sooner and more often than they do during the period immediately preceding the fight. Spotted hyenas reconcile approximately 15% of their fights, and demonstrate an overall mean CT of 11.3%. Conciliatory tendencies calculated previously for primates range from 3.1% in Japanese macaques (*Macaca fuscata*: Chaffin et al. 1995) to 51.4% in Crested macaques (*Macaca nigra*: Petit et al. 1997). Species differences in reconciliation may reflect the amount of social cohesion necessary to survive in the wild (de Waal et al. 1988). The CT of 11.3% we found in spotted hyenas falls relatively low on the CT scale observed in primates (Kappeler et al. 1992), and may reflect the fission-

fusion nature of hyena society. However, chimpanzees and bonobos also live in fission-fusion societies yet these primates reconcile far more frequently than do spotted hyenas (de Waal et al. 1979; de Waal 1986; de Waal 1987), raising the question of what might account for the low CT found in *Crocuta*.

Predation pressure on top carnivores is very low, and this is expected to reduce frequency of reconciliation (Schino 2000). Perhaps more important, however, is the difference between hyenas and chimpanzees or bonobos with respect to the intensity of feeding experienced by individual group members (Holekamp et al. 1999). Feeding competition among *Crocuta* is extraordinarily intense, with group members using their formidable weaponry on a daily basis in fights with conspecifics over ungulate carcasses. Thus, although hyenas depend in the long run on cooperation from other clan members for survival and reproduction, short-term costs of conflicts might promote unusually heavy reliance in this species on dispersive rather than non-dispersive mechanisms of conflict resolution.

East et al. (1993) found that the initiation of greetings as a form of reconciliation accounted for 8% of greetings between adult female hyenas and 9% of greetings between adult males. As in the current study, they observed that losers in fights were more likely to initiate post-fight greetings than were winners. Hofer & East (2000) found that 15% of *Crocuta* dyads engaging in conflicts were followed by friendly reunions, a result identical to that obtained in the present study. In a sample of nine hyena dyads for which Hofer & East (2000) could find matched controls within 60 days of an observed conflict, they calculated a CT of

33.3%. Our sample of 14 dyads with matched controls within 30 days of an observed conflict yielded a CT of 16.6%. Unfortunately, these sample sizes are too small to permit meaningful comparison of CTs between hyena study populations. However, our larger sample obtained using pre-conflict periods as matched controls suggests that CTs in *Crocuta* are generally substantially lower than those reported previously.

Earlier workers were unable to provide any evidence that friendly postconflict reunions actually function to restore relationships in spotted hyenas (Colmenares et al. 2000; Hofer et al. 2000). Similarly, although many primate studies have demonstrated the occurrence of post-conflict friendly reunions, very few have actually investigated the consequences of such reunions and thus justified use of the functional term 'reconciliation' (Schino 2000, but see de Waal et al. 1983; Aureli et al. 1991; Cords 1993). Our findings that both greetings and approaches occur significantly more frequently among hyenas after than before conflicts, and that these affiliative gestures are associated with reduced rates of aggression during the post-conflict interval, clearly indicate that repair of damaged relationships is indeed one function of affiliative behavior after conflicts in Crocuta. In addition to reducing the probability of subsequent aggression ((Aureli et al. 1991; Cords 1993), reconciliation in primates can also decrease stress-related behavior (Aureli et al. 1991; Das et al. 1998). Further work will be required to determine whether these effects are also observed among hyenas.

Our results are consistent with the hypothesis that reconciliation may be widespread among gregarious mammals (Rowell 2000; Schino 2000). In addition

to the spotted hyena, post-conflict behavior has now been quantitatively evaluated in three other non-primate species: bottlenose dolphins (Samuels et al. 2000), domestic goats (Schino 1998; Schino 2000), and domestic cats (van den Bos 1997). Like most primates, all of these species except cats live in stable social groups, and all except cats exhibit non-dispersive mechanisms of conflict regulation (Schino 2000). Both cats and hyenas belong to the same monophyletic superfamily (Feloidea) of mammalian carnivores, and are thus closely related (Flynn 1996). However, the fact that hyenas reconcile whereas cats do not suggests that taxonomic affiliations are less important determinants of post-conflict behavior than selection pressures associated with living in groups.

Low predation pressure is expected to increase symmetry between aggressor and victim in the initiation of reconciliation (Schino 2000). However we observed a striking asymmetry between victim and aggressor with respect to initiation of reconciliation. As is also true in many primates (Aureli et al. 1991; Aureli et al. 1991; Kappeler 1993; Aureli et al. 2000), victims in hyena fights exhibit higher CTs than do aggressors. If the purpose of early contact between opponents after a conflict is to yield information about intentions or dispositions, such information is likely to be most needed by, and most obscure to, victims of aggression (Cords 1988), since victims are more likely than aggressors to be uncertain about whether conflicts will continue. Furthermore, the fact that aggressors rarely initiate affiliative interactions after fights suggests that the longterm risk of relationship deterioration is low for winners in fights in this species,

perhaps because opportunities for losers to disperse from the clan are severely limited (Smale et al. 1997).

The vast majority of conflicts observed among free-living Crocuta occur among unrelated animals, suggesting that kin are more tolerant of each other than non-kin. Interestingly, we found that unrelated animals reconcile after fights whereas kin dyads do not. Kin associate more closely than do non-kin in this species, kin serve as frequent alliance partners, and individuals direct affiliative behavior towards kin more frequently than towards non-kin (East et al. 1993; Smale et al. 1995; Holekamp et al. 1997), as is also true in various primates (e.g. Cheney et al. 1989). Furthermore, high-ranking animals are preferred over lowerranking individuals as social companions (Seyfarth 1980; Seyfarth et al. 1984; Holekamp et al. 1997), and individual hyenas prefer to direct affiliative behavior towards high-ranking non-kin (East et al. 1993). These findings suggest that hyenas, like many primates, recognize that some group members are more valuable social partners than others. Nevertheless, neither our comparison of kin and non-kin, nor our analysis of effects of rank distance on CT, suggests that relationship value is the primary determinant of post-conflict behavior in this species. However, value of relationships with non-kin may be relatively high in this species, since non-kin often join forces to repel lions and conspecifics from ungulate carcasses, and to defend territorial boundaries (Henschel et al. 1991; Boydston et al. 2001). Thus, keeping relationships with non-kin in good repair should enhance the fitness of individual clan members, and promote reconciliation behavior.

Conciliatory tendency may be affected not only by the value of a relationship, but also by its security (Kummer 1978; Cords 1988; Aureli et al. 2000). The value of a relationship reflects the magnitude of social or ecological benefits likely to accrue from it, with highly valuable relationships most worthy of maintenance and protection. The security of a relationship reflects its predictability or resilience. The tendency to reconcile should be lowest when a relationship of low value is highly secure, and highest when a highly valued relationship is insecure (Cords 1988). Although relationships with relatives are more valuable than those with non-kin the former relationships are also likely to be highly secure. Indeed, this is suggested in our data by the rarity of attacks observed on kin. Thus, the conciliatory behavior we observed in our hyena study population suggests that relationship security is a far better predictor of CT in this species than is relationship value.



Figure 2.1. Rates at which a) greetings and approaches occurred within hyena dyads during 5 min intervals before and after fights (each bar presents data from 137 individual victims), and b) aggression occurred within individual dyads during post-fight intervals with and without greetings or friendly approaches.



Figure 2.2. Latency to first greeting or appeasement behavior after 411 fights in which both members of each dyad could subsequently be observed for 15 minutes.



Figure 2.3. Number of total first occurrences of conciliatory behavior occurring during each minute-long interval before and after 698 fights.



Figure 2.4. Percent conciliatory tendency of a) 83 individual hyenas observed to play roles in different fights of both victim and aggressor, b) 28 individual victims observed to interact in different fights with both kin and non-kin.

CHAPTER THREE

Wahaj, S. A., R. C. Van Horn, T. L. Van Horn, R. Dreyer, R. Hilgris, J. Schwarz, and K. E. Holekamp (2004). Kin discrimination in the spotted hyena (*Crocuta crocuta*): nepotism among siblings. *Behavioral Ecology and Sociobiology* 56: 237-247.

CHAPTER THREE

KIN DISCRIMINATION IN THE SPOTTED HYENA (CROCUTA CROCUTA): NEPOTISM AMONG SIBLINGS

INTRODUCTION

Kin selection theory (Hamilton 1964) predicts that genetic relatedness should influence social behavior because animals able to interact differentially with kin and non-kin would have higher inclusive fitness than animals unable to do so. The ability to discriminate kin from non-kin is thus a critical prerequisite for the operation of kin selection. Kin recognition (Holmes et al. 1983) or kin discrimination (Tang-Martinez 2001) occurs whenever kin and non-kin receive differential treatment from conspecifics and this differential treatment is based on characteristics that are correlated with genetic relatedness. The widespread occurrence of nepotism (Fletcher et al. 1987; Mateo 2002) and the rarity of close inbreeding (Hoogland 1982) suggest that many animals can recognize their relatives.

Empirical evidence from a number of species suggests that kindifferentiated behavior may be facilitated by two common mechanisms, both of which involve learning (Dawkins 1982; Holmes et al. 1983; Hepper 1986; Barnard 1990; Tang-Martinez 2001). First, in familiarity-based recognition, kin learn to recognize one another through shared association when they interact in contexts that vary with relatedness. Recognition may thus be based on the location, timing, frequency or duration of social interactions, including both direct social contact among individuals and shared association with a particular nest or burrow, or with particular conspecifics. For example, siblings born in different

breeding seasons might recognize each other as kin through shared association with a common parent (Dawkins 1982; Holmes et al. 1983; Hepper 1986; Barnard 1990). The other mechanism that often mediates kin discrimination is phenotype matching (Holmes et al. 1982; Lacy et al. 1983). If phenotypic similarity is highly correlated with genotypic similarity, then recognition may result from phenotypic comparison (Tang-Martinez 2001). Individuals that share similar phenotypes are treated as kin. Phenotypic cues from an unfamiliar conspecific may be compared to a template acquired either from a familiar relative or from the discriminating individual itself (self-referent phenotype matching: Mateo et al. 2000; Hauber et al. 2001).

Most studies of kin recognition have investigated only the ability to discriminate among maternal kin (Holmes et al. 1983; Gouzoules 1984; Fletcher et al. 1987; Walters 1987; Wilson 1987; Waldman 1988; Pfennig et al. 1995). However, in long-lived mammals showing considerable reproductive skew among males, individuals can be expected to live in groups containing paternal as well as maternal kin (Holmes 1986; Widdig et al. 2002). Nepotism among both maternal and paternal kin can potentially increase inclusive fitness, so the ability to recognize both types of kin should be adaptive. Paternal kin recognition has now been demonstrated in macaques (*Macaca mulatta*: Widdig et al. 2001), baboons (*Papio cynocephalus*: Alberts 1999); Buchan et al. 2003), and ground squirrels (*Spermophilus beldingi*: Holmes 1986; Mateo 2002). Our broad objectives here were to inquire whether recognition of paternal kin occurs in the

spotted hyena (*Crocuta crocuta*), and if so, to identify the mechanisms mediating this recognition.

Spotted hyenas are long-lived, gregarious carnivores that reside in permanent social groups, called clans. Clans are fission-fusion societies in which individuals travel, rest and forage in subgroups that can change in composition from day to day, or even from hour to hour (Kruuk 1972; Mills 1990). Most clans contain several immigrant males and multiple matrilines of philopatric females and their offspring, including individuals from a number of overlapping generations (Frank 1986; Mills 1990). Modal litter size in Crocuta is two (Kruuk 1972), but members of consecutive litters born to the same female are usually present in the clan concurrently. Social interactions within clans are strongly affected by maternal kinship (Kruuk 1972; Mills 1990) but differential treatment of maternal and paternal kin in *Crocuta* has never been studied. In other gregarious carnivores most natal group members are close kin (Gompper et al. 1996), so favoring any group member over other conspecifics would likely enhance an individual's fitness. However, Van Horn et al (2004) recently found that high levels of male-mediated gene flow among clans combined with relatively low gene flow among matrilines within a clan, reduce average relatedness among members of a spotted hyena clan to extremely low levels; mean relatedness among hyenas born in a clan is no higher than that among males immigrating into the clan from multiple neighboring groups. Because mean relatedness within a clan is so low, hyenas should be able to gain substantial fitness benefits by favoring their kin over other group members.

Spotted hyenas breed throughout the year, and both males and females mate with multiple partners (Engh et al. 2002). In addition, because females and their cubs are socially dominant to virtually all breeding males, fathers are constrained by their low social rank such that they have very few opportunities to direct nepotistic behavior toward their own offspring (Van Horn et al. 2004). Tenure in the clan held by breeding immigrant males lasts up to 9 years (Engh et al. 2002), so paternal half-siblings frequently co-exist within a clan. In conjunction with well-established matrilineal genealogies (Van Horn et al. 2004). our recent identification of 12 variable microsatellite loci in *Crocuta* allowed us to document both maternal and paternal kin relationships within one large hyena clan (Libants et al. 2000; Engh et al. 2002). Because adult male hyenas do not participate in care of young or associate regularly with their own offspring (Van Horn et al. 2004), paternal half-siblings represent a kin class in which familiarity based on direct spatial or temporal association would rarely suffice to mediate kin recognition among hyenas (Holmes et al. 1983). Thus, the ability to discriminate paternal half-siblings from non-kin would suggest self-referent phenotype matching as a mechanism of kin recognition in this species. Similarly Crocuta twin litters may be sired by either one or two males (Engh et al. 2002; East et al. 2003), producing either full-sibling littermates or half-sibling littermates reared together without unique familiarity-based cues for distinguishing between these different types of littermates. To maximize their own inclusive fitness, hyenas should treat full-sibling littermates better than half-sibling littermates if they can

distinguish between them, and self-referent phenotype matching would be the mechanism most likely to permit this.

Here we examined recognition of both maternal and paternal kin among multiple classes of siblings in free-living spotted hyenas. Our first goal was to inquire whether behavioral interactions among free-living *Crocut*a were consistent with expectations based on kin selection theory. Specifically, we tested the hypothesis that hyenas sharing larger coefficients of relatedness (Hamilton's r) would receive more affiliative behavior, and receive less aggression, than would more distantly related siblings or unrelated animals. In addition, since association patterns reveal adaptive preferences for social partners in the fission-fusion society typical of spotted hyenas (Holekamp et al. 1997; Szykman et al. 2001), we expected association patterns would vary among kin classes if kin were able to recognize one another. Our second goal was to elucidate the mechanisms enabling hyenas to recognize kin.

METHODS

Subject Animals and Study Site

Spotted hyena clans contain 10 to 90 individual members that cooperatively defend a group territory. Virtually all males disperse from their natal groups, but females are philopatric (Mills 1990; Smale et al. 1997; East et al. 2001). Adult members of each sex can be ranked in a linear dominance hierarchy, with all females able to dominate all immigrant males. Female dominance relations are stable for extended periods and across a variety of

contexts (Tilson et al. 1984; Frank 1986; Engh et al. 2000). An individual's position in the group's hierarchy determines its priority of access to food (Kruuk 1972; Frank 1986).

Female hyenas bear litters in isolated natal dens (East et al. 1989), but soon transfer their offspring to the clan's communal den, where up to 20 cubs may reside concurrently for several months. The communal den is a social focal point for clan members, and it is frequently visited by adults and by immature individuals who no longer use the den for shelter. After juveniles leave the communal den, they travel throughout the home range and feed at ungulate kills with adults. However, hyena cubs remain dependent on their mothers for milk until weaning, at 13-14 months of age (Holekamp et al. 1999).

This study took place in the Talek region of the Masai Mara National Reserve, Kenya. Subjects were members of one large *Crocuta* clan containing 60 to 80 resident hyenas, and occupying an area of 65 km². During this study the Talek clan usually contained 20-25 adult females, 8 to 14 adult immigrant males, and 35 to 45 cubs and subadults. All members of the study clan were identified by their unique spots, and sex was determined from the dimorphic glans morphology of the erect phallus (Frank et al. 1990). We estimated cub birth dates (to \pm 7 days) using methods described previously (Holekamp et al. 1996). Social ranks were determined based on wins and losses in dyadic agonistic interactions. Mother-offspring relations were established on the basis of regular nursing associations, and confirmed by genetic analysis (Engh et al. 2002). To examine predictions of kin selection theory in the study clan, we assigned

individual hyenas to kin groups, and compared measures of social behavior among groups.

Assignment of individuals to kinship groups

DNA from each Talek hyena was extracted from blood or tissue, and we genotyped individuals using eleven autosomal microsatellite loci and one Xlinked microsatellite locus as described elsewhere (Libants et al. 2000; Engh et al. 2002). All adult males present in the clan at conception of a particular litter were considered as potential fathers. We employed a maximum likelihood-based approach (Thompson 1975; Meagher 1986) to assign paternity to cubs with known, genotyped mothers using the program CERVUS (Marshall et al. 1998), but only if data were available for at least 6 autosomal loci in sire, cub, and mother genotypes (see Engh et al. 2002 for details). A male was considered the sire of a cub when CERVUS assigned him paternity at 95% confidence and he met one of the following two criteria: he was the only potential sire by simple exclusion or, in the case of a single mismatch between the multi-locus genotypes of the putative sire and cub, the software program KINSHIP (Goodnight et al. 1999) indicated that the two animals' pairwise genetic similarity (i.e., R in Queller et al. 1989) reflected sire-cub kinship at 95% confidence. Confidence in paternity and kinship was determined from the simulated distribution of the ratio of two likelihoods: the likelihood that the male was the sire of the cub (e.g., paternally related at R = 0.05), and the likelihood that the male was not the sire of the cub (e.g., paternally related by R = 0).

Our genetic analysis allowed us to assign hyenas to one of the kin groups listed in Table 3.1. Theoretical expectations regarding differential treatment of siblings are shown in Table 3.2. The subset of comparisons among groups shown in bold type in Table 3.2 allowed us to test hypotheses suggesting recognition based on familiarity or phenotype matching. We emphasize that these hypotheses need not be mutually exclusive as more than one recognition mechanism might operate in this species.

Paternal half-siblings (PHS) shared a common father, but had different mothers and no known maternal relatives in common. Animals were defined as non-kin (NK) if they were known to have different fathers and if they had no known maternal relatives in common. There was no apparent difference in body size between full- (FSL) and half-sibling (HSL) littermates. By contrast, all maternal half-siblings (MHS) and most (89.7%) paternal half-sibling were born in different years, so members of MHS and PHS pairs usually differed markedly in size until both individuals in a pair reached reproductive maturity. In addition, full- and half-sibling littermates grew up together at the communal den, whereas most members of other sibling classes did not. Thus possible confounds here were that behavioral differences between littermate and non-littermate sibling categories might reflect discrimination based on differences in body size or the amount of time spent sharing a den rather than on relatedness. To address these concerns, we compared affiliative, associative and aggressive behaviors of unrelated animals that spent at least 4 months living together concurrently at the den with those of unrelated animals that spent no time living together at the den.

We called the former cohort members (CHT) and the latter non-cohort hyenas (NCH). CHT members were generally all of the same size, whereas this was not true for NCH members. If hyenas discriminate among conspecifics based on either body size or shared time at dens rather than on relatedness, then we expected to find significant differences between CHT and NCH animals with respect to affiliative and agonistic interactions.

Behavioral Data Collection

Behavioral data were collected between June 1988 and April 1998, when observers were usually present in the study area at least 23 days/month. We observed Talek hyenas from our vehicles, mainly between 0500 and 0900 h and between 1700 and 2000 h. An observation session started when we found one or more hyenas separated from others by at least 200 m, and ended when we left that group. Observation sessions occurred at dens, at ungulate kills, and away from both dens and kills. Behavioral data presented here included those generated by both males and females monitored while they were living at the communal den and also later in life as they traveled around the clan's territory. All kin and non-kin groups contained hyenas of both sexes from birth through adulthood. We measured three classes of behaviors: affiliative behavior, association patterns, and dyadic aggression.

<u>Affiliative behavior</u>: Affiliative behaviors included playing, feeding or nursing together, and joining together to engage in coalitionary attacks on target animals. All occurrences of coalitionary aggression were recorded throughout

every observation session as critical incidents (Altmann 1974). All other affiliative behaviors were assessed using scan data collected at 15-20 minute intervals throughout each observation session. During each scan we recorded the identity and activity of every hyena present. Affiliative behaviors recorded during scans for all hyenas in all kin groups included playing or feeding together. For littermates we also recorded nursing together as an affiliative behavior. We calculated the percent of scans in which each affiliative behavior occurred within each pair of animals who were present together during at least 20 scans.

Social play behavior was recognized by the occurrence of exaggerated movements, and by the absence of aggressive postures or vocalizations characteristic of agonistic interactions. Feeding together excluded nursing, and was defined as two hyenas concurrently feeding on the same solid food item. Feeding was observed both away from the den at ungulate kills and also at the den when mothers provisioned cubs with solid food, although provisioning was very rare (Holekamp et al. 1990). Feeding competition is intense in spotted hyenas, and intolerance of concurrent feeding by conspecifics usually involves the higher-ranking member of a pair driving the subordinate individual off the food (Kruuk 1972). Thus, tolerance of concurrent feeding seemed a reasonable measure of affiliative behavior. Coalitionary aggression occurred when two or more hyenas joined together to direct aggression (defined below) against a third. Here coalition formation was considered a cooperative behavior since individuals risk injury to themselves by aiding other animals during fights (Zabel et al. 1992).

Association patterns: An association index for each pair of hyenas was calculated using the twice-weight index of (Cairns et al. 1987). Thus, to calculate an association index for a pair of animals A and B, we divided the number of observation sessions in which both A and B were present by the sum of that number plus the number of sessions in which either A or B was observed without the other being present. Young hyenas are bound to the communal den until they are 8-12 months old and their mothers visit them there daily. In addition, cubs nurse for several months after leaving the communal den, and cubs are dependent upon their mothers during the post-den period for agonistic aid, protection, and for finding food (Holekamp et al. 1997). To avoid biases involving the potential confounds of dens or mothers, both sessions at dens and sessions in which the mother of either interacting hyena was present were excluded from calculations of association indices. Both mature and immature hyenas found away from dens were included in this analysis.

Dyadic aggression: Throughout every observation session, we recorded all occurrences of dyadic aggressive behavior as critical incidents (Altmann 1974). Aggressive behaviors included head wave, lunge, aggressive posture (i.e., ears cocked forward with the tail bristled and raised), chase, displace, stand over, bite, and push. *Crocuta* littermates often fight vigorously during their first weeks of life (Frank et al. 1991). For littermate pairs we recorded whether or not aggressions occurred during nursing, but all aggression among non-littermates occurred in contexts other than nursing. The identities of all aggressors and

target animals were recorded. In contrast to coalitionary aggression, dyadic aggression involved only one aggressor and one target animal.

Data analysis

Data documenting affiliative behaviors, association indices, and dyadic aggression were compared among all sibling classes, between kin and non-kin, and also between cohort and non-cohort animals (to examine effects of differential body size on social relationships). Two types of data were used to compare behavioral interactions among various classes of siblings: scan data were used to document patterns of some affiliative behaviors (playing, feeding and nursing together) and to measure association indices, whereas critical incident data were used to measure dyadic aggression and coalition formation.

Playing, feeding and association: Playing and feeding together and association indices were analyzed using pairs of hyenas as the units of analysis. In comparing FSL and HSL, each pair of FSL (N = 7) and each pair of HSL (N = 5) contributed one data point for affiliative behaviors (playing, feeding, and nursing together) and for association. We compared FSL and HSL values using Mann-Whitney U-tests. In comparing the other eight pair types (1) FSL and MHS, 2) FSL and PHS, 3) FSL and NK, 4) MHS and HSL, 5) MHS and PHS, 6) MHS and NK, 7) PHS and NK and 8) CHT and NCH) we used non-parametric matched pairs t-tests (Wilcoxon matched-pairs signed ranks tests), with each pair of hyenas being used only once. In each case we measured the relationships of a focal individual (hyena A) with two other hyenas (hyenas B and C), and then

compared values for the A-B relationships with the values for the A-C relationships. Animals B and C in each case were matched for sex, age (<u>+</u> 1 year), and relative social rank, but differed in their kin relationships to A. For example, we compared the percent of scans during which a particular affiliative behavior was exhibited between hyena A and its full-sibling B to the percent of scans during which hyena A and its maternal half-sibling C were engaged in that same affiliative behavior. In this example, if hyena B was a male, both higher ranking and older than hyena A, then hyena C was also a male, higher ranking and older than hyena A. Note that we were not able to use this method for comparing full- and half-sibling littermates because a given hyena could not simultaneously have both a FSL and a HSL.

Association data were derived from scans performed in each observation session, based on whether or not both members of a given hyena pair were recorded as present in scans from the same observation session. We compared the association index between hyena A and B with the association index between A and C, where B and C were drawn from different kin groups. As in our analyses of affiliative behavior, we controlled for sex, body size, and rank by matching hyenas B and C as closely as possible in these respects.

Dyadic and coalitionary aggression: Data collected during critical incident sampling were used to calculate rates of both dyadic and coalitionary aggression. Rates of coalition formation within pairs were calculated by dividing the total number of coalitions formed between two animals by the number of hours during which both members of that pair were present concurrently. Rates of dyadic

aggression for each hyena A were calculated by dividing the total number of aggressive acts directed by hyena A at hyena B, divided by the total number of hours A and B spent together. We stipulated that a pair of hyenas had to be present together during at least 25 h of observation to be included in either the aggression or the coalition data set, and each pair was used only once. Since these data were not normally distributed, we compared hourly rates of aggression and coalition formation among sibling groups using a Kruskal-Wallis one-way ANOVA with relatedness class as the independent variable. We also examined aggressive interactions according to the sex composition of the pair tested (female-female, mixed sex, or male-male) within each relatedness group, using a Kruskal-Wallis one-way ANOVA. Post hoc comparisons between specific sibling groups were conducted using Mann-Whitney U tests. The sequential Bonferroni adjustment was then used to correct for multiple testing (Rice 1989). Because the only multiple comparisons performed in any of our analyses were those involving coalitionary and dyadic aggression, these were the only analyses in which Bonferroni corrections were applied. Finally, we used Mann-Whitney U tests to compare full- and half-sibling littermates with respect to rates of aggression observed only in the context of nursing, and to compare aggression rates between cohort and non-cohort hyenas.

Because the hypotheses we were testing made clear, directional predictions regarding whether or not kin should exhibit affiliative or aggressive behavior more than non-kin, all statistical tests involving these behaviors were one-tailed. However, because it was not clear *a priori* whether to expect kin to

associate more or less closely than non-kin, tests involving association data were two-tailed. Differences between groups were considered significant when $p \le 0.05$. Throughout the paper, means (<u>+</u> se) are reported.

RESULTS

Tests of predictions of kin selection theory

Four comparisons were designed to test predictions of kin selection but could not be used to differentiate kin recognition mechanisms (comparisons 1 - 4in Table 3.2). For each of these comparisons, we had four affiliative measures (play, feeding together, association, and coalition formation). Of the 16 tests that resulted, all but three showed significant kin biases in the behavior in question (Table 3.3), supporting the predictions of kin selection.

Full sibling littermates played and fed together significantly more than did either maternal half-siblings (play: Wilcoxon Z = -1.826, n = 4, p = 0.034; feeding: Wilcoxon Z = -1.826, n = 4, p = 0.034, Figure 3.1a) or paternal halfsiblings (play: Wilcoxon Z = -2.524, n = 8, p = 0.006; feeding: Wilcoxon Z = -2.10, n = 8, p = 0.018, Figure 3.1b). Full-sibling littermates also played together more than did non-kin (Wilcoxon Z = -1.859, n = 7, p = 0.032, Figure 3.1c). Hyenas associated more closely with their full-sibling littermates than with either their paternal half-siblings (Wilcoxon Z = -2.521, n = 7, p = 0.006, Figure 3.1b) or nonkin (Wilcoxon Z = -2.366, n = 7, p = 0.009, Figure 3.1c). Maternal half-siblings played (Wilcoxon Z = -1.826, n = 4, p = 0.034, Figure 3.2a) and associated

together more closely (Wilcoxon Z = -2.49, n = 11, p = 0.006, Figure 3.2a) than did non-kin.

Mean rates of coalition formation among sibling classes varied significantly overall (Kruskal Wallis T = 31.17, d.f. = 4, p < 0.001, Figure 3.3). Full-siblings formed coalitions together at higher rates than did maternal halfsiblings (Mann Whitney U = 91.5, n = 12 FSL, n = 30 MHS, p = 0.006), paternal half-siblings (Mann Whitney U = 97.0, n = 12 FSL, n = 53 PHS, p < 0.001), or non-kin (Mann Whitney U = 1403.5, n = 12 FSL, n = 131 NK, p < 0.001). Maternal half-siblings also formed coalitions together more than did non-kin (Mann Whitney U = 2548.5, n = 30 MHS, n = 131 NK, p = 0.003).

Three comparisons failed to reveal significant biases in affiliative behavior. Full-sibling littermates did not associate significantly more than maternal halfsiblings (Wilcoxon Z = -1.095, n = 4, p = 0.137, Figure 3.1a), nor did they feed together significantly more than non-kin (Wilcoxon Z = -1.521, n = 7 individuals, p = 0.064, Figure 3.1c). Maternal half-siblings also did not feed together significantly more than non-kin (Wilcoxon Z = -0.622, n = 11, p = 0.267, Figure 3.2a). However, in all three cases, results were in the direction predicted by kin selection theory.

Mean rates of dyadic aggression among sibling types varied significantly overall (Kruskal Wallis T = 10.82, d.f. = 4, p = 0.029). Interestingly however, kinship generally failed to protect hyenas from dyadic aggression. Full-sibling littermates and maternal half-siblings did not differ with respect to the rates at which they aggressed against each other (Mann Whitney U = 198, n = 12 FSL, n

= 30 MHS, p = 0.308), nor did full-siblings differ significantly in this regard from either paternal half-siblings (Mann Whitney U = 250, n = 12 FSL, n = 53 PHS, p = 0.12) or non-kin (Mann Whitney U = 682.5, n = 12 FSL, n = 131 NK, p = 0.225). Rates of dyadic aggression also failed to differ between maternal half-siblings and non-kin (Mann-Whitney U = 1961, n = 30 MHS, n = 131 NK, p = 0.493).

Tests of hypotheses suggesting kin recognition mechanisms

<u>HSL and MHS</u>: Half-sibling littermates did not differ from maternal half siblings with respect to the percent of scans in which they were observed either playing (Wilcoxon Z = -0.962, n = 5, p = 0.168, Figure 3.2b) or feeding together (Wilcoxon Z = -0.135, n = 5, p = 0.447, Figure 3.2b). Rates of coalition formation also failed to differ significantly between half-sibling littermates and maternal halfsiblings (Mann-Whitney U = 81.5, n = 5 HSL, n = 30 MHS, p = 0.374, Figure 3.3) as did rates of dyadic aggression (Mann-Whitney U = 56, n = 5 HSL, n = 30 MHS, p = 0.183). However, half-sibling littermates associated far more closely than did maternal half-siblings (Wilcoxon Z = -2.023, n =5, p = 0.022, Figure 3.2b).

<u>MHS and PHS:</u> Maternal half-siblings did not differ significantly from paternal half-siblings with respect to the percent of scans in which they played together (Wilcoxon Z = 0.0, n = 3, p = 0.5, Figure 3.2c), fed together (Wilcoxon Z = -1.461, n = 4, p = 0.07, Figure 3.2c), or associated (Wilcoxon Z = -1.461, n = 4, p = 0.07, Figure 3.2c). Furthermore, maternal half-siblings did not form coalitions together at higher rates than did paternal half-siblings (Mann-Whitney U = 917, n = 30 MHS, n = 53 PHS, p = 0.111, Figure 3.3). After Bonferroni adjustment, maternal half-siblings and paternal half-siblings also failed to differ significantly in their rates of dyadic aggression (Mann-Whitney U = 1007, n = 30 MHS, n = 53 PHS, p = 0.02).

<u>FSL and HSL</u>: Full-sibling littermates were observed playing together (Mann Whitney U = 28, n = 7 FSL and 5 HSL, p = 0.04, Figure 3.4) and feeding together on solid food (Mann Whitney U = 28, n = 7 FSL, n = 5 HSL, p = 0.044, Figure 3.4) more than were half-sibling littermates. However, association indices did not differ between full- and half-sibling littermates (Mann Whitney U = 9, n = 7 FSL, n = 4 HSL, p = 0.172, Figure 3.4). Full-sibling littermates tended to form coalitions together at higher rates than did half-sibling littermates, but this difference was not statistically significant (Mann Whitney U = 44.5, n = 7 FSL, n = 5 HSL pairs, p = 0.062, Figure 3.3).

When they were not nursing, half-sibling littermates received aggression at almost twice the rate observed among full-sibling littermates, but this difference was not statistically significant (Mann Whitney U = 19.5, n = 12 FSL, n = 5 HSL, p = 0.134). Although there was no difference in the percent of scans in which full- and half-sibling littermates were observed to be nursing concurrently (Mann Whitney U = 22, n = 12 FSL, n = 5 HSL, p = 0.232), the mean hourly rate of aggression during nursing between half-sibling littermates was nine times higher than that between full-sibling littermates. Nevertheless, variance in this measure was great, so the mean difference was not statistically significant (FSL \bar{x} = 0.048 <u>+</u> 0.02, HSL \bar{x} = 0.446 <u>+</u> 0.441, Mann Whitney U = 30, n = 12 FSL, n = 5 HSL, p = 0.5).

<u>PHS and NK</u>: Paternal half-siblings and non-kin did not differ significantly with respect to the percent of scans in which they played together (Wilcoxon Z = 0.0, n = 4, p = 0.5, Figure 3.5), nor did their association indices differ significantly (Wilcoxon Z = -0.845, n = 7, p = 0.199, Figure 3.5). However, paternal halfsiblings fed together more than did non-kin (Wilcoxon Z = -2.032, n = 11, p = 0.021, Figure 3.5). Paternal half-siblings formed coalitions at higher hourly rates than did non-kin (Mann Whitney U = 4157, n = 53 PHS, n = 131 NK, p = 0.010, Figure 3.3), but after Bonferroni adjustment this difference was not statistically significant. Paternal half-siblings displayed significantly lower rates of dyadic aggression than did non-kin (Mann Whitney U = 2519, n = 53 PHS, n = 131 NK, p = 0.002).

Effects of variables other than kinship

Effects of differential body size were investigated by comparing behavior of cohort members with that of individuals reared in different cohorts. Non-kin cohort members were not statistically distinguishable from non-kin raised in separate cohorts with respect to either playing (CHT $\bar{x} = 3.8\% \pm 1.5$, NCH $\bar{x} =$ $1.6\% \pm 1.6$, Wilcoxon Z = -1.153, n = 6, p = 0.125) or feeding together (CHT $\bar{x} =$ $0.8\% \pm 0.5$, NCH $\bar{x} = 0.3\% \pm 0.3$, Wilcoxon Z = -1.089, n = 3, p = 0.138). We also found no differences between cohort and non-cohort animals with respect to association patterns (CHT $\bar{x} = 0.039 \pm 0.007$, NCH $\bar{x} = 0.032 \pm 0.005$, Wilcoxon
Z = -0.105, n = 6, p = 0.458), rates of coalition formation (CHT \bar{x} = 0.008 <u>+</u> 0.005, NCH \bar{x} = 0.013 <u>+</u> 0.002, Mann Whitney U = 979, n = 16 CHT, n = 115 NCH, p = 0.318), or rates of dyadic aggression (CHT \bar{x} = 0.059 <u>+</u> 0.015, NCH \bar{x} = 0.068 <u>+</u> 0.006, Mann Whitney U = 967, n = 16 CHT, n = 115 NCH, p = 0.37).

We examined effects of kinship on coalition formation separately within dvads of three different sex compositions: female-female, male-male, and mixed sex. Effects of kin group were found within all three dyad types (female-female: Kruskal-Wallis T = 19.201, d.f. = 4, p = 0.001; male-male: Kruskal-Wallis T = 7.352. d.f. = 4, p = 0.061; mixed sex: Kruskal-Wallis T = 18.095, d.f. = 4, p = 0.001). When data documenting rates of coalition formation were divided up by dyad composition within kinship groups, resulting sample sizes were too small to permit all 8 possible comparisons (shown in Figure 3.3) within each dyad type. However, where sample sizes allowed comparisons, rates of coalition formation in all three dyad types showed the same pattern as those shown in Figure 3.3, in which all dyad types are represented together. For example, the rate of coalition formation in all three dyad types was higher for full-sibling littermates than for half-sibling littermates, and paternal half-siblings always formed coalitions together at higher rates than did non-kin. Thus the results shown in Figure 3.3 were not driven by the behavior of members of any one dyad type alone, as members of all dyads behaved similarly.

DISCUSSION

Evidence that kinship affects hyena social behavior

Both kin selection theory (Hamilton 1964) and mate selection theory (Bateson 1983) predict that kinship will influence social interactions among conspecifics. Kin-differentiated behavior in gregarious mammals requires significant social discrimination abilities (Sherman et al. 1997). Although earlier studies (e.g. Holekamp et al. 1999) suggested that *Crocuta* could discriminate maternal kin from non-kin, genetic data were not available until now to permit analyses of kin recognition abilities among other kin classes, including paternal kin. Full siblings, half-siblings, and non-kin each share different r-values yet all of these kin classes overlap in time and space within spotted hyena clans. Thus, if hyenas can discriminate among these relatedness classes, then they should theoretically favor their most closely related kin. Indeed, our data documenting affiliative behaviors, summarized in Table 3.3, indicate that *Crocuta* can discriminate among several different groups of siblings, and that they can also distinguish paternal kin from non-kin.

Although affiliative interactions varied here among sibling classes, patterns of dyadic aggression showed little variation. Rates of dyadic aggression were generally just as high among kin as non-kin. Thus, in this respect, spotted hyenas resemble various cercopithecine primates in which it has been found that kinship enhances cooperation but does not necessarily protect individuals from aggression (Bernstein et al. 1986; Hunte et al. 1987; Widdig et al. 2002). The only significant difference emerging in our analysis of dyadic aggression was that

paternal half-siblings received less dyadic aggression than did non-kin. This result is interesting because we have found in a separate study (Van Horn et al. 2004) that young hyenas direct significantly less intense aggression at their sires than at unrelated adult control males. Thus, whereas maternal kinship does not necessarily protect hyenas from aggression, reducing the frequency or intensity of aggression may represent one form of nepotism among paternal kin in *Crocuta*. High frequencies of agonistic interaction with kin in some species might be attributed to more frequent social interaction in general, and consequently to increased opportunities to come into conflict with kin (Bernstein et al. 1986). However, this cannot explain our results since we controlled for the amount of time pairs spent together in our analysis of aggression rates.

In virtually all analyses represented here, sample sizes were small, variance was great, and statistical power was low. Our sample sizes were severely limited by availability of genetic data and by our efforts to carefully match animals for comparison based on sex, age, and social status. Nevertheless differential treatment of various kin classes clearly occurred among our study animals. This differential treatment occurred in accordance with predictions of kin selection theory, and could not be explained by effects of sex, body size, or social rank. The robust differences we observed in affiliative interactions among full-siblings, half siblings, and non-kin (Table 3.3) support acceptance of the hypothesis that hyenas can discriminate among individuals sharing different coefficients of relatedness.

Evidence supporting the familiarity hypothesis

Evidence of kin discrimination has been reported for a wide variety of species (Waldman et al. 1979; Gouzoules 1984; Fletcher et al. 1987; Waldman 1988; Pfennig 1999; Silk 2002; van der Jeugd et al. 2002), but the mechanisms by which kin recognition is mediated are known only for a small subset of these. One possibility is that individuals identify relatives on the basis of familiarity engendered by shared association with each other or with a common parent (Walters 1987). Hyena cubs associate very closely with their mothers for the first several months of life (Holekamp et al. 1997). Furthermore, maternal kin associate more closely than do non-kin (Holekamp et al. 1997), so hyena cubs are often in proximity to older siblings and other maternal relatives. That spotted hyenas base recognition of maternal kin on familiarity is suggested here by two lines of evidence. First, although half-sibling littermates and maternal halfsiblings share the same coefficient of relatedness, half-sibling littermates grow up together at the den, whereas maternal half-siblings do not because they are born in different years. Here half-sibling littermates associated more closely than did maternal half-siblings, suggesting that familiarity cues experienced during communal rearing affect patterns of affiliation among siblings once all these animals are independent of the communal den.

Hyenas may also use familiarity cues to distinguish between maternal half-siblings and paternal half-siblings, even though they share the same relatedness coefficient with animals in both groups. Here, although we found no differences between maternal half-siblings and paternal half-siblings that were

statistically significant, maternal half siblings nevertheless tended to be favored over paternal half-siblings in all types of interactions measured except play (Table 3.3). There is no paternal care in this species, females often mate with multiple males, and paternal kin do not associate closely, so paternal halfsiblings have no access to shared social cues of relatedness. However, as also occurs in baboons (Smith et al. 2003), maternal kin in hyenas can use social cues based on shared experience with a common mother to govern their affiliative behavior. Though our results suggest that *Crocuta* can recognize paternal kin, maternal kinship may nonetheless be more important to hyenas than paternal kinship. Maternal kinship explains a great deal of the variance in social relationships among cercopithecine primates even when paternal relationships are known (Smith et al. 2003).

Evidence supporting the phenotype matching hypothesis

Phenotype matching has been suggested as an important mechanism of kin recognition in peacocks (*Pavo cristatus*, Petrie et al. 1999), beavers (*Castor canadensis*, Sun et al. 1997), ground squirrels (*Spermophilus beldingi*, Sherman 1981; Holmes et al. 1982; Holmes et al. 1983; Holmes 1986; Holmes 1986), macaques (*Macaca mulatta*, Widdig et al. 2001), and baboons (*Papio cynocephalus*, Smith et al. 2003). Phenotype matching is especially likely when multiple paternity prevents littermates from experiencing any obvious differences with respect to familiarity cues on which to base discriminations between full-siblings and half-siblings (Holmes et al. 1982). This appears to be the case in

Crocuta. Four lines of evidence suggest that hyenas allocate both affiliative and aggressive behaviors according to kinship in the absence of social cues of relatedness, suggesting self-referent phenotype matching as a mechanism for kin discrimination.

First, although full- and half-sibling littermates share common pre-natal and post-natal environments, full-sibling littermates showed more affiliative behavior than did half-sibling littermates. In addition, rates of aggression in both nursing and non-nursing contexts were higher between half-sibling littermates than between full-sibling littermates. The consistent trends observed in affiliation and aggressive behavior between full-sibling littermates and half-sibling littermates cannot be mediated by familiarity-based mechanisms, and instead support an hypothesis invoking phenotype matching (Hauber et al. 2001).

Second, paternal half-siblings are more closely related than non-kin, but neither have access to social cues of relatedness in *Crocuta*, thus making paternal kin recognition through familiarity improbable. However, paternal halfsiblings were observed feeding together during a greater percent of scans, formed coalitions together at higher rates, and were less frequently the targets of dyadic aggression than were non-kin.

Third, although we observed directional trends suggesting greater nepotism among maternal half-siblings than paternal half-siblings, we found no significant differences between these groups of siblings. This similarity between maternal half-siblings and paternal half-siblings is predicted by the phenotype matching hypothesis but not by the familiarity hypothesis.

Finally, half-sibling littermates and maternal half-siblings in *Crocuta* share the same coefficient of relatedness (r = 0.25) but differ with respect to the den environments and social cues they experience. We observed no differences between half-sibling littermates and maternal half-siblings with respect to playing together, feeding together, rates of forming coalitions together, or rates of dyadic aggression. The lack of difference in these behaviors between half-sibling littermates and maternal half-siblings is more parsimoniously explained by the phenotype matching hypothesis than by the familiarity hypothesis.

Familiarity-based and phenotype matching mechanisms of kin recognition are not mutually exclusive, and more than one mechanism has been suggested to operate in a variety of species, including ground squirrels (Sherman 1980; Holmes et al. 1982; Holmes 1986; Schwagmeyer 1988), mice (Kareem et al. 1982), and various primates (Widdig et al. 2001; Silk 2002; Widdig et al. 2002). Similarly both mechanisms appear to operate in spotted hyenas. Earlier observations suggested that hyenas distinguish among individual conspecifics based on vocalizations (Holekamp et al. 1999), odors (Hofer et al. 2001), and visual cues (Kruuk 1972). Further study will be required to ascertain which of these sensory modalities might modulate kin recognition in *Crocuta*.

Group	Abbreviation	Mother	Father	L	Share Den?	Body Size?
Full-sib Littermates	FSL	same	same	0.50	yes	same
Half-sib Littermates	HSL	same	different	0.25	yes	same
Maternal Half sibs	SHW	same	different	0.25	no	different
Paternal Half sibs	SHd	different	same	0.25	ou	different
Non-kin	NK	different	different	<0.125	no	different
Cohort Members	CHT	different	different	<0.125	yes	same
Non-cohort Hyenas	NCH	different	different	<0.125	no	different

unal	e
"r."	san e.
d by re co	/ the e siz
cate r moi	sam
indi ne ol	/ the
ess is at o	appr
edne ently	vere e roi
relat	oup v ler al
nt of r cor	h oth
<i>fficiel</i> jethe	r eac f eac
g toc	hs of
dean i livin	mont
r 3. N onths	few
apte 4 m	siners nin a
n Ch east	mer vitt
ired inter	borr
sper	ir noi Juals
os co den"	ner a ndivia
groul ig a (wner nt. Ir
-kin harir	
l non as "s	
and ated	rly de
ndica	idy si ig ea
e 3.1 Ials ii	durin
Labl Anim	size .

Familiarity	Phenotype Matching
1. FSL > MHS	1. FSL > MHS
2. FSL > PHS	2. FSL > PHS
3. FSL > NK	3. FSL > NK
4. MHS > NK	4. MHS > NK
5. HSL > MHS	5. HSL = MHS
6. MHS > PHS	6. MHS = PHS
7. FSL = HSL	7. FSL > HSL
8. PHS = NK	8. PHS > NK

Table 3.2. Predictions for interactions among spotted hyenas generated by kin selection theory (in regular font) and by hypotheses suggesting two possible mechanisms of kin recognition (in bold font), familiarity-based recognition and phenotype matching. ">" denotes the prediction that greater affiliation, higher rates of coalition formation, and lower rates of dyadic aggression should be seen in one group than the other if kin recognition occurs via that mechanism, whereas "=" denotes a prediction that the two groups should treat each other similarly. Predictions in bold face indicate those that differ between the familiarity hypothesis and phenotype-matching hypothesis of kin recognition; in the remaining (non-bolded) cases, predictions of both hypotheses are the same.

Preponderance of Evidence	supports kin selection	supports kin selection	supports kin selection	supports kin selection	familiarity/ phenotype matching	familiarity/ phenotype matching	phenotype matching	phenotype matching
Aggression	FSL = MHS	FSL > PHS	FSL = NK	MHS = NK	HSL = MHS	SH4 < SHM	HSL > FSL	NK > PHS*
Coalitions	FSL > MHS*	FSL > PHS*	FSL > NK*	MHS > NK*	SHM = JSH	SH4 < SHW	FSL > HSL	PHS > NK*
A.I.	FSL > MHS	FSL > PHS*	FSL > NK*	MHS > NK*	HSL > MHS*	SH4 < SHW	FSL = HSL	PHS = NK
Feeding	FSL > MHS*	FSL > PHS*	FSL > NK	MHS = NK	HSL = MHS	SH4 < SHW	FSL > HSL*	PHS > NK*
Playing	FSL > MHS*	FSL > PHS*	FSL > NK*	MHS > NK*	HSL = MHS	SHd = SHW	FSL > HSL*	PHS = NK
Comparison	1. FSL:MHS	2. FSL:PHS	3. FSL:NK	4. MHS:NK	5. HSL:MHS	6. MHS:PHS	7. FSL:HSL	8. PHS:NK

predictions of hypotheses suggesting mechanisms by which hyenas might recognize kin. Asterisks indicate P < 0.05. Comparisons 1 – 4 (in regular font) test predictions of kin selection theory, and comparisons 5 – 8 (in bold font) test Table 3.3. Summary of results from Chapter 3, with statistically significant differences indicated by asterisks.

Figure 3.1. Mean percent of scans in which individual hyenas were observed playing and feeding together with a) full-sibling littermates and maternal half-siblings, b) full-sibling littermates and paternal half-siblings, and c) full-sibling littermates and non-kin. Mean association indices are also shown for each set of animals. All pairs represented here were matched for sex, age, and relative social rank. Sample sizes represent numbers of individuals. Statistically significant differences are indicated with an asterisk.

Figure 3.1



Figure 3.2. Mean percent of scans in which individual hyenas were observed playing and feeding together with a) maternal half-siblings and non-kin, b) maternal half-siblings and half-sibling littermates, c) maternal and paternal half-siblings, and. Mean association indices between individual hyenas and members of both comparison groups are also shown. All pairs represented here were matched for sex, age, and relative social rank. Other notation is as in Figure 3.1.





Figure 3.3. Mean hourly rates at which coalitions formed within dyads composed of full-sibling littermates, half-sibling littermates, maternal half-siblings, paternal half-siblings, and non-kin. Sample sizes represent numbers of dyads. Statistically significant differences are indicated with asterisks over brackets.



Figure 3.4. Mean percent of scans in which pairs of full- and half-sibling littermates were observed playing and feeding together. Mean association indices between full- and half-sibling littermates are also shown. Sample sizes represent numbers of sibling dyads. Statistically significant differences are indicated with an asterisk.



Figure 3.5. Mean percent of scans in which individual hyenas were observed playing and feeding together with their paternal half-siblings and unrelated animals. Mean association indices are also shown. All pairs represented here were matched for sex, age, and relative social rank. Other notation is as in Figure 3.1.

CHAPTER FOUR

FACTORS INFLUENCING SIBLING AGGRESSION IN THE SPOTTED HYENA (CROCUTA CROCUTA)

INTRODUCTION

Inclusive fitness theory (Hamilton 1964) explains how costly altruistic behaviors can increase in frequency within populations through compensatory benefits received by kin. However, Hamilton's rule also specifies the evolutionary conditions under which selfish behavior, including sibling rivalry, is expected to occur among close kin (Mock et al. 1997). Intense aggression directed at siblings is one form of sibling rivalry that is commonly observed in animals. Aggression among siblings is often severe and sometimes lethal. Kin selection theory predicts that animals' behavior should reflect a balance between direct and indirect components of inclusive fitness, and that this balance should determine when, if ever, it might pay to attack or kill siblings to gain direct fitness at the cost of reduced indirect fitness.

Whereas siblicidal aggression is a common and well-documented phenomenon in many birds (Mock 1984; Drummond et al. 1986; Mock et al. 1997; Loughhead et al. 1999), the topic has been little studied in mammals, with its occurrence documented only among domestic pigs (*Sus scrofa*, Fraser et al. 1991) and inferred among spotted hyenas (*Crocuta crocuta*, Frank et al. 1991; Hofer et al. 1997; Golla et al. 1999; Smale et al. 1999). Among domestic cats, dogs, and pigs, poor nutrition during gestation can retard intrauterine growth in the last offspring within a clutch or litter (Robinson et al. 1999), and the 'runt' of a litter may lose out in post-natal sibling competition. Aggression among siblings

has been described in Arctic foxes (*Alopex lagopus* Macpherson 1969), red foxes (*Vulpes vulpes*, Henry 1985; Henry 1985), coyotes (*Canis latrans*, Bekoff 1981), and Galápagos fur seals (*Arctocephalus galapagoensis*, Trillmich 1986), but the functional significance of this aggression remains unclear.

Sibling rivalry has been defined as "any features of animals or plants that have the effect of promoting individual survival and/or reproduction at the expense of current and future siblings (Mock et al. 1997, p. 8)." Siblicide represents an extreme form of sibling rivalry in which sibling competition results in death (Mock et al. 1990). Although siblicide may results from overt aggression, fighting may not be the only form of sibling competition. That is, siblicide can also occur when one offspring monopolizes resources, ensuring mortality among its siblings. Mortality through non-aggressive siblicide is much less certain than through overt aggression (Mock et al. 1997). The focus of this chapter is on sibling rivalry among spotted hyena cubs expressed through overt aggression, since aggression directed at siblings here represents a vivid and easily quantifiable behavioral indicator of selfishness. We also hope to shed light on the factors that influence this fighting among hyena siblings.

Spotted hyenas are precocial at birth (Pournelle 1965). Litter size in this species is small, usually one or two cubs, and rarely three. Adult female *Crocuta* usually have only 2 functional teats, so nursing more than two cubs concurrently is generally impossible. In contrast to other neonatal carnivores, hyena cubs are born with their eyes open, coordinated locomotor capabilities, and fully erupted canine and incisor teeth (Frank et al. 1991). Among captive hyenas, intense

fighting between littermates begins within minutes of birth (Frank et al. 1991) and this fighting continues for the next several days (Frank et al. 1991; Smale et al. 1995; Drea et al. 1996). In the wild, neonatal spotted hyenas spend most of their time underground (East et al. 1989), making behavioral observations of neonatal cubs there difficult and rare. Although fighting is often observed in the wild between littermates when they emerge from the den, direct observations of fatal sibling aggression have never been made among wild hyenas. However, indirect evidence suggests that intense early sibling aggression might function in nature to kill siblings, resulting in either obligate (Frank et al. 1991; Frank 1996) or facultative siblicide (Hofer et al. 1997; Golla et al. 1999; Smale et al. 1999; Wachter et al. 2002). Siblicide is considered 'obligate' when aggression within a brood or litter is almost always fatal to subordinates, as occurs in many avian species such as eagles, herons, and egrets (reviewed in Mock et al. 1997). In 'facultative' siblicide, however, siblings adjust competition intensity as costs and benefits of intrabrood aggression vary with current environmental conditions (Mock et al. 1997). Facultative siblicide has been documented in blue-footed boobies (Sula nebouxi), ospreys (Pandion haliaetus), cattle egrets (Bubulcus ibis) and great blue herons(Ardea herodias fannini) (reviewed in Mock et al. 1997).

Frank et al. (1991) invoked three lines of indirect evidence to infer the occurrence of obligate siblicide among spotted hyena cubs. First, they observed intense fighting among captive littermates and severe wounding and emaciation among some wild-caught cubs from twin litters. Second, they found that litter

sizes observed in the wild were significantly smaller than those observed in captivity, suggesting that litter reduction might routinely occur in the wild due to siblicide. Although obligate siblicide is known to occur in several avian species (Mock et al. 1997), obligate siblicide had not previously been suggested to occur in any mammal. Third, Frank et al. (1991) observed significantly more same-sex twin litters than mixed-sex twin litters in the wild than expected based on chance expectations, and they inferred based on this that siblicide was contingent on litter sex composition. They also observed an excess of female singletons, suggesting that these females were originally from all-female twin litters that were reduced to singletons through siblicide. The sex-bias in the obligate model of hyena siblicide proposed by Frank et al. (1991) made siblicidal aggression among spotted hyenas unique in the animal kingdom, as frequency or intensity of attacks on siblings are not known to vary with sibling sex in any other vertebrate species. Frank et al. (1991) failed to clearly establish that siblicide was responsible for these sex biases and small litter sizes, as they never conclusively refuted alternative explanations for these patterns. Larger litter size in captive than wild hyenas might be due to differences in prenatal factors (e.g., stress and diet quality) or postnatal factors (e.g., predation in nature) rather than to obligate siblicide. A bias towards production of mixed-sex litters might not be the result of obligate siblicide in same-sex litters, but rather due to female manipulation of sex ratios according to environmental conditions (Holekamp et al. 1995). In addition, the function of intense early intra-litter aggression in hyenas might not serve to eliminate a sibling, but rather to establish intra-litter rank relationships very early

in life, as these would strongly affect future competitive outcomes while still maximizing inclusive fitness. Furthermore, workers studying hyenas after Frank et al. (1991) found no evidence for obligate siblicide among wild spotted hyenas, and concluded that skewed ratios of litter sex compositions indicated only the occurrence of facultative siblicide in this species (Hofer et al. 1997; Smale et al. 1999). Further support for the facultative siblicide hypothesis came from studies examining rates of intra-litter aggression between hyena cubs (Smale et al. 1995; Golla et al. 1999; Wachter et al. 2002). Here it was argued that, when environmental conditions are poor, rates of intra-litter aggression are high and the dominant sibling can monopolize milk from both the mother's teats, eventually causing the subordinate cub to starve.

Although the debate persists over whether overt sibling aggression in spotted hyenas might result in obligate or facultative siblicide, all investigators agree that early sibling aggression in this species is unusually intense and frequent. The goals of the current study, were therefore to determine what factors influence the occurrence and frequency of aggression among *Crocuta* siblings. Specifically, using data on sibling aggression occurring upon emergence from the den collected systematically in the wild over a 15 year period, we asked how rates of sibling aggression were influenced by cub age, litter composition, maternal rank, local prey abundance, identity of the target of aggression, and the specific context in which aggression occurs.

METHODS

Study Site and Subjects

This study took place in the Talek region of the Masai Mara National Reserve, Kenya, in the northern portion of the Serengeti ecosystem. This region consists of open rolling grasslands grazed year-round by several different ungulate species, the most numerous of which are Thompson's gazelles (*Gazella thomsoni*), topi (*Damaliscus korrigum*) and impala (*Aepyceros melampus*). Each year the resident ungulate populations are joined for 3 or 4 months by large migratory herds of wildebeest (*Connochaetes taurinus*) and zebra (*Equus burchelli*). The migratory herds typically arrive in the Talek area in July or August and depart in October or November. When both resident and migratory ungulates are present, superabundant food resources are generally available to Talek hyenas such that feeding competition is greatly relaxed among them (Holekamp et al. 1996; Holekamp et al. 1999).

Social groups, or clans, of spotted hyenas contain multiple adult males and several matrilines of adult female kin with offspring. Males disperse from their natal groups after puberty, whereas females are usually philopatric (Frank 1986; Henschel et al. 1987; Mills 1990; Smale et al. 1997). Adults can be ranked in a strict linear dominance hierarchy with members of the same matriline occupying adjacent rank positions. Female *Crocuta* are dominant to immigrant males, and offspring attain ranks just below those of their mothers (Holekamp et al. 1991; Engh et al. 2000; Engh et al. 2003). Rank relations, particularly those among females, remain stable for extended periods and across a variety of

contexts (Kruuk 1972; Tilson et al. 1984; Frank 1986; Smale et al. 1993). An individual's position in the group's hierarchy strongly determines its priority of access to food (Kruuk 1972; Frank 1986).

Throughout the year, female hyenas bear their litters in isolated natal dens (East et al. 1989). When cubs reach 2 to 4 weeks of age, females transfer their offspring to the clan's communal den where several mothers maintain their cubs together for 8 to 12 months (Kruuk 1972). Spotted hyena cubs typically leave the communal den at approximately 8 months of age and begin traveling throughout the home range and feeding at ungulate kills with adults. However, hyena cubs remain dependent on their mothers for milk until weaning, which takes place, on average in our study population, at 13 to 14 months of age (Holekamp et al. 1996).

Subjects here were members of one large *Crocuta* clan that usually contained 60 to 80 hyenas who defend a group territory of approximately 65 km². All adult members of the study clan were identified by their unique spot patterns, and young cubs that had not yet developed spot patterns were identified by unique scarring, bald patches, ear notches, size differences, molt patterns, or a combination of these features. Sex was determined from the dimorphic glans morphology of the erect phallus (Frank et al. 1990). By two months of age, the glans of the male's phallus assumes a distinctly different shape from that of the female (Drea et al. 1998). That is, the male's phallus is pointed at its tip and has a constriction above the glans whereas the shape of the female's pseudopenis is blunt at the tip and lacks a constriction. Cubs regularly develop erections during

greeting ceremonies, which occur virtually every time a new hyena arrives at the den. Young hyenas were sexed here repeatedly by multiple observers, and sex could be assigned with complete certainty by the time cubs were 2-3 months old. However, on several occasions, cubs died before they could be sexed. Only litters in which the sexes were known for all siblings were included in our dataset.

We estimated cub birth dates (to +7 days) by using their pelage, size, and other aspects of their appearance and behavior when cubs were first observed above ground (Holekamp et al. 1996). Mother-offspring relations were established on the basis of regular nursing associations. Members of twin litters may be sired by either one or two fathers, but monozygotic twins have never been documented in spotted hyenas (R. C. Van Horn, pers. comm.). Social ranks were determined based on wins and losses in dyadic agonistic interactions. Outcomes of these interactions were organized into a matrix from which each individual hyena could be assigned a social rank in the clan's dominance hierarchy (Martin et al. 1988). Intra-litter ranks were determined based on outcomes of dyadic aggression between littermates; when one cub aggressed against its littermate, which responded by avoidance and submissive behavior, we described the aggressor as dominating its littermate. By the time twin litters emerge above ground in nature, sibling rank relationships are wellestablished such that aggression within litters is exhibited almost exclusively by the dominant cub.

Data Collection

Behavioral data and measures of prey abundance were collected between June 1988 and September 2003. Throughout this period, observers were present in the study area at least 23 days/month except for April 1991, when observers were present for only 14 days. We observed hyenas daily from our vehicles, which we used as mobile blinds, mainly between 0600 and 0900 h and between 1700 and 1900 h. An observation session started when we found two or more hyenas separated from other conspecifics by at least 200m, and terminated when we left that group. Observation sessions occurred at natal and communal dens, at ungulate kills, and away from both dens and kills. Throughout each observation session, we recorded the identities of all individual hyenas present at 20 to 30 minute intervals.

During the study period, we monitored behavioral interactions within 71 twin litters (18 all-female litters, 34 mixed-sex litters, and 19 all-male litters) in which both members of the litter were present together during at least 25 hours of observation during the co-existence of those two littermates. Data were available during each monthly age interval throughout the first year of life for 24 of these 71 twin litters, including 7 all-female litters, 16 mixed-sex litters, and one all-male litter. These 24 twin litters were used in analyses of how sibling aggression varies with age, how shifts occur over time with respect to the contexts in which aggression is observed, and in comparisons of aggression directed at siblings and unrelated peers as this changes over time. Both members of 46 of the 71 twin litters (11 all-female litters, 26 mixed-sex litters, and

9 all-male litters) were observed during the 1-2 month age interval for at least
150 min together, and these 46 litters were used in all analyses involving only the
1-2 month age interval, which started at birth and ended at 2 months of age.

Throughout every observation session, we recorded all occurrences of aggressive behavior as critical incidents (all occurrence sampling, Altmann 1974), and the identities of all aggressors and target animals were also recorded. Although social play behavior can resemble aggressive behavior among cubs. the former often involves play solicitation postures and never results in pain- or fear-induced vocalizations. Occurrence of these vocalizations, or the active flight of one opponent, was used here to distinguish true aggression from play-fighting. Aggressive behaviors included head wave, lunge, aggressive posture (i.e., ears cocked forward while the tail was bristled and raised), chase, displace, stand over, bite, bite-shake and push. The specific context in which each aggressive interaction occurred was classified as occurring either over "milk", over "solid foods" or in "other" contexts involving neither milk nor solid foods. Aggression over milk and solid food was associated with conflicts between littermates over access to the mother's nipples during nursing or access to meat or bones, respectively. Aggressive interactions occurring in contexts unrelated to 'milk' or 'solid foods' were often unprovoked, or occurred in response to affiliative behavior directed by of one littermate towards the other. Dyadic aggression involved only one aggressor and one target animal. Coalitionary aggression was not included in the data set. Rates of aggression within pairs were calculated by dividing the total number of aggressive interactions observed between two

animals during a particular age interval by the total number of hours in which both members of that pair were observed concurrently during that age interval. Rate data for each age interval were based on the following mean number of hours of observation per litter: for 1-2 month age interval, 6.38 ± 0.77 hours of observation; for 3-4 month age interval, 20.95 ± 1.62 hours of observation; for the 5-6 month age interval, 24.60 ± 1.72 hours of observation; for the 7-8 month age interval, 19.33 ± 1.44 hours of observation; for the 9-10 month age interval, 13.74 ± 1.21 hours of observation; and for the 11-12 month age interval, 9.14 ± 1.04 hours of observation.

Local availability of food to Talek hyenas was estimated by counting all prey animals found within 100m of 4 km-long transect lines in two different areas of the Talek clan home range (Holekamp et al., 1999). Both transects were run twice each month, between 0800 and 1000h. During any age interval, the aggression rate calculated for any particular pair of siblings could be associated with the average number of prey counted per census for all censuses performed between the starting and ending dates of that age interval for that sibling pair. On average 4.8 ± 0.02 prey censuses were conducted during each 2 month age interval. Monthly variation in prey counts has been described by Holekamp et al. (1993). The number of prey animals counted per census ranged from 61 to 958, with a mean of 353.17 ± 24.45 . Since births are not synchronized among female *Crocuta* (Szykman 2001), cub age is not correlated with prey abundance in this species.

Data Analysis

Rates of dyadic aggression were analyzed using the pair as the unit of analysis, with each pair used only once in the entire data set. Since our data were not normally distributed, we compared hourly rates of aggression between sibling pairs using non-parametric statistics. Effects of age were examined by partitioning age into 2-month intervals up to one year (1-2 months, 3-4 months, 5-6 months, 7-8 months, 9-10 months and 11-12 months). Using the 24 twin litters observed throughout the first year of life, we employed a Friedman's repeatedmeasures ANOVA to determine how aggression rates varied with age. Post hoc comparisons were performed using Wilcoxon matched-pairs signed-ranks tests. The sequential Bonferroni adjustment was then used to correct for multiple testing (Rice 1989).

To determine how age-related shifts occurred in the contexts in which we observed intra-litter aggression, we divided age into 2 periods of 1-6 months and 7-12 months and compared the distribution of aggressive interactions between each of the two age intervals within the contexts of 'milk', 'solid foods', and 'other' using a Wilcoxon matched-pairs signed-ranks tests. As in our analysis of age effects, effects of the context in which aggression occurred were examined using the 24 twin litters for which we had data throughout the first year of life.

We evaluated effects of composition of twin litters on rates of aggression using a Kruskal-Wallis one-way ANOVA to compare all-female litters, mixed-sex litters, and all-male litters, and a Mann-Whitney U-test to compare same-sex to mixed-sex litters. We inquired how rates of aggression differed between same-

sex and mixed-sex sibling pairs within twin litters that were female-dominated and male-dominated using a Mann-Whitney U-test.

In analyses examining effects of maternal rank on rates of aggression between littermates, we assigned each litter the rank of its mother during the year in which that litter was born. Since the total number of adult females present in the clan varied over time, rank was standardized from 0 to 1, with 1 assigned to the highest-ranking, and 0 to the lowest-ranking sibling pair born within each year. High-ranking littermates were defined as those having a standardized rank of 0.5 or greater, while low-ranking littermates had a standardized rank less than 0.5. Effects of maternal rank on hourly rates of aggression were examined in two ways. First, we evaluated hourly rates of aggression in relation to standardized rank using a Spearman's Rank correlation. Second, we assigned litters to high- and low-rank categories, and compared hourly rates of aggression between high- and low-ranking litters using a Mann-Whitney U test. Within high- and low-ranking litters, we compared aggression rates observed between mixed-sex and same-sex littermates using Mann-Whitney U-tests. Rates of aggression were evaluated in relation to prey abundance using a Spearman's Rank correlation. Differences between same and mixed-sex litters in relation to levels of prey abundance were analyzed using a Mann-Whitney U-test.

To determine how targeting of aggression varied during early ontogeny, we used 21 of the 24 twin litters observed throughout the first year of life and employed a Friedman's repeated-measures ANOVA to compare the percent of

aggressive acts cubs directed towards siblings among 2-month age intervals. The same analysis was conducted with the percent of aggressive acts directed towards unrelated cohort peers. Cohort peers were defined as unrelated animals that spent at least 4 months living together concurrently at the clan's communal den. Whereas individual cubs may have only one twin littermate, they may have up to 12 unrelated cohort peers with a mean of 3.52 + 0.58 peers per individual cub. Post hoc comparisons were not made among age intervals since our interest was in whether or not there was an ontogenetic shift in percent of aggressions directed toward siblings and cohort peers. However, we did compare the percent of aggressive acts a cub directed towards its siblings with the percent of aggressive acts directed at unrelated peers during each 2-month age interval using a Wilcoxon matched-pairs signed-ranks tests. Although information about paternity was unavailable for many hyenas used as subjects in this study, we defined unrelated animals as having coefficients of relatedness (r, Hamilton 1964) less than 0.125, calculated from matrilineal pedigrees as in Holekamp et al. (1999). Differences between groups in all analyses were considered significant when $p \le 0.05$. Throughout the paper, means (+ se) are reported. All statistical tests were performed using Statistica 6.1 software.

RESULTS

Effects of Age

Rates of intra-litter aggression were highest and most variable during the first 2 months of life, and aggression rates subsequently dropped dramatically (Figure 4.1). A Friedman's ANOVA on rates of aggression within the 24 litters in

which both cubs survived to 12 months revealed a significant effect of age ($F_{5,23}$ = 16.41, P = 0.006). Rates of aggression dropped significantly between the 1-2 and 3-4 month age intervals (Wilcoxon Z = 2.78, n = 24, P = 0.005), but there was no significant difference in aggression rates between the 3-4 month and 5-6 month intervals (Wilcoxon Z = 1.35, n = 24, P = 0.177). By 5-6 months of age, rates of aggression had stabilized at low levels and did not change again significantly.

Effects of the context in which aggression occurs

Using the 24 twin pairs in which both siblings survived to 12 months, we found that siblings engaged in a significantly higher percentage of aggressions while fighting over milk during the 1-6 month age interval than during the 7-12 month age interval (Figure 4.2; Wilcoxon Z = 3.41, n = 24, P < 0.001). However, siblings engaged in a significantly higher percentage of aggressions over solid food during the 7-12 month age interval than during the 1-6 month age interval (Figure 4.2; Wilcoxon Z = 2.81, n = 24, P = 0.005). Aggression directed toward siblings in 'other' contexts did not vary significantly with age (Figure 4.2; Wilcoxon Z = 1.94, n = 24, P = 0.06).

Effects of litter composition and sex of dominant cub

Since rates of aggression were highest and most variable during the 1-2 month age interval, we limited our subsequent analyses of factors affecting variability in intra-litter aggression to this first age interval. Although mixed-sex

twins tended to exhibit higher rates of aggression than did either type of samesex twins during the 1-2 month age interval, rates of aggression did not vary significantly with litter composition (Kruskal-Wallis T = 4.65, n = 11 all-female, 26 mixed-sex, and 9 all-male litter-mates, P = 0.098). However, when all-female and all-male pairs were lumped together into the category of same-sexed twins, we found that mixed-sex litter-mates had significantly higher rates of aggression than did same-sex litter-mates during the 1-2 month age interval (Figure 4.3; Mann-Whitney U = 178, n = 20 same-sex litter-mates, 26 mixed-sex litter-mates, P = 0.05).

To evaluate the effects of the sex of the dominant sibling within each litter during the 1-2 month age interval, we compared aggression rates from litters in which a male was the dominant cub with litters in which a female dominated its litter-mate. Within mixed-sex litters, there were no significant differences in rates of aggression between litters in which females were the dominant cubs and those in which males were dominant (Figure 4.3; Mann-Whitney U = 38, n = 22 dominant females, 4 dominant males, P = 0.67). Similarly, within same-sex litters, aggression rates did not vary with the sex of the dominant cub (Figure 4.3; Mann-Whitney U = 37, n = 11 dominant females, 9 dominant males, P = 0.28). Within twin litters in which the dominant cub was male, we found no significant difference between same-sex and mixed-sex litters in the rate of hourly aggression during the 1-2 month age interval (Figure 4.3; Mann-Whitney U = 69, n = 4 mixed-sex, 9 same-sexed litter-mates, P = 0.52). However, within twin litters in which the dominant cub was female, early aggression rates were higher

within mixed than same-sex litters (Figure 4.3; Mann-Whitney U = 14, n = 22 mixed-sex, 11 same-sexed litter-mates, P = 0.035).

Effects of maternal rank

Variability in intra-litter aggression during the 1-2 month interval was not correlated with maternal rank (Spearman $r_s = -0.0126$, n = 46 pairs, P = 0.934). The mean aggression rate within low-ranking litters was twice that within high-ranking litters during the 1-2 month age interval, but this difference was not statistically significant (Mann-Whitney U = 263.5, n = 24 high-ranking litter-mates, 22 low-ranking litter-mates, P = 0.991). We also examined effects on rates of intra-litter aggression of rank and litter composition together. Within high-ranking litters, we found no difference in aggression rates between same-sexed and mixed-sexed litters during the 1-2 month age interval (Figure 4.4; Mann-Whitney U = 58, n = 10 same-sexed, 14 mixed-sex litter-mates, P = 0.46). However, within low-ranking litters, aggression occurred at higher rates within mixed- than same-sex litters during the 1-2 month interval (Figure 4.4; Mann-Whitney U = 33, n = 10 same-sexed, 12 month interval (Figure 4.4; Mann-Whitney U = 33, n = 10 same-sexed, 12 mixed-sexed litter-mates, P = 0.05).

Effects of prey abundance

Hourly rates of intra-litter aggression during the 1-2 month age interval decreased as local prey abundance increased (Figure 4.5; Spearman $r_s = -0.294$, n = 46, P = 0.049). To determine whether local prey abundance differentially influenced aggression rates among high and low-ranking litters, we also

evaluated the relationship between prey and rates of aggression for high and low-ranking litter-mates separately. Rates of aggression among high-ranking litter-mates did not vary significantly with prey abundance during the 1-2 month age interval (Spearman $r_s = -0.128$, n = 24, P = 0.56). Rates of aggression among low-ranking litter-mates declined as prey abundance increased, but this trend was only marginally significant (Spearman $r_s = -0.398$, n = 22, P = 0.06). To determine whether the higher rates of aggressions found in mixed-sex litters was due to lower levels of prey abundance, we compared levels of prey abundance during the 1-2 month age interval between same-sex and mixed-sex litters and found there to be no significant difference (Mann-Whitney U = 586, n =37 same-sexed, 34 mixed-sex litter-mates, P = 0.62).

Sibling aggression compared with aggression toward unrelated peers

The percentage of all aggressive acts that hyenas directed towards siblings decreased with age (Figure 4.6; $F_{5,20} = 22.68$, P < 0.001). Despite the fact that siblings associated with each other more closely than did unrelated peers throughout the first year of life, hyenas directed a higher percentage of all their aggressive acts towards their unrelated peers as they grew older (Figure 4.6; $F_{5,20} = 22.68$, P < 0.001). Cubs directed a significantly higher percentage of their total aggressions toward their siblings than toward unrelated peers during the 1-2 month age interval (Figure 4.6; Wilcoxon Z = 2.67, n = 21, P = 0.008) and during the 3-4 month age interval (Figure 4.6; Wilcoxon Z = 2.79, n = 21, P = 0.005). Within the age intervals of 5-6 and 7-8 months, cubs did not direct

significantly different proportions of their aggression towards litter-mates and unrelated peers (5-6 months: Wilcoxon Z = 0.369, n = 21, P = 0.711; 7-8 months: Wilcoxon Z = 0.533, n = 21, P = 0.593). However, by 9-10 months, hyenas directed significantly more aggression toward unrelated peers than their litter-mates (Wilcoxon Z = 2.416, n = 21, P = 0.015). We found no significant difference in the percentage of aggressions directed towards litter-mates and unrelated peers in the 11-12 month age interval (Wilcoxon Z = 0.254, n = 21, P = 0.798).

DISCUSSION

Variables affecting sibling aggression

Overall, we found that age, context of aggression, litter composition, local prey abundance and the identity of the target of aggression all play important roles in shaping patterns of intra-litter aggression among spotted hyena cubs. Thus, we have replicated and extended results reported by Smale et al. (1995) in that we observed the same patterns of ontogenetic variation as did these earlier workers with respect to both hourly rates of intra-litter aggression and the contexts in which intra-litter fighting occurs. The current study also showed that rates of intra-litter aggression were affected by litter sex composition and local prey abundance, variables not previously examined by Smale et al. (1995).

As in earlier studies of this species (Frank et al. 1991; Smale et al. 1995; Drea et al. 1996; Golla et al. 1999), spotted hyena cubs in our study exhibited the highest rates of aggression during the first 2 months of life, with aggression rates
declining thereafter. Intra-litter dominance determines priority of access to the mother's teats and the dominant cub can potentially monopolize the supply of milk. Thus, the early establishment of intra-litter rank relations can confer important benefits to dominant cubs, such as faster cub growth (Hofer et al. 1993). Frank et al. (1991) found that rates of intra-litter aggression decreased in captivity once intra-litter dominance was established. In captivity, dominance was established within the first 8 days of life (Smale et al. 1995). All rank relationships among spotted hyenas other than those between litter-mates are determined by social variables such as maternal rank and immigration status, and rank among non-sibling youngsters depends at first on whether or not their mothers and other conspecifics are present during fights (Frank 1986; Holekamp et al. 1993; Smale et al. 1993). However, wild neonates establish rank relationships with their siblings in isolated natal dens away from external influences, so intra-litter rank relationships can only be determined by cubs' relative fighting ability (Smale et al. 1995). The importance of early establishment of dominance status, and the fact that this status can only be determined by fighting suggests that the intense intra-litter aggression observed during the 1-2 month age interval in this study represents an early struggle to establish and maintain intra-litter dominance between siblings, rather than a routine attempt to kill one's litter-mate as suggested by Frank et al. (1991). The intensity of early fighting observed between litter-mates in both the wild and captivity reflects the huge fitness advantage the winner in these fights will enjoy if environmental conditions deteriorate to the point at which the mother can only

provide enough milk to support a single cub. Dominant cubs can displace their subordinate litter-mates from access to the mothers' teats until weaning, and from access to solid food indefinitely (Smale et al. 1995). Given that rates of aggression are quite low in Talek hyenas after the first 2 months of life, we found it surprising that Hofer and East (1997) observed siblicide in a litter already nine months old (273 days). Perhaps the commuting system of Serengeti spotted hyenas, in which mothers take extended foraging trips outside of the clan's territory, favors prolonged sibling aggression not seen in the Masai Mara.

Intra-litter aggression among spotted hyenas is primarily related to competition among litter-mates over access to milk or solid food. During the first 6 months of life, cubs in this study typically fought over access to the mother's nipples. However, by months 7-12, cubs fought primarily over solid food, even though weaning in this population does not occur until ~ 13 months of age. Smale et al. (1995) found that over 80% of intra-litter aggressions in spotted hyena cubs occurred over competition for milk or food. Similarly, in this study, over 65% of intra-litter aggression occurred in the context of competition over access to milk or food, suggesting that rates of aggression might vary with resource abundance. Indeed, we found that rates of aggression during the first 2 months of life decreased as local prey abundance increased, and that this relationship was more apparent in offspring of low- than high-ranking mothers. Golla et al. (1999) found that levels of aggression between hyena siblings in the wild decreased as maternal provisioning rates increased, further suggesting that resource availability influences sibling competition. Wachter et al. (2002)

compared intra-litter aggression between populations of spotted hyenas inhabiting prey-rich and prey-poor areas of Tanzania, and found that litter-mates fought less in the population co-existing with abundant prey. All these data are consistent with the hypothesis that prey scarcity may lead to deficiency in the milk supply of the lactating female, enhancing rates and intensities of aggression between her cubs for access to her teats.

Perhaps the best examples of food availability influencing sibling aggression come from observations and field experiments with birds. Avian models of facultative siblicide predict that the amount of food supplied by the parent should influence offspring aggression. This "food amount hypothesis" (Mock et al. 1987) is supported by avian studies demonstrating a causal relationship between food deprivation and sibling aggression. Observational studies of birds have shown increased fighting during periods of low food availability, increased attack rates during meals and decreased attack rates after meals (reviewed in Mock et al. 1987; Drummond 2001). Field experiments manipulating the feeding of blue-footed booby chicks have also found that rates of aggression increased when chicks are food-deprived (Drummond et al. 1989; Nunez et al. 1996). To date, the data from spotted hyenas suggest that, like these avian species, their behavior conforms to predictions of Mock's (1987) "food amount hypothesis" rather than to the predictions of the obligate siblicide hypothesis.

Given that local prey abundance influenced sibling aggression in the current study, and that social rank determines priority of access to food in

Crocuta (Kruuk 1972), we found it surprising that maternal rank did not significantly influence sibling aggression here. Since high-ranking females are usually able to provide sufficient milk to support both offspring in their twin litters, the need for aggression between high-ranking litter-mates should be less than that between low-ranking litter-mates. In this study, low-ranking litter-mates demonstrated twice the mean aggression rates of high-ranking litters, yet this difference was not statistically significant due to large variance and small sample sizes. Furthermore, the negative relationship between prey abundance and rates of intra-litter aggression was stronger within low-ranking than high-ranking litters. Thus, trends in our data suggest that maternal rank might indeed influence intra-litter aggression. Golla et al. (1999) and Smale et al. (1995) found that rates of sibling aggression increased as maternal social status declined, suggesting that lower rates of milk production among low-ranking mothers result in higher rates of sibling aggression.

Both the Smale et al. (1995) study and the current study took place in the Talek region of the Masai Mara National Reserve, Kenya, where prey are generally abundant. Although the time periods of the two studies differed, the same methods for estimating local prey abundance were used during both study periods and mean levels of prey abundance between the two study periods did not significantly differ (Mann-Whitney U = 15292, P = 0.35). Thus, the relationship between intralitter aggression and maternal rank observed in the Smale et al. (1995) could not be attributed to a lower mean abundance of local prey than that recorded in the current study. Rate data from our study were

based on only half the number of hours of observation per litter as those used by Smale et al. (1995). Therefore, another possibility is that our less-rigorous criterion for time cubs needed to spend together to be included in the data set might account for the difference in results between our studies. However, even when we limited our data set to the six litters that were observed for the same number of hours as were cubs observed by Smale et al. (1995), we still found no significant relationship between rates of aggression and maternal rank during the 1-2 month age interval (Spearman $r_s = -0.183$, n = 14 pairs, P = 0.531).

Sex-biased siblicide

The obligate siblicide model proposed by Frank et al. (1991) assumes that same-sexed hyena litters routinely fight until one sibling dies because same-sex twins are evenly matched in competitive ability, but that competition is relaxed among mixed-sex litters. Although Hofer and East (1997) and Golla et al. (1999) found no support for the obligate siblicide hypothesis in spotted hyenas, they observed higher rates of aggression and/or facultative siblicide in same- than mixed-sex litters, particularly among all-female litters. In the current study, we found no significant difference in rates of aggression between all-female, mixed-sex, and all-male litters and this same negative result was also obtained by Smale et al. (1995) and Drea et al. (1996). However, we were surprised to find here that, when all-female and all-male litters were pooled, mixed-sex litters, particularly those produced by low-ranking females, engaged in aggression at higher rates than did same-sexed litters. In fact, the highest rates of aggression

during the 1-2 month age interval were observed in low-ranking, mixed-sex litters, particularly when females rather than males were the dominant cubs. This result contradicts predictions of an hypothesis suggesting that adult female Crocuta might be producing mixed-sex twins to avoid competitive symmetries and prolonged fighting between same-sex twins (Smale et al. 1999). Since higher rates of aggression in mixed-sex litters were not associated with lower levels of prey abundance than those found in same-sex litters, this difference could not be attributed to variation in prey abundance. Perhaps one reason that previous studies in the Serengeti (Hofer and East 1997; Golla et al. 1999) found higher rates of aggression or facultative siblicide in all-female litters was because the Serengeti area is characterized by lower levels of prey abundance than in the Masai Mara (current study). When levels of prey are low, competition between siblings in all-female litters might be higher since females will remain in the natal clan and grow up as competitors. However, where relatively abundant prey are available throughout the year, such as in the Masai Mara, females might compete more with their brothers than their sisters since their brothers will not remain in the natal clan and become important allies for them.

Frank et al. (1991) suggested that, since females acquire their mother's rank in the social hierarchy and remain in the natal clan their entire lives, elimination of a close-ranking competitor would confer selective advantages to surviving cubs from all-female litters. However, to explain siblicidal aggression within male-male litters, Frank et al. (1991) proposed a different adaptive explanation, arguing that elimination of a close-ranking brother would result in

more rapid weight gain during the first year of life, which in turn would improve a male's chance of survival during dispersal. Sex-biased siblicide among spotted hyenas seems unlikely considering the lack of parsimony in the preceding explanations, our observation here of higher aggression rates among same- than mixed-sex twins, and the fact that sex-biased siblicide has not been recorded in any other animal species to date. In addition, hyena litter-mates are often tremendously helpful to each other regardless of their sex (Smale et al. 1995; Wahaj et al. 2004), so routine killing of same-sex litter-mates would appear to be substantial. For example, spotted hyena siblings are each other's closest associates and most frequent partners during coalition formation (Smale et al. 1995; Wahaj et al. 2004). Thus, although same-sex siblings may be each other's closest closest-ranking competitors, they are also each other's most important allies.

During the first 6 months of life, hyena litter-mates in this study engaged in more aggressions over milk than in any other context, and during the first 4 months of life they fought more with their litter-mates than with unrelated peers. However, by 7-12 months of age, cubs began fighting over access to solid food, and by the 9th and 10th months of life, they directed more aggression toward their peers than toward their litter-mates. After cubs leave the communal den at approximately 8 months of age, both siblings and unrelated peers are potential competitors over access to solid foods. Since intra-litter dominance is firmly established by 7-12 months, cubs no longer need to fight with their siblings and begin competing with other peers over access to carcasses. This ontogenetic

progression from fighting with siblings to fighting with peers might correspond to developmental changes in the need to establish rank relations with clan members other than siblings (Drea et al. 1996).

In addition to the factors found in the current study, other variables that appear to influence rates of sibling aggression are maternal hormone levels during fetal development (Dloniak 2004) and genetic relatedness within twin litters (Wahaj et al. 2004). Dloniak (2004) showed that high-ranking female spotted hyenas have higher fecal androgen concentrations than do low-ranking females during the second half of gestation, and that this variance in maternal fecal androgen levels is related to offspring behavioral phenotype. Cubs born to females excreting higher fecal androgen concentrations during late pregnancy show higher rates of aggression toward unrelated peers than do cubs born to females with lower fecal androgen concentrations. However, Dloniak (2004) found no relationship between maternal androgen levels and intra-litter aggression, so prenatal androgens do not appear to affect aggression rates between siblings in spotted hyenas.

Relatedness between litter-mates also influences rates of intra-litter aggression. Female spotted hyenas often mate with multiple partners and twin litters may be sired by either one or two males, resulting in full-sibling and halfsibling litter-mates (Engh et al. 2002; East et al. 2003). Wahaj et al. (2004) found that rates of aggression during nursing between half-sibling littermates were nine times higher than that between full-sibling litter-mates. Similarly, Hofer et al. (2004) found that rates of aggression between half-sibling litter-mates were

higher than between full-sibling twins, and that this difference was substantially exaggerated under conditions of limited access to resources. Perhaps variation in mean relatedness within rank categories explains why Smale et al. (1995) found higher rates of aggression in lower-ranking litters, but this study failed to find rank-related variation in intralitter aggression.



Figure 4.1. Mean hourly rates of intra-litter aggression during each two month age interval in the first year of life. The sample represented by each data point includes 24 litters in which both cubs survived to 12 months. Statistically significant differences are denoted with an asterisk.



Figure 4.2. Mean percent of aggressive interactions between siblings during the 1-6 (black bars) and 7-12 (gray bars) month age intervals that we observed in the contexts of 'milk', 'solid foods', and contexts 'other' than milk or solid foods. The sample represented in each pair of bars is 24 litters in which both cubs survived to 12 months. Statistically significant differences are denoted with asterisks.



Figure 4.3. Mean hourly rates of aggression during the 1-2 month age interval within same and mixed-sex litters in which females (black bars) and males (gray bars) were the dominant cubs. Sample sizes above bars represent number of litters in each group. Other notations are as in Figure 4.2.



Figure 4.4. Mean hourly rates of aggression during the 1-2 month age interval within same-sex (gray bars) and mixed-sex litters (black bars) born to mothers of high and low rank. Other notations are as in Figure 4.3.



Figure 4.5. Hourly rates of intra-litter aggression during the 1-2 month age interval as a function of local prey abundance. Sampled litters include 24 high-ranking litters and 22 low-ranking litters.



Figure 4.6. Mean percent of all aggressive interactions during each two month age interval in which hyenas directed aggression toward their litter-mates (black bars) or towards unrelated peers within their cohorts (gray bars). The sample here includes 21 litters in which both cubs within each twin litter survived to 12 months. Total aggressive interactions included 354 fights directed toward siblings and 237 fights directed toward cohort peers during the first 12 months of life. Statistically significant differences within each age interval are indicated by asterisks.

CHAPTER FIVE

USE OF ULTRASONOGRAPHY TO TEST OBLIGATE AND FACULTATIVE MODELS OF SIBLICIDE IN THE SPOTTED HYENA (*CROCUTA CROCUTA*)

INTRODUCTION

Whereas siblicidal aggression is a common phenomenon in many birds (Mock 1984; Drummond et al. 1986; Mock et al. 1997; Loughhead et al. 1999), the topic has been little studied in mammals, with its occurrence documented only among young pigs (Sus scrofa: Fraser et al. 1991) and inferred among spotted hyenas (Crocuta crocuta: Frank et al. 1991; Hofer et al. 1997; Golla et al. 1999). In birds, sibling rivalry can be severe, and it is often lethal (Drummond 2001). Spotted hyenas have been described as a bizarre exception to the general rule that mammalian sibling rivalry is less deadly than sibling conflict among birds (Mock et al. 1997). Modal litter size among spotted hyenas is two, and vicious fighting among neonatal litter-mates has been documented by Frank et al. (1991). These authors suggested that the function of this early sibling aggression in hyenas is to kill one's litter-mate. This suggestion has been widely adopted, and is now presented as fact in various textbooks on animal behavior in general (e.g. Alcock 2001; Goodenough et al. 2001), books on sibling rivalry in particular (Mock et al. 1997), and also in a number of films and articles in the popular press (Sherman 1992; Stevens 1993; Slack 1999; McDonald 2001).

Siblicide is considered 'obligate' when aggression within a brood or litter is almost always fatal to subordinates, as is true in many avian species (e.g. eagles, herons, and egrets: reviewed in Mock et al. 1997). By contrast, in 'facultative' siblicide, siblings adjust the intensity of intrabrood aggression as

costs and benefits of this aggression vary with current environmental conditions (Mock et al. 1997). Here inclusive fitness benefits of permitting sibling survival generally outweigh direct fitness costs of resource competition with siblings except under environmental conditions so challenging that only a single offspring has any chance of survival.

Female spotted hyenas give birth to one or two cubs, and rarely three. Adult female hyenas usually have only two functional teats, so nursing more than two cubs concurrently is generally impossible. In the wild, female *Crocuta* bear their litters in isolated natal dens or burrows (East et al. 1989) and transfer them to the clan's communal den when the cubs reach 2 to 4 weeks of age. The small diameter of both natal and communal den holes prevents adults from entering, so dens are accessible only to the neonates. Although neonates usually come to the mouth of the den to nurse, observers may not see them there for several weeks because they remain hidden in the den entrance whenever the mother lies there to suckle her young (East et al. 1989).

The difficulty of observing free-living neonatal spotted hyenas has made direct observation of siblicide impossible in the wild. However, indirect evidence for obligate siblicide in this species has emerged in the work of Frank et al. (1991). These workers concluded that 25% of all same-sex hyena cubs born routinely succumb to fatal sibling aggression. This conclusion was based on three lines of indirect evidence. First, neonatal spotted hyenas are precocial and born with fully erupted incisors and canines. Intense aggression between captive littermates, involving the use of these weapons and occurring immediately after

birth, suggested that this early aggression might function to kill siblings (Frank et al. 1991). Second, the litter sizes observed by Frank et al. (1991) in the wild were significantly smaller than those they observed among captive hyenas. suggesting that litter reduction must occur routinely in the wild. Specifically. among litters born to multiparous captive females, the ratio of singletons: twins: triplets was 1:10:2 at birth, and mean litter size was 2.08 (in 13 litters). By contrast, the ratio of singletons: twins: triplets was 42:57:0 in the field when cubs were first brought from the natal den to the clan's communal den at 2-4 weeks of age, and mean litter size was 1.58 (in 99 litters; Frank et al. 1991). Third, samesex twin litters were rare in wild populations when compared to captive populations. That is, the ratio of same-sex: mixed-sex twin litters was 5:5 in captivity, but only 5:28 and 1:8 in wild populations in eastern Africa (Frank et al. 1991) and the Kalahari (Mills 1990), respectively. Frank et al. (1991) concluded that this difference between captive and field observations resulted from selective loss of cubs in the wild due to habitual siblicide within same-sex litters. Specifically, they suggested the following: "Siblicide appears to kill nearly 25% of spotted hyena offspring: at birth, 50% of litters are same-sex, and half of those individuals succumb" (Frank et al. 1991, page 704). Such a sex-bias in siblicidal behavior has not been observed or suggested to occur in any other animal species studied to date.

Smale et al. (1999) found that sex ratios and litter sizes in free-living *Crocuta* varied with environmental conditions, and argued that their results conformed better to a facultative model of siblicide in spotted hyenas than to an

obligate model. However, their study could not address the question of why litter sizes observed by Frank et al. (1991) appeared to differ between captive and wild hyenas. In order to test the possibility that differences in litter sizes observed in captive and field settings might be due to differing circumstances before birth, rather than siblicide after birth, Smale et al. (1999) suggested using ultrasonography in pregnant hyenas to obtain early estimates of litter size in wild hyenas, combined with intensive monitoring of behavioral interactions among den-dwelling cubs as soon as possible after birth. Wahaj and Holekamp (in prep) adopted the latter suggestion and examined factors influencing sibling aggression during the first two months of life in wild spotted hyena cubs. The primary goal of the current study is to test predictions of obligate and facultative models of siblicide using ultrasonography and observational data to compare litter size, sex composition and litter reduction between captive and wild hyenas.

The obligate and facultative models of siblicide generate different suites of predictions regarding what we should observe as we examine litters in wild and captive populations of spotted hyenas. First, as noted by Frank et al. (1991), the obligate model predicts that 25% of offspring die in the wild. However, if siblicide is facultative, litter reduction should be far less common than the 25% predicted by the obligate model. Second, Frank et al. (1991) argued that the few same-sex litters observed in their earlier field work resulted from habitual siblicide within triplet litters, and that singletons resulted from habitual siblicide within same-sex twin litters. By contrast, the facultative model predicts that siblicide should only occur routinely when a mother produces more offspring than she can support.

Thus, the facultative model indicates we should be more likely to observe siblicide in prey-poor than prey-rich environments, and we should observe siblicide frequently when a female produces a larger number of cubs that her number of functional teats, such as when females give birth to triplet litters or when females with only one intact teat give birth to twins. Both of these latter types of cases are unusual, and in both mortality due to siblicide should occur irrespective of offspring sex. Third, the sex-bias in the obligate model predicts that sex ratios in singleton litters and sex compositions of twin litters should differ from chance expectations due to litter reduction among same-sex twins, whereas the facultative model predicts that litter reductions should occur within both same- and mixed-sex litters, and result in offspring sex ratios and litter compositions that do not differ from chance expectations. Fourth, according to the obligate siblicide hypothesis, hyena litter sizes in captivity should be larger than in wild populations since researchers and/or the mother (because she has access to her cubs) can intervene in captivity to prevent litter reduction via siblicidal aggression. Otherwise, however, litter sizes and frequencies of litter reductions should be similar between captive and wild populations. In addition, the facultative model predicts that intra-litter aggression in the wild should be more intense during periods of resource scarcity than during periods of resource abundance, whereas the obligate model predicts no such variation in the intensity of this aggression. Finally, the obligate model of siblicide predicts that cubs from mixed-sex litters should have higher survivorship than cubs from same-sex litters, and that cubs whose same-sex litter-mates survive should have

lower survivorship than should cubs whose same-sex siblings die very young. The facultative model predicts no difference in survivorship between cubs from same- and mixed-sex litters or between cubs whose litter-mates survive and those whose siblings die at an early age.

Although our primary goal here was to test predictions of obligate and facultative models of siblicide in the spotted hyena, we also hoped to use ultrasonography to inquire about potential mechanisms of litter reduction other than siblicide, focusing on prenatal mechanisms in particular. Two obvious possibilities are resorption of embryos *in utero* and stillbirths. Female spotted hyenas are unique among mammals in having external genitalia that are highly masculinized. That is, the clitoris is enlarged to form a fully erectile pseudopenis and the vaginal labia are fused to form a structure that remarkably resembles the scrotal sac of the male (Neaves et al. 1980). Because the female hyena gives birth through her peniform clitoris, complications during the birth process may result in fetal mortality (Frank et al. 1994; Frank et al. 1995). Females giving birth for the first time, in particular, may experience unusually high mortality of their neonates due to dystocia (Frank et al. 1995). Here, although we were unable to evaluate stillbirths in free-living hyenas, resorptions could be observed in both wild and captive females, and stillbirths could be evaluated in captive females.

METHODS

Field Study

Field data were collected between July 1988 and May 2004 from one large social group, or clan, of spotted hyenas inhabiting the Talek region of the Masai Mara National Reserve, Kenya. Hyena clans contain multiple adult males and several matrilines of adult female kin with their offspring. Members of both sexes are capable of breeding at approximately 24 months of age, although most individuals delay first reproduction for several more months (Matthews 1939; Holekamp et al. 1996). Males disperse from their natal groups after puberty. whereas females are usually philopatric (Frank 1986; Henschel et al. 1987; Mills 1990; Smale et al. 1997). Adults can be ranked in a strict linear dominance hierarchy, with members of the same matriline occupying adjacent rank positions. Female Crocuta are dominant to immigrant males, and offspring attain ranks just below those of their mothers (Holekamp et al. 1991; Engh et al. 2000; Engh et al. 2003). Rank relations, particularly those among females, remain stable for extended periods and across a variety of contexts (Kruuk 1972; Tilson et al. 1984; Frank 1986; Smale et al. 1993). An individual's position in the group's hierarchy strongly determines its priority of access to food (Kruuk 1972; Frank 1986).

Subjects here were members of a *Crocuta* clan that usually contained 60 to 80 hyenas, and defended a group territory of approximately 65 km² (Boydston et al. 2001). All adult members of the study clan were identified by their unique spot patterns, and young cubs that had not yet developed spots were identified

by unique scarring, bald patches, ear notches, size differences, molt patterns, or a combination of these features. Sex was determined from the dimorphic glans morphology of the erect phallus (Frank et al. 1990). By two months of age, the glans of the male's phallus assumes a distinctly different shape from that of the female (Drea et al. 1998). That is, the male's phallus is pointed at its tip and has a constriction above the glans whereas the female's pseudopenis is blunt at the tip and lacks a constriction. Cubs regularly develop erections during greeting ceremonies, which occur virtually every time a new hyena arrives at the den. Young hyenas were sexed here repeatedly by multiple independent observers, and sex could be assigned with complete certainty by the time cubs were 2-3 months old. However, on several occasions, cubs died before they could be sexed. A disappearance date for each cub was assigned as the date on which it was last seen if it did not survive to adulthood.

Spotted hyenas breed throughout the year with the gestation period lasting 110 days (Schneider 1926; Kruuk 1972). We estimated cub birth dates (to \pm 7 days) by using their pelage, size, and other aspects of their appearance and behavior when cubs were first observed above ground (Holekamp et al. 1996). We assigned the date on which a cub was first seen above ground as its 'date first seen', although it's actual date of birth might have been several weeks earlier.

Mother-offspring relations were established on the basis of regular nursing associations. Females that had given birth at least once (multiparous females) could be distinguished from nulliparous females (who had never previously given

birth) based on the presence of pink scar tissue on the posterior surface of the erect phallus (Frank et al. 1994). This scarring results from tearing of the phallus at first parturition, and it remains visible throughout the lifetime of the animal. Social ranks were determined based on wins and losses in dyadic agonistic interactions. Outcomes of these interactions were organized into a matrix from which each female hyena could be assigned a social rank in the clan's dominance hierarchy (Martin et al. 1988).

Since very little is known about the length or timing of the estrous cycle in female spotted hyenas, and since pregnancy cannot be identified through assessment of the female's physical state in this species, we relied on the abrupt cessation of male courtship behavior to provide behavioral indicators as to when females became pregnant. That is, females were usually followed by an entourage of adult males for some weeks before estrus, but this entourage abruptly disappeared when a female conceived (Szykman 2001). Once a female was considered pregnant, she was immobilized with an intramuscular injection of Telazol (6.5mg/kg) delivered from a CO2-powered rifle in a lightweight plastic syringe. When anaesthetized, females underwent a transabdominal ultrasound using a real-time HITACHI EUB-405 scanner with a 5 MHz curved-array transducer. Animals were scanned while lying on their backs in the supine position after we removed their fur from the lower abdomen with electric clippers. Ultrasound data were collected in the field from August 1999 through May 2004. Multiple ultrasounds within a single pregnancy were not possible in wild hyenas due to both the difficulty of darting any particular female repeatedly within a

narrow window of time, and our reluctance to stress pregnant females with multiple dartings.

Fetuses and their heartbeats were identified using methods described by Place et al. (2002). Briefly, using the bladder as a landmark, we located the bifurcation of the uterine body and followed each of the two horns anteriorly to search for the presence of fetuses. Fetuses were recognized during early pregnancy by the presence of a gestational sac within a thickened endometrial cavity (Figure 5.1a). Fetal cardiac activity can be clearly discerned as a rapid fluttering within the fetus as early as 24 days post-mating (M. Weldele and N. Place – pers. communication). Thirty days after mating, development of spine. cranium, and long bones begin, and skeletal elements can be easily discerned on sonographic images (Figure 5.1b). Circumference of the gestational sac (in young fetuses) and fetal femur lengths (in older fetuses) were measured to estimate gestational age (Place et al. 2002), which later allowed us to intensify monitoring of pregnant females during a narrow window of time around the expected date of parturition. All immobilized females were radio collared (Telonics Inc., Mesa, AZ) and tracked daily to facilitate discovery and observation of neonatal cubs. Two different sets of litter size data from wild female hyenas are reported in this chapter: a) the subset of female hyenas that were examined via ultrasonography, and b) all female hyenas, including those with litters that were and were not observed in utero with ultrasound.

Detailed demographic records for the Talek hyena clan were collected throughout the study period and have been maintained since early 1988.

Demographic data were used to calculate survivorship tables during the first two years of life for individuals in a) litters of varying size, b) twin litters of varying sex compositions and c) twin litters in which one sibling died before three months of age compared to those in which both siblings lived at least three months. Data for survivorship tables were not censored.

Captive Study

Pregnant spotted hyenas were studied at the Field Station for the Study of Behavior, Ecology, and Reproduction at the University of California, Berkeley from October 1987 through March 2003. A description of the Berkeley hyena colony is presented in Frank et al. (1989) and captive husbandry is described in Berger et al. (1992). Pregnancies in captivity were monitored by transabdominal ultrasonography using a real-time Aloka SSD-500 scanner with a 5 MHz curvedarray transducer. Prior to each examination, animals were immobilized with an intramuscular injection of Ketaset (4 - 6 mg/kg) and xylazine (1 mg/kg)administered via blow-dart. Detection of fetuses was as in field studies, although captive hyenas often underwent multiple (mean = 1.96 ± 0.15) ultrasounds per pregnancy, whereas wild hyenas underwent only one. For cases in which more than one ultrasound was conducted during a particular captive pregnancy, but the number of fetuses observed varied among examinations, the ultrasound showing the greatest number of fetuses was used in calculating litter size in *utero*. Exact dates on which conception occurred were often known for captive females because mating usually occurred in the presence of human observers.

In addition, gestational age could straightforwardly be estimated based on femur length measurements made on fetuses via ultrasonography (Place et al. 2002). Staff members are almost always present during captive births to aid or intervene in the event of birth complications. Therefore, in contrast to the situation in the field study, neonatal litter sizes presented for captive hyenas were true litter sizes at birth as there was no time or opportunity for mortality to reduce litters after parturition. Captive mothers give birth in pens (2 X 2 m) with straw bedding and a plastic 200-litre barrel. Mothers are accessible to the infants 24 h a day, which is not the case in nature. Although severe fighting between sibling neonates has been observed in captivity, siblicide has never been observed there because cubs are always separated before they can inflict mortal injury on one another.

Long-term goals of the Berkeley hyena project include experimental treatments of pregnant spotted hyenas with anti-androgens and letrazole (an aromatase inhibitor) as well as performing fetectomies (early caesarian sections) to study development of the external genitalia. Females born to mothers that had been treated with anti-androgens were not considered at all in our dataset. In order to determine which captive pregnancies should be included in our comparisons with those in wild hyenas, we compared litter size at birth in multiparous females that had never received any sort of treatment (n = 12) with litter size at birth from each of the following groups: females who were themselves treated during pregnancy with anti-androgens or letrozole (n = 12), females that had previously had a fetectomy performed on them during an earlier pregnancy (n = 13), and females on which an amniocentesis procedure was

performed during the current pregnancy (n = 5). Mean litter size in all but the last of these groups was significantly smaller than in untreated females ($F_{3, 39} = 3.6$, P = 0.04, followed by Tukey's posthoc pairwise comparisons), so all these other treatment groups were excluded from all further analyses. Together, females receiving no treatment and females on which amniocentesis was performed were referred to as "untreated" captive females, and their litters comprised our dataset.

Terminology

The number of fetuses observed "*in utero*" refers to the number of fetuses detected via ultrasonography during pregnancy in both captive and wild hyenas. The term "neonatal" refers to the post-partum number of neonates in captivity, but in the wild this term refers to the number of cubs present when a litter was first seen above ground. In both cases, "neonatal" litter size indicates the number of cubs observed alive as soon after parturition as possible. Resorptions of fetuses were detected when either a second ultrasound during a particular pregnancy confirmed a reduction in the number of fetuses *in utero* (captive study only) or a second or third trimester fetus lacked a heartbeat (both captive and wild studies). Stillbirths could only be positively identified in captive hyenas, because sources of early mortality are difficult to identify in the field. In both wild and captive studies, "litter reduction" was defined as a decrease in litter size from the number of fetuses observed *in utero* to the number of living "neonatal" cubs observed.

Data Analysis

Results are reported for both primiparous and multiparous females. However, to make our results comparable to those of Frank et al. (1991), in some analyses we limited our dataset to only multiparous females. Sample sizes of litters observed in utero and those observed as neonates sometimes differed in the wild because some ultrasounded females kept their litters so well hidden that we never had opportunities to observe these cubs as neonates. In addition, some captive litters were born to females that were never ultrasounded. Independent sample t-tests were used to compare litter sizes between captive and wild populations, and between primiparous and multiparous females. Chisquare tests were used to compare litter compositions, and these were adjusted for small sample sizes as necessary using Yate's correction. Litter reductions were assessed by matched comparison of number of fetuses observed in utero to the number of cubs observed alive at the end of the same pregnancy either at parturition (captivity) or when cubs first emerged above ground (field study) at approximately six weeks of age (mean = 42.9 ± 6.8 days, n = 27 cubs in the wild). For these matched comparisons we used dependent sample t-tests. We used a Spearman's Rank correlation to assess the relationship between mean litter sizes born to females in the wild and the social ranks of those females. A one-way ANOVA was used to determine whether litter size varied with age at which litters were first seen above ground. Binomial tests were used to determine whether observed sex ratios differed from those based on chance

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expectations. In survivorship analyses, proportions of individuals surviving were compared among multiple groups using a multiple-sample test, in which a score was assigned to each survival time using Mantel's procedure (Mantel 1967), and then a Chi-square value was computed based on the sum of these scores for each group. Cox's F tests were used in survivorship analyses to compare survival between only two groups. Despite small sample sizes and low statistical power, differences between groups were considered significant when $P \le 0.05$. Throughout the paper, means (\pm se) are reported. All statistical tests were two-tailed and performed using Statistica 6.1 software.

RESULTS

Sample Sizes

<u>Field Study</u>: Twenty-two females were immobilized and ultrasounded during pregnancy in the wild, including 2 primiparous and 20 multiparous females. For 18 of these 22 females, we were able to observe their litters above ground within the first few weeks of life, but four ultrasounded females maintained their litters for extended periods in dense thickets or otherwise made it impossible for us to observe their cubs as neonates. Of the 18 observable females, 2 were primiparous and 16 were multiparous. In the larger sample of all wild females for which litter size was known (including both those who were ultrasounded and those who were not), we had 241 litters, of which 44 were from primiparous females and 192 from multiparous females. For mothers of five of these 241 litters, parity was not known.

<u>Captive Study</u>: Twenty-seven "untreated" females were immobilized and ultrasounded during pregnancy, including 11 primiparous and 16 multiparous captive females. Thirty-two litters were born in the captive colony, but mothers of five of these litters were not ultrasounded prior to birth. Of these 32 captive litters, 15 were born to primiparous females and 17 to multiparous females.

Litter Composition and Litter Size

Litter composition in utero did not differ overall between captive and wild hyenas (Figure 5.2; Yate's χ^2 = 0.265, d.f. = 2, P > 0.8), nor did it differ when we compared only captive and wild hyenas that had been examined during pregnancy with ultrasonography (Figure 5.2; Yate's $\chi^2 = 0.884$, d.f. = 2, P > 0.5). When we compared the ratios of singleton: twin: triplet litters at birth between untreated captive females (litter composition at birth was 11:11:3) and our larger sample of all wild hyenas (litter composition at emergence was 123:126:2), we found a significant difference between these litter compositions (Yate's χ^2 = 11.78, d.f. = 2, P < 0.01). However, when triplet litters were eliminated from the neonatal dataset, we found no difference between captive and wild hyenas with respect to the proportions of singletons and twin litters observed (Yate's χ^2 = 0.03, d.f. = 1, P > 0.98). These data suggest that perhaps captive hyperas have disproportionately large numbers of triplet litters compared to wild hyenas. We observed no difference in composition between litters observed in utero and those observed again later as neonates in either captive (Figure 5.2a; Yate's χ^2

= 1.84, d.f. = 2, P > 0.30) or wild hyenas (Figure 5.2b; Yate's χ^2 = 0.39, d.f. = 2, P > 0.80).

Considering both primiparous and multiparous females together that were examined via ultrasonography, we found no difference between captive and wild hyenas in mean size of litters in utero (captive: n = 27, mean = 1.93 + 0.12; wild: n = 22, mean = 1.91 ± 0.11 ; t = 0.102, P = 0.92), or in mean size of neonatal litters (captive: n = 32, mean = 1.31 + 0.16; wild: n = 18, mean = 1.50 + 0.20; t = -0.702, P = 0.49). In order to fairly compare neonatal litter size between captive and wild hyenas that were never examined via ultrasonography, we limited the dataset of captive hyenas to only those 25 cases in which litter size was greater than zero (i.e., cases were excluded in which ultrasonography indicated occurrence of stillbirths or resorptions such that no cubs were born alive) since litters in which all cubs are stillborn cannot be detected in wild litters not observed with ultrasound. Mean neonatal litter size in captive females here did not differ significantly from that observed in the larger sample of all wild females (captive: n = 25, mean = 1.68 + 0.14; wild: n = 251, mean $= 1.52 \pm 0.03$; t = 1.34, P = 0.18). Mean neonatal litter size also did not differ between wild females that were ultrasounded and wild females that were not ultrasounded (wild ultrasounded litters: n = 18, mean = 1.50 ± 0.20 ; all wild litters: n = 241, mean = 1.52 ± 0.03 ; t = 0.09, P = 0.92). Since the age at which cubs are first observed differs between captive and wild hyenas, we also compared mean neonatal litter size born to the 25 captive hyenas with that observed in a subset of 25 wild hyenas whose cubs were seen soonest after birth (i.e., the earliest dates first seen), but we found no

difference (mean age first seen in the wild for this subset was 4.12 ± 0.52 days, mean litter size for both wild and captive populations was 1.68, t = 0.0, P = 1.0).

Litter Composition and Litter Size among Multiparous Females

We next restricted our analyses only to multiparous females to compare our findings with those reported by Frank et al. (1991). Here again we found no difference in litter composition between captive and wild hyenas either in utero (captive litter composition = 2:11:3, wild litter composition = 2:16:2; Yate's χ^2 = 0.185. d.f. = 2. P > 0.9) or among neonates (captive litter composition = 4:8:3. wild litter composition = 2:10:1; Yate's χ^2 = 0.60, d.f. = 2, P > 0.7). When only multiparous females were considered, litter composition differed between captives and our larger sample of all wild hyenas (captive litter composition = 4:8:3, wild litter composition = 87:103:2; Yate's χ^2 = 16.93, d.f. = 1, P < 0.001), but again this difference vanished when triplets were excluded from the dataset (Yate's $\chi^2 = 0.29$, d.f. = 2, P > 0.8). We found no difference in litter composition between uterine and neonatal observations of multiparous females either in captivity (Yate's $\chi^2 = 0.57$, d.f. = 2, P > 0.70) or in the wild (Yate's $\chi^2 = 0.31$, d.f. = 2, P > 0.80).

Among ultrasounded females, we also failed to find any significant difference between captive and wild hyenas in mean litter sizes observed either *in utero* (captive: n = 16, mean = 2.06 ± 0.13 ; wild: n = 20, mean = 2.00 ± 0.10 ; t = 0.36, P = 0.72) or as neonates (captive: n = 17, mean = 1.61 ± 0.23 ; wild: n = 16, mean = 1.5 ± 0.22 ; t = 0.45, P = 0.65). Thus, our results here differed from

those obtained earlier by Frank et al. (1991). However, when we compared multiparous captive females with our larger sample of wild multiparous females that were not ultrasounded, we found neonatal litter size to be significantly larger in captivity than in the wild (Table 5.1; t = -2.63, P = 0.009). Thus, these results were the same as those reported earlier by Frank et al. (1991).

Litter Reduction

Significantly more fetuses were observed *in utero* than as neonates among captive females (Figure 5.3; t = 2.8, n = 27, P = 0.01). Specifically, nine of 27 litters were reduced in captive females. Among captive multiparous females, five of 16 litters were reduced, and here again we observed significantly more fetuses *in utero* than living neonates (t = 2.41, n = 16 pairs, P = 0.03). Thus, roughly one third of captive litters were reduced, and none of those reductions were due to siblicide.

When we compared the number of fetuses observed *in utero* in the wild to the number of cubs in litters observed later as neonates, we found that five of 18 litters were reduced. Because siblicide could not account for reductions in either singleton litters or in litters from which all cubs disappeared, only two of the five cases of litter reduction we observed in ultrasounded wild hyenas could have possibly been attributable to siblicide. In both of these cases, twin litters *in utero* appeared above ground as singletons. Thus, as in captivity, we found that significantly more fetuses were observed *in utero* than as neonates in the wild (Figure 5.3; t = 2.36, n = 18, P = 0.03). Although siblicide is not permitted in

captivity, the proportion of litters in which litter reduction occurred did not differ between captive and wild hyenas (Yate's $\chi^2 = 0.004$, d.f. = 1, P > 0.95). Among wild multiparous females, five of 18 litters were reduced and more fetuses were observed than neonates within pregnancies (t = 2.41, n = 16 pairs, P = 0.03).

Possible Causes of Litter Reduction

In captivity, 80% of litter reductions were due to stillbirths and 20% were due to resorptions. Of 32 captive pregnancies, 9 (28%) produced at least one stillborn cub, and fetal resorptions occurred in 2 (6.3%) of the 32 pregnancies.

In wild spotted hyenas, litter reductions might have been due to stillbirths, resorptions, predation, disease, infanticide or siblicide. Although it was impossible for us to detect stillbirths in nature, we did observe one fetus being resorbed in a wild female. There was substantial variation in the age at which cubs were first observed above ground, and the longer it took us to see wild litters, the smaller they tended to be (Figure 5.4; $F_{2, 216} = 3.18$, P = 0.04). Singleton litters were first observed above ground substantially later than twin (LSD post-hoc test, P = 0.04) or triplet litters, though the small sample of triplet litters failed to yield significant differences in age at first sighting from those of smaller litters (LSD post-hoc test, P = 0.09 for singleton vs. triplet and P = 0.19 for twin vs. triplet).

In our large sample of wild females, we observed 32 cases of litter reduction from twin to singleton litters in 102 twin litters and half of these 32 cases occurred before the first three months of life. Six of these reductions were
most likely due to siblicide. For example, each of two adult females in our study population sustained severe damage to one of her teats during fights, leaving only one functional teat intact. Each time either of these females produced twins (three times for each female), the subordinate cub was viciously attacked by the dominant whenever it attempted to nurse. These subordinate cubs exhibited many wounds and scars, grew far more slowly than their dominant siblings and inevitably disappeared within the first few months of life (mean age at death for these six cubs was 118.4 ± 39.8 days). In both triplet litters born in the wild, the third-ranking cub disappeared at a very young age (at 4 and 29 days after birth, respectively), after being severely attacked by both its dominant litter-mates.

Effects of Maternal Parity and Social Rank

In captivity, litter size *in utero* did not vary significantly with parity (t = 1.50, P = 0.15), but neonatal litter sizes were larger among multiparous females than among primiparous females (Figure 5.5; t = 2.48, P = 0.02). Among primiparous females in captivity, seven of 15 pregnancies produced stillborn cubs. Among captive multiparous females, 2 stillbirths and 2 resorptions were observed in 17 pregnancies. Although primiparous females tended to have more stillbirths and resorptions than multiparous females, this difference was not significant among captive females (Yate's $\chi^2 = 1.0$, d.f. = 1, P > 0.7).

Only two primiparous females were ultrasounded in the wild and each contained only one fetus *in utero*, whereas mean litter size *in utero* for multiparous females was 2.0 ± 0.10 (n = 20 females). Among ultrasounded wild

hyenas, these same two primiparous females had neonatal litter sizes of only one cub while mean neonatal litter size from multiparous females was $1.92 \pm$ 0.14 (n = 13 females). Because only two primiparous females were ultrasounded in the wild, it was impossible to use statistical tests to fairly compare mean litter size *in utero* between primiparous females in the wild and captivity. However, among our larger sample of 236 wild hyenas, we observed no significant difference in neonatal litter sizes between primiparous and multiparous females (Figure 5.5; t = 1.46, P = 0.147). Thus, it appears that litter sizes in the two wild primiparous females in which we observed singletons fetuses *in utero* were not representative of litter sizes in other primiparous females in the natural habitat. We found no relationship between mean litter size and maternal social rank among our large sample of wild female hyenas (F_{23,219} = 0.65, Spearman r_s = 0.033, n = 24 ranks, P = 0.875).

Sex Ratios

We observed no significant difference between proportion of male and female cubs produced in all litters considered together, in singletons considered alone, or in twin litters considered alone among either captive or wild hyenas (Table 5.2). The ratio of all-male:mixed-sex:all-female twin litters born in captivity was 3:5:4 and this did not differ from chance expectations (Yate's $\chi^2 = 0.21$, d.f. = 1, P > 0.5). Similarly, ratios in twin litters born to wild hyenas did not differ from chance expectations (Figure 5.6a; Yate's $\chi^2 = 0.95$, d.f. = 1, P > 0.5), nor did they differ from these ratios observed in captive hyenas (Yate's $\chi^2 = 0.55$, d.f. = 2, P > 0.7).

Within 33 twin litters born to multiparous females, Frank et al. (1991) observed a ratio of 4:28:1 (all-male:mixed-sex:all-female), which differed significantly from chance expectations (Yate's $\chi^2 = 14.56$, d.f. = 1, P < 0.001). Among multiparous females from the current study, we observed a ratio of 17:43:22 at emergence, which did not differ from chance expectations (Yate's χ^2 = 0.54, d.f. = 1, P > 0.3), but was significantly different from the ratio observed by Frank et al. (1991) (Figure 5.6b; Yate's $\chi^2 = 9.56$, d.f. = 2, P < 0.01).

Survivorship Among Wild Hyena Cubs

Among wild hyenas, we observed significant differences in the proportion of individuals surviving to reproductive maturity (24 mo. of age) among 112 singleton cubs, 208 cubs from twin litters, and 6 cubs from triplet litters (Figure 5.7a; $\chi^2 = 13.34$, d.f. = 3, P = 0.001). Cubs from twin litters experienced higher survivorship than those from singleton (Cox's F_{224, 416} = 1.29, P = 0.01) or triplet litters (Cox's F_{12, 416} = 7.33, P < 0.001), and cubs born as singletons also survived better than did cubs from triplet litters (Cox's F_{224, 12} = 4.10, P = 0.009). Of 146 cubs from twin litters in which the sex of both siblings could be determined, we found no significant variation in survivorship among individuals from all-female, mixed-sex, and all-male litters (Figure 5.7b; n = 26 cubs from allfemale litters, 84 cubs from mixed-sex litters, 36 cubs from all-male litters; $\chi^2 =$ 1.59, d.f. = 3, P = 0.45). Within twin litters, individuals experienced better survivorship when their siblings survived to at least three months of age than when their siblings disappeared before three months of age (Figure 5.7c; n = 142 individuals whose siblings survived longer than 3 months, n = 32 individuals whose siblings disappeared before 3 months of age, Cox's $F_{284, 64}$ = 2.11, P < 0.001). These data strongly suggest habitual siblicide would be maladaptive in these animals.

DISCUSSION

Several lines of evidence presented here are inconsistent with predictions of the obligate siblicide hypothesis. First, possible siblicide in the wild could account at most for reduction of cub number in only 2 of 18 (11.1%) ultrasounded litters. This is considerably less than the 25% predicted by the obligate siblicide model, especially considering that 37% of litters in captivity were reduced strictly due to stillbirths and resorptions. We observed resorption in one of 18 wild litters, which is the same proportion (6%) as that observed among captive litters (2 of 32). Stillbirths occurred in 28% of 30 captive litters. If rates of stillbirth were similar in captivity and the wild, we would have expected reductions due to stillbirths in five of 17 wild litters. In fact, four wild litters were smaller when observed as neonates above ground than when observed *in utero*. Thus, if indeed rates at which stillbirths occur are roughly the same in the wild and in captivity, then further litter reduction due to siblicide must be extremely rare in our wild population at the present time.

Second, frequency of litter reductions in captivity, where siblicide could not occur, did not differ from that in our wild study population. Third, habitual siblicide did not reduce triplets to same-sex twins as suggested by Frank et al. (1991), since all members of both wild triplet litters observed here died before five months of age. Fourth, the lack of sex bias in compositions of twin or singleton litters in either the wild or captivity suggests that litter reductions here were not occurring exclusively within same-sex litters in the wild. In addition, probability of survival among members of twin litters did not vary with sex composition of these litters. Finally, habitual siblicide could not be adaptive in spotted hyenas since, not only did cubs from twin litters, but also twins whose siblings died early experienced significantly worse survivorship than did those whose siblings survived for at least three months. These results unambiguously contradict predictions of the obligate siblicide hypothesis.

On the other hand, a large body of evidence now exists that facultative siblicide does occur among free-living spotted hyenas. Hofer and East (1997) inferred that facultative siblicide might be occurring in all-female twin litters based on occurrence of significantly fewer all-female litters than expected by chance, and a female bias within singleton litters. Golla et al. (1999) found support for the facultative siblicide hypothesis by demonstrating that rates of aggression between litter-mates increased as maternal rank and cub growth rates decreased. Similarly, Smale et al. (1995) observed that rates of aggression within twin litters increased as maternal rank decreased. Smale et al. (1999)

found that offspring sex ratios varied as environmental conditions changed over a 10 year study period, but that mean litter size remained constant, a result consistent with the facultative model of siblicide. Most recently, Wahaj and Holekamp (in prep) observed that sibling aggression increased as local prey abundance declined. Thus, several lines of evidence from a number of different researchers indicate that siblicide may occur facultatively within hyena litters.

We agree with the assertion made by Frank et al. (1991) that fighting between neonatal litter-mates is unusually intense in spotted hyenas relative to sibling fighting observed in most other mammals. However, we disagree with Frank et al. (1991) regarding the function of this early aggression. Whereas Frank et al. (1991) argued that neonatal fighting functions to kill siblings within same-sex litters, we agree with the alternative suggestion put forward by Smale et al. (1995), that the function of neonatal fighting is simply to establish a rank relationship between litter-mates. Once this rank relationship has been established, there is little need for further aggression unless the mother simply cannot support all the cubs to which she has given birth, as frequently occurs in triplet litters. Even in captivity, medical treatment of sibling-inflicted wounds was only required for members of triplet litters (Frank et al. 1991).

Frank et al. (1991) observed larger neonatal litters among multiparous captive females than litter sizes at emergence in wild *Crocuta*. Data from the current study replicate that earlier result. However, litters were first observed on the day of birth in captivity in both the current study and that of Frank et al. (1991), and some weeks after birth in the wild, where mortality might have

occurred that was due to a number of sources other than siblicide. We found here that the longer it took us to first observe litters in the wild, the likelier it was that we would observe singletons. We saw triplet litters unusually early in life because these cubs were typically so hungry that, even at very young ages, they followed their mothers some meters away from den holes as they attempted to continue nursing. Siblicide did appear to reduce triplet litters, and litters born to mothers possessing only one functional teat. But aside from these cases, we have no more reason to believe cub disappearances were due to siblicide than to predation, disease or a number of other mortality sources.

Between 1979 and 1984, Frank et al. (1991) observed four cases of early litter reduction due to siblicide in the field, from twins to singletons. Between 1988 and 2003, we observed 32 cases in which cubs originally seen as twins were reduced to singletons within the first three months of life. In addition, all triplet litters we observed in the field died early. Among ultrasounded wild hyenas, 28% of litters observed *in utero* were reduced. In captivity, 33% of litters observed *in utero* were reduced by the end of the parturition process. Thus, litter reduction occurs commonly in both captive and wild hyenas. In captivity, siblicide cannot account for any litter reduction at all, and it also appears that very few cases of litter reduction could be attributed to siblicide in our wild study population during the current study period. Birth complications, especially among primiparous spotted hyenas, appear to be a common cause of litter reduction (Frank et al. 1995). In a zoo in Amsterdam, 35% (18 of 51) of cubs were stillborn or died immediately after birth from cases other than siblicide (Kranendonk et al.

1982). In the captive Berkeley colony, Frank et al. (1995) reported that eleven of 18 neonates (61%) born to primiparous females were stillborn and four of 11 (36.4%) primiparous females experienced dystocia (difficult parturition), whereas only two of 30 (7%) multiparous females had stillbirths. In another study, five of eight (63%) primiparous female *Crocuta* experienced birth complications (Frank et al. 1994). In addition to giving birth through a narrow penile clitoris, female spotted hyenas possess abnormally long birth canals and fetuses have short umbilical cords, so infants not born immediately after placental detachment will die of anoxia in the birth canal (Frank et al. 1995). This risk of oxygen deprivation appears to be greater in primiparous females where labour takes longer since the clitoris must stretch for the first time to accommodate the fetus (Frank et al. 1994). Thus, litter reductions appear to be caused more commonly by dystocia than by siblicide in *Crocuta*, especially among primiparous females. We failed to observe parity influencing litter sizes among our larger sample of wild females and perhaps this was because other sources of mortality in the wild (such as predation and disease) were diluting any effects of parity.

Frank et al. (1991, page 704) claimed a "marked difference in survival [occurred] between same- and mixed-sex litters," whereas our survival analysis showed that mixed-sex litters survive no better than same-sex litters of either type. Frank et al. (1991) clearly observed a bias towards mixed-sex twin litters, and they attributed this finding to obligate siblicide within same-sex litters. If siblicide is truly sex-biased and independent of local environmental conditions, then sex composition of twin litters in hyena populations should deviate from

binomial expectations, vielding an overabundance of mixed-sex litters and too few same-sex litters. However, our long-term study failed to find a sex bias in twin litters, and our ratios of the various types of twin litters differed significantly from those reported by Frank et al. (1991). Secondary sex ratios in Crocuta can apparently be adjusted according to ecological conditions (Holekamp et al. 1995; Smale et al. 1999). Studies conducted in areas where hyenas are challenged by relatively low prey abundance, have found a bias towards production of mixedsex twins (Mills 1990; Hofer et al. 1997). By contrast, siblicide and sex biases within twin litters were absent in spotted hyenas from Ngorongoro Crater, an area characterized by year-round abundant prey (Wachter et al. 2002). Thus, one hypothesis to account for the difference in composition in twin litters between the current study and that of Frank et al. (1991) is that perhaps a combination of low prey availability and large clan size accounted for the bias towards mixed-sex twin litters during the study period of Frank el al. (1991). Whereas clan size hovered around the local carrying capacity throughout the study period of Frank et al. (1991), clan size was well below carrying capacity just as often as it was at or above carrying capacity during our own extended study period in the same population (Boydston 2000). On the other hand, as S. E. Glickman (pers. comm.) has pointed out, although physiological mechanisms are known by which offspring sex ratios can be biased either towards males or towards females (e.g. alteration of vaginal PH, etc.), no mechanism is currently known by which adult females might increase the probability of producing mixed-sex twins. Unless a female Crocuta can separately adjust conditions within the two horns of her

bicornuate uterus to promote the occurrence of mixed-sex twins, it remains possible that the peculiar sex compositions in twin litters observed in Frank et al. (1991) were caused by frequent occurrence of facultative siblicide within samesex litters during their earlier study period.

The intensive fighting that occurs between neonatal spotted hyena siblings has received a great deal of attention from the media, and the lay public now generally believe that hyena cubs routinely behave like Cain and Abel. This view adversely affects our ability to protect these and other members of the family Hyaenidae (East et al. 1998). Our data convincingly show that the normal function of early sibling aggression in this species cannot be to kill one's littermate. We therefore hope our data will change public attitudes towards these much maligned animals, and facilitate their conservation.

Population	Mean Litter Size <u>+</u> se	N
Frank et al. (1991) - captive	2.08 <u>+</u> 0.14	13
Frank et al. (1991) - field	1.58 <u>+</u> 0.05	99
This study - captive	1.93 <u>+</u> 0.18	15
This study - field	1.56 <u>+</u> 0.04	192

Table 5.1. Neonatal litter sizes born to multiparous females that were directly observed in Chapter 5 compared to those observed earlier by Frank et al. (1991), who used only multiparous females in their analysis. "N" indicates the number of litters born.

Population	# Males	# Females	N	P (binomial test)
Wild (overall)	140	128	268	0.25
Captive (overall)	21	20	41	0.50
Wild (in singleton litters)	38	43	81	0.66
Captive (in singleton litters)	5	4	9	0.50
Wild (in twin litters)	89	97	186	0.61
Captive (in twin litters)	11	13	24	0.42

Table 5.2. Sex ratios among litters born to wild and captive hyenas.



Figure 5.1. Sonographic images of a) early pregnancy (gestational age here was 43 days) indicated by the presence of a gestational sac and thickened endometrial cavity. The gray donut-shaped outer circle is the endometrial wall of the uterine horn and the dark hypoechoic center represents the amniotic fluid and the developing fetus, b) late pregnancy (gestational age here was 97 days) indicated by skeletal development as well as an observable heartbeat. The vertebrae and ribs of the fetus are indicated by the hyperchoic white bands across the screen. The arrow points to the fluttering cardiac region.



Figure 5.2. Percent of all litters that contained one, two or three cubs when observed *in utero* and again as neonates in a) captive and b) wild hyenas examined during pregnancy with ultrasonography. Numbers above bars represent numbers of litters of each type.



Figure 5.3. Matched pairs comparisons within pregnancies of litter size before and after birth in captive and wild hyena populations. Numbers above bars represent numbers of pregnant females examined via ultrasonography. Significant differences are indicated with asterisks.



Figure 5.4. Mean age at which cubs were observed above ground in wild litters for which we had an opportunity to see cubs. Numbers above bars represent litters in each group. Significant differences are noted with an asterisk.



Figure 5.5. Mean litter sizes before and after birth compared between primiparous and multiparous hyenas in captivity and in the wild. Neonatal litter sizes are reported for both ultrasounded and non-ultrasounded hyenas. Numbers above bars represent litters in each group. Significant differences are indicated with asterisks.



Figure 5.6. Percent of wild twin litters of each type born to a) both multiparous and primiparous females in the current study compared to expectations based on a binomial distribution and b) multiparous females from the current study compared to those monitored by Frank et al. (1991). Sample sizes above bars represent number of litters of each type observed.

Figure 5.7. Survivorship of a) 112 singleton cubs, 208 cubs from twin litters, and 6 cubs from triplet litters; b) 26 cubs from all-female litters, 84 cubs from mixed-sex litters, and 36 cubs from all-male litters, and c) 142 individuals from twin litters whose litter-mate lived longer than 3 months and 32 individuals whose twin died before 3 months of age.











Age (months)

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